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Proximate and phytochemical analysis of *Acanthus ilicifolius* hot water extract from three different growing locations

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Abstract. *Batubara R, Hanum TI, Affandi O, Nurminah M, Winarni I, Hannum S, Yasmin OY, Chery SC, Dalimunte A. 2025. Proximate and phytochemical analysis of* Acanthus ilicifolius *hot water extract from three different growing locations. Biodiversitas 26: 1641-1651.* Jeruju (*Acanthus ilicifolius*) is a tide-dependent plant and often obtains more freshwater supply. The chemical composition of this plant needs to be explored so that consumers can obtain the required information, particularly regarding the use of *A. ilicifolius* hot water extract. Therefore, this research aimed to determine proximate and phytochemical content of *A. ilicifolius* from three different growing locations. Proximate analysis was carried out by testing water, ash, fat, and protein composition, while phytochemical tests and GC-MS (Gas Chromatography Mass Spectrometry) were used to determine the number and names of chemical compounds, respectively. The results showed that ash, fat, and protein composition of *A. ilicifolius* from the three sample locations were not statistically different. Based on SNI 3945-2016 and SNI 01-4453-1998, water content met the standard of 2.11-7.47%, while ash content did not fulfill the requirement of 0.56-0.59%. Fat and protein content ranging from 8.58 to 10.22% and 19 to 19.95% had no standard specification. Phytochemical test results of hot water extract showed that the compounds from the three sample locations were the same, including flavonoids, glycosides, steroids, triterpenoids, saponins, and tannins. Based on GC-MS, the main compound in hot water extract of *A. ilicifolius* sampled from Pasar Rawa Village was 2(3H)-Benzoxazolone. In comparison, the main compounds from Percut Village and Paya Pasir Village were found to be dodecanoic acid, 1,2,3-propanediol ester, and dodecyl palmitate.

Keywords: *Acanthus ilicifolius* leaves, compounds, hot water extract, phytochemical, proximate

INTRODUCTION

Mangrove plants are extensively used by communities as food, shelter (Kusmana 2018), and traditional medicine (Herningtyas et al. 2023). Various parts of mangrove, such as leaves, stems, and roots, are traditionally used to treat several diseases, including headaches, asthma, rheumatism, and skin infections. A commonly used species is Jeruju (*Acanthus ilicifolius* L.), which thrives in the center-to-rear zones of mangrove ecosystems (Sandrawati et al. 2023). According to World Health Organization (WHO), over 80% of the global population uses plants as medicine, particularly mangrove (Rahmaniah et al. 2019). In Indonesia, surrounding communities often use *A. ilicifolius* for beverages, a habit influenced by their regular consumption of tea or coffee before starting daily activities (Batubara et al. 2022). As a natural ingredient, tea is widely consumed due to the bioactive flavonoids, which enhance both taste and consumer satisfaction (Fibrianto et al. 2021). Green tea contains antioxidants such as tannins, polyphenols, and flavonoids, which are also present in the bark and leaves of mangrove plants. These compounds can be extracted with hot water to produce natural antioxidants, providing health benefits without harmful effects (Hinokidani et al. 2022).

Acanthus ilicifolius leaves are rich in phytochemicals, including alkaloids, flavonoids, terpenoids, saponins, and phenols (Paul and Ramasubbu 2017). The secondary metabolites, such as alkaloids, saponins, terpenoids, and flavonoids, also show significant cytotoxic activity against cancer cells (Arunita et al. 2023). Methanol and ethanol extracts of *A. ilicifolius* contain steroids, alkaloids, flavonoids, glycosides, tannins, and reducing sugars (Pothiraj et al. 2021; Naher et al. 2022). Specifically, chemical compounds isolated from *A. ilicifolius* include 6-hydroxy-benzoxazolinone, methoxy-4-hydroxy-methyl benzoate, and (Z)-4-coumaric acid 4-O-D-glucopyranoside (Parthiban et al. 2021). Previous research on the methanol extract of the leaves has also identified seven bioactive compounds (Bora et al. 2017).

The traditional use and pharmacological benefits of *A. ilicifolius* have been identified, particularly in managing allergies and helminthiasis (Sardar et al. 2018; Le et al. 2022). Ethanol, water, and n-hexane extracts of *A.*

ilicifolius show anthelmintic activity against *Pheretima posthuma* and *Ascaridia galli* (Husori et al. 2018). Polysaccharides in hot water extract have shown antioxidant activity (Zhang et al. 2014) capable of neutralizing free radicals that are naturally produced as metabolic byproducts (Rahmaniah et al. 2019). Nugraha et al. (2023) also reported the potential as an anticancer agent, with antioxidants playing an essential role in inhibiting carcinogenesis. These compounds are correlated with other bioactivities, including in-vitro and in-vivo cytotoxicity against tumor cells and anticarcinogenic properties. Proximate content in *A. ilicifolius* varies by altitude and other factors such as soil conditions, location, and genetics (Hernández et al. 2024*;* Ojo et al. 2024).

Based on the description, *A. ilicifolius* has been widely used for food and medicine, but there is a lack of specific research on how growing conditions affect proximate content. Therefore, this research aimed to analyze proximate content and chemical compounds in hot water extracts of *A. ilicifolius* from different locations, providing valuable insights into the chemical diversity based on varying growth environments.

MATERIALS AND METHODS

Time and place

The sample was callected in several locations, including (i) Pasar Rawa Village, Gebang, Langkat District (4.008392° N, 98.402903°E), (ii) Percut Village, Percut Sei Tuan, Deli Serdang District (3.714447°N, 98.789514°E), and (iii) Paya Pasir Village, Medan Marelan, Medan City, North Sumatra, Indonesia (3.729168° N, 98.662846° E) (Figure 1). The sample collection occurred during the same period in 2021. The Pharmaceutical Biology Laboratory, Faculty of Pharmacy Universitas Sumatera Utara (USU), Indonesia, performed the characterization and phytochemical screening. The Forest Products Technology Laboratory, Faculty of Forestry, USU, Indonesia, performed extraction and determined the moisture content. Subsequently, GC-MS (Gas Chromatography-Mass Spectrometry) examination was carried out at the Medan Customs Laboratory.

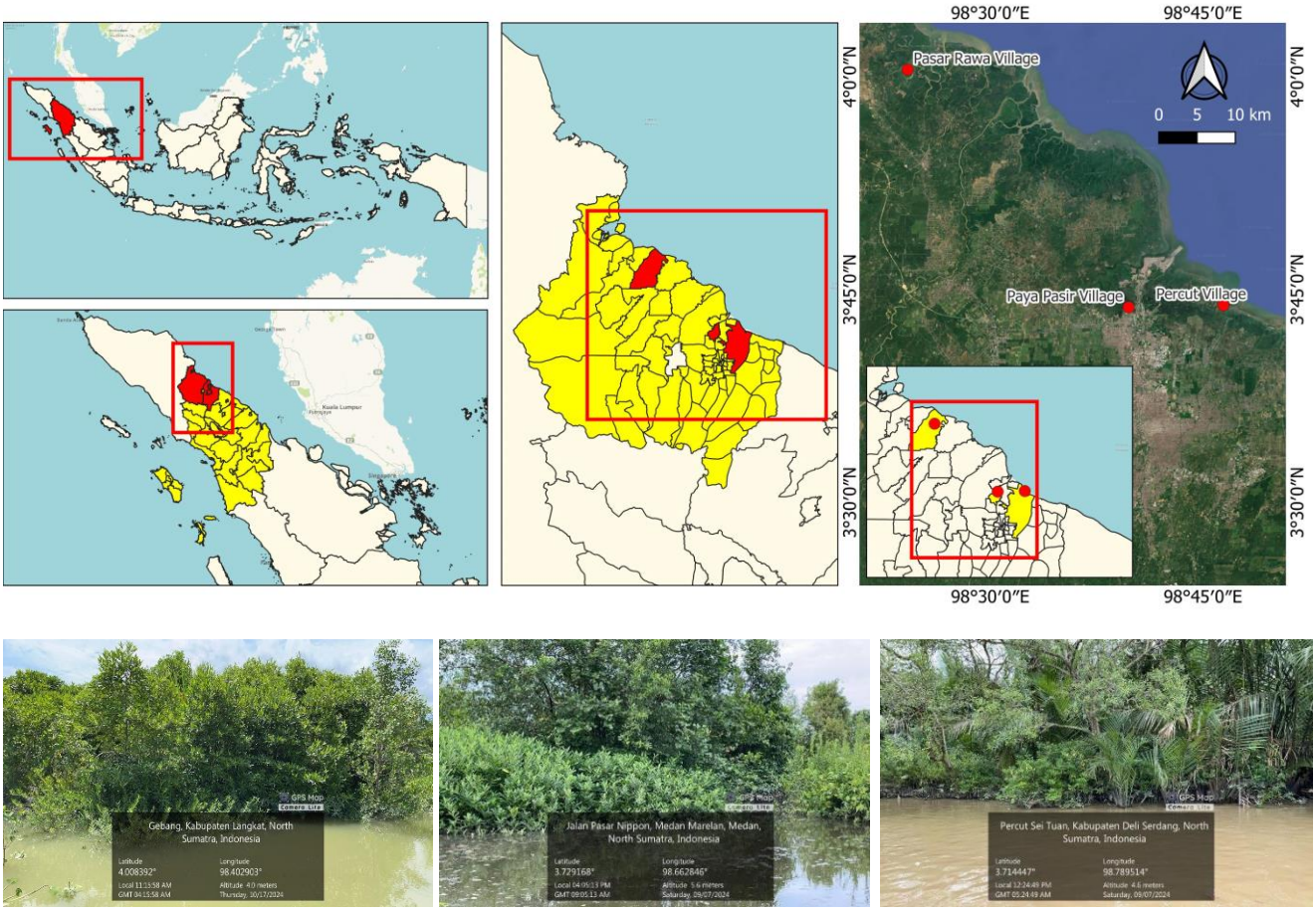

Figure 1. Location of research sample collection in (i) Pasar Rawa Village, Gebang, Langkat District, (ii) Percut Village, Percut Sei Tuan, Deli Serdang District, and (iii) Paya Pasir Village, Medan Marelan, Medan City of North Sumatra, Indonesia

Materials identification

The identification of samples was carried out using reference books to ensure accurate classification at USU Medan Laboratory, part of the Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Indonesia.

Raw material preparation

The fresh leaves of *A. ilicifolius* in Figure 2 were carefully washed under running water to remove dirt and impurities. The samples were placed on parchment paper until the water was absorbed, dried at a controlled temperature of 40-50°C and the powder form was obtained using a grinder (Nurdiani et al. 2023).

Proximate analysis

Moisture and ash content was determined using SNI 01-2891-1992 (BPS 1992) method. Specifically, the moisture content of the sample was determined by drying in an oven at 105°C until a constant weight was achieved and calculated by the formula:

$$Moisture\ Content\ (\%) = \frac{Initial\ weight\ of\ sample\ -\ Final\ weight\ of\ dried\ sample}{Initial\ weight\ of\ sample} x\ 100$$

For ash content, the sample was placed in a crucible and burnt in a muffle furnace at 550°C until all organic matter was burnt, leaving only inorganic residues. Ash content was calculated by the formula:

$$Ash\ Content\ (\%) = \frac{Weight\ of\ ash}{Initial\ weight\ of\ sample} x\ 100$$

Protein content was determined using the SNI 01-0008-1987 (BPS 1987) and the total nitrogen was measured with the Kjeldahl method. This process included digestion of the sample with sulfuric acid in the presence of a catalyst to convert the nitrogen to ammonium sulfate. The ammonium was distilled into a boric acid solution and the amount of nitrogen was determined through titration. Protein content was calculated by the formula:

$$Protein\ Content\ (\%) = Nitrogen\ Content\ (\%) \times 6.25$$

Fat content was evaluated using the MPOB K.1.3.2004 method through solvent extraction, typically with petroleum ether in a Soxhlet apparatus. The dried sample was placed in a thimble and the solvent was continuously cycled through the sample, extracting fat over several hours. After completing the extraction process, the solvent was evaporated and the remaining fat was weighed.

$$Fat\ Content\ (\%) = \frac{Weight\ of\ extracted\ fat}{Initial\ weight\ of\ sample} x\ 100$$

Preparation of *Acanthus ilicifolius* hot water extract

The extract was prepared by brewing 5 g of A. ilicifolius simplicia powder in 250 mL of hot water for 7 minutes. The results of the brewing were filtered using a rotary evaporator at 40°C to obtain a concentrated mass of 10 mL, which was used for phytochemical screening and GC-MS test.

Phytochemical screening

Phytochemical screening is qualitative chemical research of physiologically active chemicals found in *A. ilicifolius* hot water extract. According to the Indonesian Ministry of Health (2000) and Harborne (1987), phytochemical screening is performed to discover alkaloids, glycosides, flavonoids, steroids/terpenoids, tannins, and saponins. In this research, alkaloids were detected using Mayer's method, where the plant extract was treated with hydrochloric acid and Mayer's reagent. The formation of a creamy white precipitate showed the presence of alkaloids. Glycosides were identified through the Borntrager's test for anthraquinone glycosides, where the extract was boiled with sulfuric acid, followed by chloroform addition and ammonia treatment. A pink or red color in the chloroform layer confirmed glycosides.

Flavonoids were tested using the Shinoda test, where the extract was mixed with ethanol, magnesium ribbon, and hydrochloric acid. Based on the results, the appearance of pink or red coloration confirms flavonoids. Steroids were detected with the Salkowski test, where chloroform and sulfuric acid were used and positive results were confirmed by a reddish-brown ring at the interface.

Tannins were identified through the ferric chloride test, where a blue-black or greenish-black color signals hydrolyzable or condensed tannins. Saponins were detected using the foam test, as confirmed by persistent froth upon vigorous shaking with distilled water.

Figure 2. The fresh leaves of *Acanthus ilicifolius* in A. Pasar Rawa Village; B. Percut Village; C. Paya Pasir Village of North Sumatra, Indonesia

GC-MS analysis

GC-MS analysis was carried out at the Medan Customs Laboratory, with instrument's model number GCMS 7890B. Prepared samples were injected into GC inlet using an auto-injector. The inlet was heated to 250-300°C, and the sample was vaporized immediately upon entry. The detector captured ions, generating a unique mass spectrum for each compound. A chromatogram was produced, showing peaks representing each compound and its corresponding mass spectrum.

RESULTS AND DISCUSSION

Proximate analysis of *Acanthus ilicifolius*

Table 1 shows the results of proximate analysis of *A. ilicifolius* leaves, with moisture content of all samples less than 10%, fulfilling simplicia standard. Based on SNI 3945-2016 (BPS 2016) and SNI 01-4453-1998 (BPS 1998), the moisture content percentage of green tea and powdered green tea was below 8%, showing that the three samples tested met the standard. The ash concentration of the three samples was not different, ranging from 0.56 to 0.59%, which did not meet the standard requirement of 4-8% according to SNI 3945-2016 (BPS 2016) and SNI 01-4453-1998 (BPS 1998).

Ash content represents the quantity of metals in plants, while acid-insoluble ash shows the presence of silicates. Generally, metals and silicates are derived from soil and water taken up by plant tissues. Ash content of a sample is determined to identify the amount of internal (physiological ash) and exterior minerals (non-physiological ash) present inside or outside the plant tissue (WHO 2011). In this research, ash content value obtained was satisfactory, which was less than the 8% required for dry simplicia. Analysis of variance (ANOVA) results showed that the differences in the three growing locations of *A. ilicifolius* were not statistically significant at the 95% confidence range for assessing the moisture and ash content of simplicia.

The results showed that fat content of *A. ilicifolius* was not considerably different. The percentage of fat content in green tea has not been set in SNI 3945-2016 (BPS 2016) and SNI 01-4453-1998 (BPS 1998). This is because plant genetic variables (clones) and environmental circumstances have a substantial impact on differences in fat content. Generally, fat is formed by ester complexes of glycerol and fatty acids, with phospholipid compounds, sterols, and pigments playing a significant role in plant and animal tissues (Sumanti et al. 2022). According to the BPOM (2009), fat content criteria is termed low-fat when the value is less than 3%. Fatty acid-derived compounds found in *A. ilicifolius* have been reported (36), which consisted of palmitic acid and octadecanoic acid.

The sample from Percut Village had the greatest protein content, as presented in Table 1, although statistical analysis showed no significant differences. The percentage of green tea protein content has not been determined in SNI 3945-2016 (BPS 2016) and SNI 01-4453-1998 (BPS 1998). Proteins play various roles in plants, serving as catalysts in the reaction process, enzymes for microtubule and microfilament proteins (actin), as well as performing structural functions in ribosomes. Furthermore, proteins facilitate transport during photosynthesis and respiration, serving as food reserves, specifically as amino acid reserves for seedlings after germination (Tillman et al. 2005). The standard protein content of food products regulated by the BPOM (2009) is not less than 2%.

Based on the test results, all samples tend to have relatively the simplicia values except for moisture content as observed in Pasar Rawa Village, which is lower. The variation in result can be caused by various factors, including temperature and drying methods. Purnomo et al. (2023) reported that differences in simplicia drying temperatures and methods affected moisture content, where lower temperatures required a longer time to cause a significant reduction. However, previous research regarding differences in moisture content of simplicia for plants with the same drying method was not found.

Based on previous research, the nutritional value of *A. ilicifolius* is 72.32 (0.64%) water content, 44.72 (21.79%) fiber content, 43.83 (0.34%) protein, 0.58 (0.40%) fat, and 5.03 (0.15%) total ash content, with 76.63 (8.64 g/mL) level of antioxidant activity (Basyuni et al. 2019). Khadeeja et al. (2023) showed that the highest antioxidant content was found in methanol extract with a percentage of 43.2%, followed by water extract at 37.5%, while ethanol and chloroform extract had low percentages. According to Andriani et al. (2020), the presence of antioxidants in *A. ilicifolius* extract antioxidant levels was considered very strong, strong, moderate, weak, and inactive when IC50 value was <10, 10-50, 50-100, 100-250, and >250 µg/mL, respectively.

Other results showed that the nutritional composition of *A. ilicifolius* was 72.32% water, 5.03% ash, 0.58% fat, 43.83% protein, 44.72% fiber, and 76.63 g/ml antioxidant. Zhang et al. (2022) stated that antioxidant content could limit free radical production, reducing the development of oxidative stress. According to Adriani et al. (2020), antioxidant content of *A. ilicifolius* extract was relatively high due to the presence of flavonoids capable of inhibiting free radicals by single electron transfer.

Table 1. Results of proximate analysis of *Acanthus ilicifolius*

Characteristics	Proximate Analysis Result (%)		
	Pasar Rawa Village	Percut Village	Paya Pasir Village
Moisture content	2.11 ± 1.37[a]	7.47 ± 3.28 [a]	4.49 ± 1.53 [a]
Ash content	0.56 ± 0.02 [a]	0.59 ± 0.02 [a]	0.56 ± 0.08 [a]
Fat content	10.22 ± 1.60 [a]	8.58 ± 1.43 [a]	9.60 ± 1.78 [a]
Protein content	19.00 ± 0.80 [a]	19.95 ± 3.89 [a]	19.16 ± 3.54 [a]

Note: Numbers followed by the same letter are not considerably different

Phytochemical screening results

Phytochemical screening results in Table 2 showed no variation in the concentration of chemical compound groups. Specifically, *A. ilicifolius* extract contained the same class of secondary metabolites, including glycosides, flavonoids, steroids, triterpenoids, saponins, and tannins. This research showed that the location of growth did not affect the type of chemical compounds. The three locations were selected because traditional communities had used *A. ilicifolius* as raw material for beverages by brewing with hot water. Firdaus et al. (2023) reported that different high-altitude growing places, specifically environmental conditions, affected the content of secondary metabolite compounds in plants. In this research, the three sampling locations have an altitude between 0-6 meters above sea level, showing that differences in places with different heights did not cause variation in secondary metabolite content in plants. Santoso et al. (2020) identified the medical properties of *A. ilicifolius* in relieving joint pain or rheumatism due to the presence of saponins, flavonoids, and terpenoids which functioned as anti-inflammatory.

One of the compounds found in *A. ilicifolius* was tannins, which were widely recognized as dissolved compounds in tea. The community uses *A. ilicifolius* leaves as a raw material for water extract brewing, thereby benefiting from tannin compounds, which have complex layers functioning as an antioxidant. For gastric diseases, tannins can protect the stomach by increasing defense against irritants and accelerating the tissue repair process due to the content of anti-inflammatory activity (Rizeki et al. 2019). Antioxidant capacity is derived from the phenolic content and several other phytochemicals. According to Indriani et al. (2020), the methanolic *A. ilicifolius* showed the highest antioxidant activity compared to other solvent extracts such as ethanol, chloroform, and ethyl acetate. Antioxidant activity of *A. ilicifolius* extract is related to the capability as radical scavenger by transferring proton to free radical. Antiradical efficiency capacity of *A. ilicifolius* can be classified as medium, although it shows potential as a good and natural antioxidant agent.

Tannins are included in the group of secondary metabolite compounds, which provide significant antibacterial activity (Natarajan et al. 2023). Based on the results, the highest antibacterial activity was seen in *E. coli* and *B. subtilis.* Prananingrum et al. (2022) showed that *A. ilicifolius* extract had significant antifungal properties. Furthermore, a 4% extract of *A. ilicifolius* was shown to inhibit the growth of *C. albanicans* in the specimen and could increase depending on the dose. The presence of flavonoids, alkaloids, glycosides, polyphenols, tannins, and steroids contained in *A. ilicifolius* extract plays a role in antifungal activity. This plant also contains saponins, which are bitter-tasting chemicals, imparting the taste of tea. *Acanthus ilicifolius* extract has anticancer activity obtained from flavonoids, saponins, and terpenoids, with cytotoxic activity value in the isolate showing IC_{50} value of 88.89 ug/mL (Arunita et al. 2023). Sardar et al. (2018) reported the presence of antioxidant, anti-inflammatory, antinociceptive, anticancer, anti-asthma, antimicrobial, anti-diabetic, anti-hyperglycemia, osteoblastic, and activity against neurological disorders.

In traditional medicine, *A. ilicifolius* is used to treat headaches, asthma, rheumatism, skin infections, dyspepsia, paralysis, asthma, and as an antidote to snake bite (Chatterjee et al. 2023). In modern medicine, phytochemical compounds in *A. ilicifolius* are shown to have antioxidant, anti-inflammatory, antimicrobial, and anticancer activities. Flavonoids can inhibit free radicals, while saponins and terpenoids contribute to anti-inflammatory and cytotoxic activities against cancer cells. The leaves extract also shows potential as an effective antifungal and antibacterial agent (Sardar et al. 2018).

GC-MS

Analysis results in Tables 3, 4, and 5 show the chemical composition of *A. ilicifolius* based on the chromatograms in Figures 3, 4, and 5. GC-MS chromatograms show peaks indicating the detection of phytochemicals with different retention times and area percentages. Identification of chemical compounds using the same solvent and detection method shows that secondary metabolites produced by plants vary significantly based on their environmental conditions. The chromatogram shows compounds found in *A. ilicifolius* from three different locations.

Regarding Pasar Rawa Village sample, 74 chemical compounds were detected, mainly consisting of 2(3H)-benzoxazolone ($C_7H_5NO_2$) functioning as both allelochemical and phytoalexin. The compound 2(3H)-benzoxazolone (C7H5NO2) is a class of alkaloids compounds from *A. ilicifolius* extracts, with various types including 2-benzoxazolinone (Minocha and Tiwari 1981; Murty et al. 1984), Acanthiline A (pyrido[1,2-a] indole); 4-hydroxybenzoxazole-2-one (Long et al. 2009), or The Benzoxazinoids reported in *A. ilicifolius* included Benzoxazin-3-one, benzoxazinoid glucosides (Kokpol et al. 1986), with ilicifolius, namely Benzoxazin-3-one, benzoxazinoid glucosides (Kokpol et al. 1986). Several investigations reported the activity of 2(3H)-benzoxazolone derivatives as antimicrobial (Koksal et al. 2002), analgesics (Gokhan et al. 1999), anti-inflammatory (Paramashivappa et al. 2003), anticonvulsant (Ucar et al. 2003), and immunodeficiency virus (HIV) reverse transcriptase (Deng et al. 2006) activities. Moreover, phytoalexin compound is a functional food for humans, characterized by antioxidant, and anti-inflammatory activity. This compound is capable of lowering cholesterol and has anticancer activity that is capable of improving human health (Dawid and Hille 2018).

Table 2. Phytochemical screening results of *Acanthus ilicifolius*

Compounds	Sample origin		
	Pasar Rawa Village	Percut Village	Paya Pasir Village
Alkaloids	-	-	-
Glycosides	+	+	+
Flavonoids	+	+	+
Steroids/triterpenoids	+	+	+
Saponins	+	+	+
Tannin	+	+	+

Note: +: positive for compound, -: negative for compound

Saranya et al. (2015) stated that *A. ilicifolius* extract could reduce blood sugar levels and increase regeneration of pancreatic cells, showing potential for use in treating diabetes. Furthermore, hot water extract of *A. ilicifolius* reduced pain and purify red blood cells. This research showed that *A. ilicifolius* had the potential to be used as a functional food and beverage to provide various benefits for the body.

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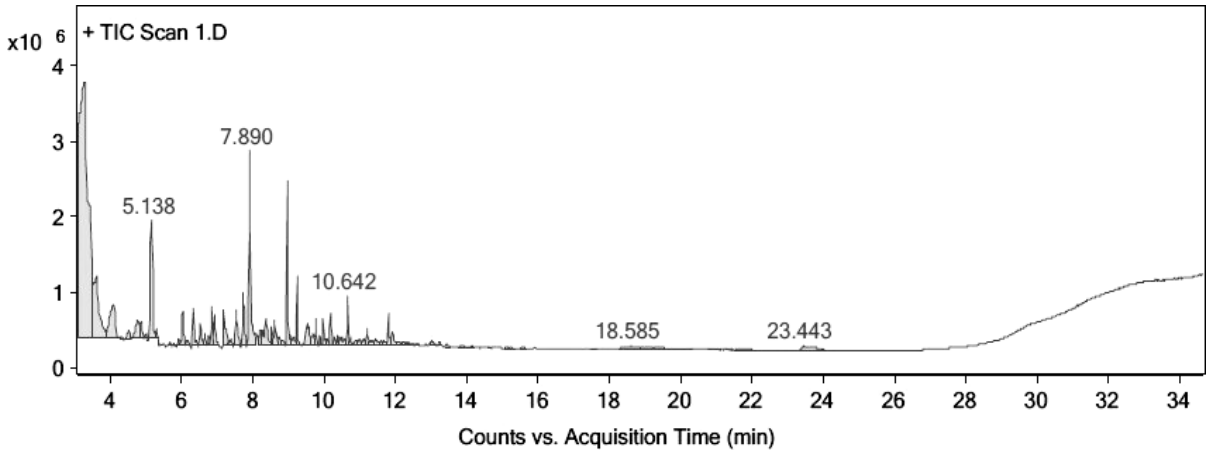

Figure 3. Chromatogram of GC-MS test results of Pasar Rawa Village *Acanthus ilicifolius* hot water extract

Table 3. GC-MS analysis of *Acanthus ilicifolius* from Pasar Rawa Village shows compounds

Retention time (RT)	Compound name	Formula
3.253	N, N-Dimethylglycine	$C_4H_9NO_2$
3.604	1,2,3-Propanetriol	$C_3H_8O_3$
4.066	5-Ethyl-2-furaldehyde	$C_7H_8O_2$
4.306	1,4,7-Trioxonin, (Z,Z,Z)-	$C_6H_6O_3$
4.51	Methyl nicotinate	$C_7H_7NO_2$
4.75	N,N-Dimethyl-l-leucine	$C_8H_{17}NO_2$
4.861	3-Dimethylamino-2,2-dimethyl-1- Propanol	$C_7H_{17}NO$
4.99	Benzenamine, N,N,3-trimethyl-	$C_9H_{13}N$
5.138	Benzofuran, 2,3-dihydro-	C_8H_8O
5.285	Flamenol	$C_7H_8O_3$
5.673	1,4-Benzenediol, 2-methoxy-	$C_7H_8O_3$
5.747	1,4-Benzenediol	$C_6H_6O_2$
5.913	Tetraethoxymethane	$C_9H_{20}O_4$
6.024	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$
6.172	3-Buten-2-one, 4-(5-methyl-2- furanyl)-, (E)-	$C_9H_{10}O_2$
6.32	Phenol, 2,6-dimethoxy-	$C_8H_{10}O_3$
6.505	DL-Proline, 5-oxo-, methyl ester	$C_6H_9NO_3$
6.634	1,3-Dioxolane, 2-ethenyl-4-methyl-	$C_6H_{10}O_2$
6.745	3-Pyridinecarboxamide	$C_6H_6N_2O$
6.837	3-Amino-5-(2-furyl)pyrazole	$C_7H_7N_3O$
6.911	Cyclododecane	$C_{12}H_{24}$
7.169	Benzaldehyde, 2-hydroxy-6-methyl-	$C_8H_8O_2$
7.373	2,4-Dihydroxy-5,6-dimethylpyrimidine	$C_6H_8N_2O_2$
7.52	.beta.-D-Glucopyranose, 1,6-anhydro-	$C_6H_{10}O_5$
7.705	3-Keto-4-aza-2,3-dihydrobenzopyran	$C_8H_7NO_2$
7.890	2(3H)-Benzoxazolone	$C_7H_5NO_2$
8.185	Metacetamol	$C_8H_9NO_2$
8.352	Heptanoic acid, 6-oxo-	$C_7H_{12}O_3$
8.518	Benzothiazole, 2-(methylthio)-	$C_8H_7NS_2$
8.592	3-Methyl-Adenine	$C_6H_7N_5$
8.684	Alanine, 3-(benzyloxy)-, L-	$C_{10}H_{13}NO_3$

8.777	2-Propenoic acid, 3-(4- hydroxyphenyl)-, methyl ester	$C_{10}H_{10}O_3$
8.943	6-Propylbenzo[1,3]dioxol-5-ylamine	$C_{10}H_{13}NO_2$
9.054	2-Furanol, tetrahydro-2,3-dimethyl-, trans-	$C_6H_{12}O_2$
9.22	L-Phenylalanine, N-acetyl-, methyl ester	$C_{12}H_{15}NO_3$
9.515	2-Propenoic acid, 3-(4- hydroxyphenyl)-, methyl ester	$C_{10}H_{10}O_3$
9.682	dihydro - sinapyl alcohol	$C_{11}H_{16}O_4$
9.756	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	$C_{11}H_{16}O_3$
9.866	(S,E)-4-Hydroxy-3,5,5-trimethyl-4-(3- oxobut-1-en-1-yl)cyclohex-2-enone	$C_{13}H_{18}O_3$
9.959	5-Amino-1,4-dihydro-quinoxaline-2,3- dione	$C_8H_7N_3O_2$
10.07	2-(Dimethylaminomethyl)-2-(isopropylsulfanylmethyl)-2- methyl)propan-1-ol	$C_{10}H_{23}NOS$
10.162	Butanoic acid, 4-(4- hydroxyphenylamino)-4-oxo-	$C_{10}H_{11}NO_4$
10.273	1,6-Octadien-3-ol, 3,7-dimethyl-	$C_{10}H_{18}O$
10.365	Methyl 3-(4'-hydroxyphenyl)prop-2- enoate (Identity ?)	$C_{10}H_{10}O_3$
10.439	Spiro[9-Hydro-10-oxophenanthrene-9- oxirane]	$C_{15}H_{10}O_2$
10.642	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$
10.735	Bicyclo[5.2.0]nonane, 4-methylene-2,8,8-trimethyl-2-vinyl-	$C_{15}H_{24}$
10.864	2-(Dimethylamino)ethanol, TMS derivative	$C_7H_{19}NOSi$
10.975	(-)-(R)-1-(2,4,6-Trimethylphenoxy)-2- propanamine	$C_{12}H_{19}NO$
11.086	4-Oxa-1-azabicyclo[3.2.0]heptan-2- one, 6,6-dimethoxy-5-(4- methoxyphenyl)-	$C_{14}H_{17}NO_5$
11.196	trans-Sinapyl alcohol	$C_{11}H_{14}O_4$
11.307	1,2,3,4-tetrahydro-5,8-dihydroxy-1-(4- hydroxybenzyl)-isoquinoline	$C_{16}H_{17}NO_3$
11.418	1,2,4-Triazolo[4,3-a]pyrimidinium, 2,3- dihydro-1,5,7-trimethyl-3-oxo-, hydroxide, inner salt	$C_8H_{10}N_4O$
11.584	syn,syn-2-(.alpha.-Dimethylamino-2- chlorobenzyl)cyclohexan-1-amine	$C_{15}H_{23}ClN_2$
11.658	Tricyclo[3.3.1.0(2,8)]nona-3,6-diene-2,6-diol, 4,8-dimethyl-, diacetate, (.+- .)-	$C_{15}H_{18}O_4$
11.787	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$
11.88	.beta.-D-Mannofuranoside, 1-O-(10- undecenyl)-	$C_{17}H_{32}O_6$
12.046	6-Methyl-2-methoxycarbonyl-4,6-dihydrothieno[3,4-b]thiophene 5,5- dioxide	$C_9H_{10}O_4S_2$
12.212	N-trimethylammonio-2- hydroxypropanamidate	$C_6H4N_2O_2$
12.6	2-Methyl-2H-benzo[b][1,4]oxazin-3(4H)-one	$C_9H_9NO_2$
12.988	Benzyl .beta.-d-glucoside	$C_{13}H_{18}O_6$
13.228	Octadecanoic acid, 3-oxo-, methyl ester	$C_{19}H_{36}O_3$
13.413	(2R)-2-Hydroxy-2-methylpentanoic acid	$C_6H_{12}O_3$
13.819	(1'S,5R)-5-Aminomethyl-3-(1'- phenylethyl)-1,3-oxazolidin-2-one	$C_{12}H_{16}N_2O_2$
13.986	2-Oxoisoindolo[2,1-a]indole	$C_{15}H_9NO$
14.115	N-acetyltryptophan-methyl ester	$C14H_{16}N_2O_3$
15.075	cis-2-(Bromomethyl)-4,4-dimethyl-5- methoxytetrahydrofuran	$C_8H_{15}BrO_2$
17.514	1- Iodoheptacyclo[6.6.0.0(2,6).0(3,13).0(4,11).0(5,9).0(10,14)]tetradecane	$C_{14}H_{15}I$
18.585	1-Naphthaleneacetic acid, 2- (acetyloxy)decahydro-2,5,5,8a-tetramethyl-7-oxo-, [1R- (1.alpha.,2.beta.,4a.beta.,8a.alpha.)]-	$C_{18}H_{28}O_5$
18.991	3-chlor-endo-6,syn-7- diiodbicyclo[3.1.1]hept-3-en-2-yl-4- nitrobenzoate	$C_{14}H10ClI_2NO_4$
19.361	3-chlor-endo-6,syn-7- diiodbicyclo[3.1.1]hept-3-en-2-yl-4- nitrobenzoate	$C_{14}H_{10}ClI_2NO_4$
20.802	4-cyano-1-methyl-5-pyrazol-methylcarboxyla	$C_7H_7N_3O_2$
21.91	2-(5-Hexenyl)-2,5-dihydrofuran	$C_{10}H_{16}O$
23.443	syn-2,3-Dithia-9-[(p-nitrobenzoyl)oxy]bicyclo[4.2.1]nonane S-oxide	$C_{14}H_{15}NO_5S_2$

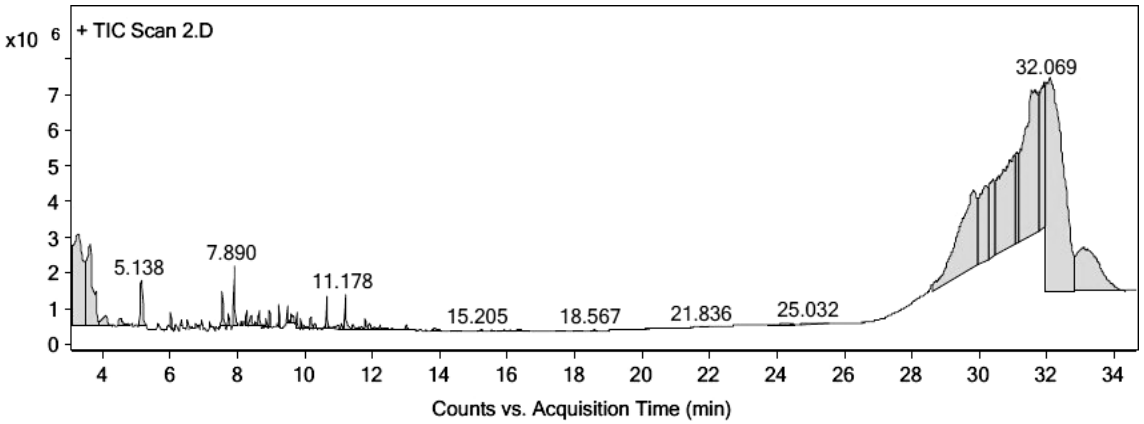

Figure 4. Chromatogram of GC-MS test results of Percut Village *Acanthus ilicifolius* hot water extract

Table 4. GC-MS analysis of *Acanthus ilicifolius* from Percut Village shows compounds

Retention time (RT)	Compound name	Formula
3.272	N,N-Dimethylglycine	$C_4H_9NO_2$
3.623	N,N-Dimethylglycine	$C_4H_9NO_2$
4.085	1-Propanone, 1-(2-furanyl)-	$C_7H_8O_2$
4.509	4H-Pyran-4-one, 2,3-dihydro-3,5- dihydroxy-6-methyl-	$C_6H_8O_4$
5.138	Benzofuran, 2,3-dihydro-	C_8H_8O
5.636	1,2,3-Butanetriol	$C_4H_{10}O_3$
6.006	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$
6.32	Phenol, 2,6-dimethoxy-	$C_8H_{10}O_3$
6.504	DL-Proline, 5-oxo-, methyl ester	$C_6H_9NO_3$
7.169	2-Hydroxy-5-methylisophthalaldehyde	$C_9H_8O_3$
7.262	3-Pentanol, 2,3,4-trimethyl-	$C_8H_{18}O$
7.539	D-Allose	$C_6H_{12}O_6$
7.724	Benzoic acid, 5-hydroxy-2-methoxy-, methyl ester	$C_9H_{10}O_4$
7.89	2(3H)-Benzoxazolone	$C_7H_5NO_2$
8.093	Acetophenone, 3',4'-dimethoxy-	$C_{10}H_{12}O_3$
8.259	Megastigmatrienone 2	$C_{13}H_{18}O$
8.389	Thymidine	$C_{10}H_{14}N_2O_5$
8.518	2-Buten-1-one, 1-(3-hydroxy-2,6,6- trimethyl-1-cyclohexen-1-yl)-, (E)-	$C_{13}H_{20}O_2$
8.629	Megastigmatrienone	$C_{13}H_{18}O$
8.758	beta-D-Glucopyranoside, methyl	$C_7H_{14}O_6$
8.832	Furan-2-carboxaldehyde, 5-(1- piperidyl)-	$C_{10}H_{13}NO_2$
8.924	1-Amino-1-(3,4- methylenedioxyphenyl)propane	$C_{10}H_{13}NO_2$
9.201	L-Phenylalanine, N-acetyl-, methyl ester	$C_{12}H_{15}NO_3$
9.46	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2- methoxyphenol	$C_{10}H_{12}O_3$
9.571	1-Adamantanecarboxylic acid, 2- ethylcyclohexyl ester	$C_{19}H_{30}O_2$
9.737	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-o	$C_{11}H_{16}O_3$
9.866	(S,E)-4-Hydroxy-3,5,5-trimethyl-4-(3- oxobut-1-en-1-yl)cyclohex-2-enone	$C_{13}H_{18}O_3$
9.959	5-Amino-1,4-dihydro-quinoxaline-2,3- dione	$C_8H_7N_3O_2$
10.07	2,5-Bis(N,N-dimethylaminomethyl)pyrrole	$C_{10}H_{19}N_3$
10.162	Butanoic acid, 4-(4- hydroxyphenylamino)-4-oxo-	$C_{10}H_{11}NO_4$
10.273	Benzenamine, 4-(hexyloxy)-	$C_{12}H_{19}NO$
10.402	3 - hydroxy – propanal	$C_3H_6O_2$
10.55	3-Methyl-Adenine	$C_6H_7N_5$
10.624	Methyl palmitate	$C_{17}H_{34}O_2$
10.679	7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3- hydroxy-1-butenyl)-1,5,5-trimethyl-	$C_{13}H_{22}O_3$
11.067	Hexadecanoic acid, ethyl ester	C18H36O2
11.178	Cyclohexanecarboxylic acid, 2- phenylhydrazide	$C_{13}H_{18}N_2O$
11.418	Decanoic acid	$C_{10}H_{20}O_2$
11.566	Methyl exo-6- Formylbicyclo[3.1.0]hexane-6- carboxylate	$C_9H_{12}O_3$
11.658	(S)-(+)-1-(2-Pyrrolidinylmethyl)- pyrrolidine	$C_9H_{18}N_2$
11.769	9-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$
11.88	.beta.-D-Mannofuranoside, 1-O-(10- undecenyl)-	$C_{17}H_{32}O_6$
12.046	6-Methyl-2-methoxycarbonyl-4,6- dihydrothieno[3,4-b]thiophene 5,5- dioxide	$C_9H_{10}O_4S_2$
12.212	Ethyl (9E)-9-Octadecanoate	$C_{20}H_{38}O_2$
12.415	10,11-dihydro-11-oxopyrido[2,3- b]benzo[f][1,4]diazapin	$C_{12}H_9N_3O$
12.582	2-Methyl-4-benzyl-5-(N-(p-benzyloxyphenyl)ethyl)acetamido)oxazole	$C_{28}H_{28}N_2O_3$
12.988	Benzyl .beta.-d-glucoside	$C_{13}H_{18}O_6$
13.801	3-Cyano-3-octyl-1,4-cyclohexadiene	$C_{15}H_{23}N$
14.429	Methyl [4-hydroxy-5-oxofuran-2(5H)- ylidene]acetate	$C_7H_6O_5$
15.205	1-docosanol	$C_{22}H_{46}O$
15.519	Octadecanoic acid, 2-hydroxy-1- (hydroxymethyl)ethyl ester	$C_{21}H_{42}O_4$
15.87	2'-O-Methyl-Adenosine	$C_{11}H_{15}N_5O_4$
16.073	Pyrrolo[3,4-c]pyrrole-1-carboxylic acid, octahydro-3-(4-nitrophenyl)-4,6-dioxo-5-phenyl-1-(phenylmethyl)-, methyl ester, (1.alpha.,3.alpha.,3a.beta.,6a.beta.)- (.+-.)-	$C_{27}H_{23}N_3O_6$
16.313	Caruilignan D	$C_{14}H_{16}O_6$
17.551	Bis(1-adamantyl)acetyl chloride	C22H31ClO
18.567	2-Methyl-2-cyclopenten-1-ol	$C_6H_{10}O$
24.182	Glyceryl Tridodecanoate	$C_{57}H_{10}O_6$
25.032	2-[(5E)-3-(trimethylsilyl)-5- heptenyl]cyclohexanone	$C_{16}H_{30}Osi$
29.816	Dodecanoic acid, 1,2,3-propanetriyl ester	$C_{39}H_{74}O_6$
30.185	Glyceryl Tricodecanoate	$C_{57}H_{10}O_6$
30.998	Dodecanoic acid, 1,2,3-propanetriyl ester	$C_{39}H_{74}O_6$
31.645	Dodecanoic acid, 1,2,3-propanetriyl ester	$C_{39}H_{74}O_6$
32.069	Dodecanoic acid, 1,2,3-propanetriyl ester	$C_{39}H_{74}O_6$
33.104	Dodecanoic acid, 1,2,3-propanetriyl ester	$C_{39}H_{74}O_6$

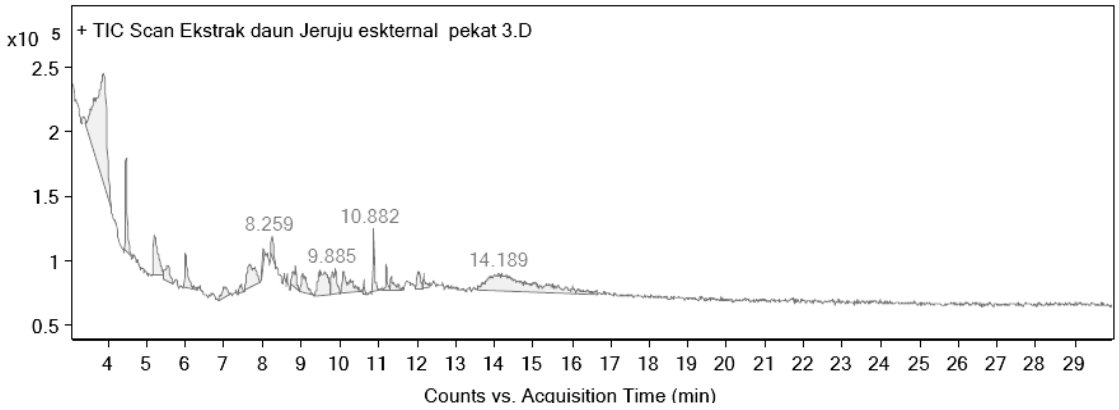

Figure 5. Chromatogram of GC-MS test results of Paya Pasir Village *Acanthus ilicifolius* hot water extract

Table 5. GC-MS Analysis of *Acanthus ilicifolius* from Paya Pasir Village Shows Compounds

Retention time (RT)	Compound name	Formula
4.473	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	$C_6H_8O_4$
5.193	4-vinylphenol	C_8H_8O
6.006	4-vinyl-guaiacol	$C_9H_{10}O_2$
7.022	2-Methylene-4-phenybut-3-yne-1-ol	$C_{11}H_{10}O$
7.447	3-Butyn-2-ol, 2-methyl-	C_5H_8O
7.668	Desulphosinigrin	$C_{10}H_{17}NO_6S$
8.019	3H-Benzooxazol-2-one	$C_7H_5NO_2$
8.259	4-hydroxy-3-methoxy-benzoic acid	$C_8H_8O_4$
9.035	anti-7-Isopropylbicyclo[2.2.1]heptane-2,3-dione-(Z)-monotosyl-hydrazone	$C_{17}H_{22}N_2O_3S$
9.497	2-(.alpha.-Ethoxyacetyl)pyridine	$C_9H_{11}NO_2$
9.885	2,6-Di-t-butyl-4-acetoxyphenol	$C_{16}H_{24}O_3$
10.107	Benzoic acid, 4-hydroxy-3,5-dimethoxy-	$C_9H_{10}O_5$
10.624	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$
10.882	Dodecylpalmitate	$C_{28}H_{56}O_2$
11.196	(1'R,6S)-2-Methyl-6-(4-methylcyclohexa-2,4-dienyl)hept-2-en-4-one	$C_{15}H_{22}O$
12.175	Dimethyl 2-Isobutyl-c-5-[2'-(1",3"- Dioxolan-2"-yl)ethyl]pyrrolidin-r-2,c-4-Dicarboxylate	$C_{17}H_{29}NO_6$

Regarding hot water extract, samples from Percut Village had the highest compound consisting of 64, followed by Pasar Rawa and Paya Pasir Villages comprising 58 and 16 compounds, respectively. A total of 138 compounds were identified on the three samples tested, comprising only 13.04% similar components. The results of phytochemical screening did not show significant differences in secondary metabolite compounds in samples from three different locations. However, *A. ilicifolius* used had highly varying components based on GC-MS test. Rahmiyani et al. (2023) reported that some compounds identified in GC-MS test could be different at various sampling locations, with significant changes over time. Based on quantity, differences occurred due to variations in soil chemical parameters at the growing location. In Percut Village location, *A. ilicifolius* plants were taken from between mangrove stands that were constantly inundated with seawater, directly adjacent to the sea. Furthermore, in Paya Pasir Village, *A. ilicifolius* plants were taken from land and converted into ponds. When the 2(3H)-benzoxazolone was high, seawater entered the location where *A. ilicifolius* grew and inundated the plants. Meanwhile, *A. ilicifolius* from Pasar Rawa Village was obtained from mangrove forest stands whose conditions were influenced by tides, as shown in Figure 1. Based on the growing conditions, the soil samples from the research location differed from the physical, chemical, and biological properties of the soil.

The main compound in the sample from Percut Village was Dodecanoic acid, 1,2,3-propanetriyl ester, which provided mild aroma and sweet flavor. Dodecanoic acid, a 1,2,3-propanetriyl ester compound is categorized as the main compound based on the highest chromatogram peak in Figure 4, which is at a retention time of 32.069 minutes. According to GC-MS test results, the data obtained did not show the percentage of each component, thereby limiting further review. In this research, dodecyl palmitate is the major component in Paya Pasir Village, widely recognized as a good source of calories with antioxidant potential. Mangrove hot water extract production from *A. ilicifolius* has been established in various Indonesian settlements. This is consistent with the customs of the surrounding

population, which often drinks tea or coffee before engaging in daily activities. Similarly, in the mangrove beach area at North Sumatra, *A. ilicifolius* hot water extract is available and can be enjoyed by visitors. This hot water extract is produced innovatively with various flavors to increase enjoyment such as lemongrass, ginger, pandanus, and bunga telang flavors. This plant is used in India and China for treatments such as dyspepsia, paralysis, and asthma, with leaves possessing therapeutic properties for treating poison from snake bites (Chatterjee et al. 2023).

In conclusion, this research showed that proximate content of *A. ilicifolius* and a class of chemical compounds did not change between the three growing locations. However, further investigation showed a significant variation in the chemical compounds found in *A. ilicifolius* leaves.

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REFERENCES

Andriani D, Syamsulina R, Widyasari P. 2020. Identification of compounds isolated from a methanolic extract of *Acanthus ilicifolius* leaves and evaluation of their antifungal and antioxidant activity. Biodiversitas 21 (6): 2521-2525. DOI: 10.13057/biodiv/d210625.

Arunita AV, Rahayu HSE, Pribadi P. 2023. The effectiveness of jeruju plant extract *(Acanthus ilicifolius)* as anticancer: Literature review. Gaster 21 (1): 91-99. DOI: 10.30787/gaster.v21i1.865.

Badan Pengawas Obat Dan Makanan Republik Indonesia [BPOM]. 2009. Informasi Nilai Gizi Produk Pangan. [Indonesian]

Badan Pusat Statistik [BPS]. 1987. SNI 01-0008-1987. Bungkil Inti Kelapa Sawit. Badan Standardisasi Nasional, Jakarta. [Indonesian]

Badan Pusat Statistik [BPS]. 1992. SNI 01-2891-1992. Cara Uji Makanan dan Minuman. Badan Standardisasi Nasional, Jakarta. [Indonesian]

Badan Pusat Statistik [BPS]. 1998. SNI 01-4453-1998. Teh Hijau Bubuk. Badan Standardisasi Nasional, Jakarta. [Indonesian]

Badan Pusat Statistik [BPS]. 2016. SNI 3945:2016. Teh Hijau. Badan Standardisasi Nasional, Jakarta. [Indonesian]

Basyuni M, Siagian B. Slamet N, Sulistiyono LA, Putri E, Yusraini, Lesmana. 2019. Leaf nutrition content and organoleptic of Jeruju (*Acanthus ilicifolius* L) and processed products in Lubuk Kertang Village, North Sumatera. IOP Conf Ser: Earth Environ Sci 374 (1): 012052. DOI: 10.1088/1755-1315/374/1/012052.

Batubara R, Aulia K, Hanum TI, Affandi O, Ulfa M. 2022. Antioxidant activity and microbial contamination of jeruju leaf tea with ginger and lemongrass variant packaging in storage period. IOP Conf Ser: Earth Environ Sci 1115 (1): 012102. DOI: 10.1088/1755-1315/1115/1/012102.

Bora R, Adhikari PP, Das AK, Raaman N, Sharma GD. 2017. Ethnomedicinal, phytochemical, and pharmacological aspects of Genus *Acanthus*. Intl J Pharm Pharm Sci 9 (12): 18-25. DOI: 10.22159/ijpps.2017v9i12.22386.

Chatterjee T, Chakrabarty S, Roy S, Biswas O, Mitra B, Chaudhuri P. 2023. Spatial and temporal variation of floral visitors and their visitation pattern on *Acanthus ilicifolius* L.: a case study from the mangrove ecosystem of Indian Sundarbans. J Insect Conserv 27 (3): 1-11. DOI: 10.1007/s10841-023-00472-0.

Dawid C, Hille KM. 2018. Functional metabolomics—a useful tool to characterize stress-induced metabolome alterations opening new avenues towards tailoring food crop quality. Agronomy 8 (8): 138. DOI: 10.3390/agronomy8080138.

Deng BL, Cullen MD, Zhou Z, Hartman TL, Buckheit Jr RW, Pannecouque C, De Clercq E, Fanwick EP, Cushman M. 2006. Synthesis and anti-HIV activity of new alkenyldiarylmethane (ADAM) non-nucleoside reverse transcriptase inhibitors (NNRTIs) incorporating benzoxazolone and benzisoxazole rings. Bioorg Med Chem 14 (7): 2366-2374. DOI: 10.1016/j.bmc.2005.11.014.

Fibrianto K, Muthifalyza R, Yuwono SS. 2021. Consumer study of Indonesian coffee leaves tea as traditional drinks. IOP Conf Ser Earth Environ Sci 924 (1): 012030. DOI: 10.1088/1755-1315/924/1/012030.

Firdaus IWAK, Rahmah A, Apriasari ML, Wasiaturrahmah Y. 2023. Quantitative phytochemical test of methanol extract of tabat barito leaves (*Ficus deltoidea* Jack.). Dentino 8 (1): 76-80. DOI: 10.20527/dentino.v8i1.16085.g9023.

Gokhan N, Erdogan H, Durlu T, Demirdamar R. 1999. Analgesic activity of acylated 2- benzoxazolinone derivatives. Il Farmaco 54 (1-2): 112-115. DOI: 10.1016/S0014-827X(98)00111-6.

Harborne JB. 1987. Metode fitokimia: penuntun cara modern menganalisis tumbuhan. Penerbit ITB, Bandung. [Indonesian]

Hernández PA, Samaniego I, Leguizamo A, Garcia MJB, Yuste MCA. 2024. Nutritional and functional properties of quinoa (*Chenopodium quinoa* Willd.) chimborazo ecotype: insights into chemical composition. Agriculture 14: 396. DOI: 10.3390/agriculture14030396.

Herningtyas W, Rahmila YI, Pujiono E. 2023. Mangrove plants as traditional medicine by local coastal communities in Indonesia. IOP Conf Ser: Earth Environ Sci 1266 (1): 012006. DOI: 10.1088/1755-1315/1266/1/012006.

Hinokidani K, Aoki R, Irie M, Nakanisha Y. 2022. Usability of mangrove plant leaves as tea materials: A comparison study on phenolic content and antioxidant capacities with commercial teas. Biocatalysis Agric Biotechnol 40: 102307. DOI: 10.1016/j.bcab.2022.102307.

Husori DI, Sumardi, Heron T, Selviani G, Sri RN. 2018. In Vitro anthelmintic activity of *Acanthus Ilicifolius* leaves on *Ascaridia galli* and *Pheretima posthuma*. J Appl Pharm Sci 8 (2): 164-167. DOI: 10.7324/JAPS.2018.8224.

Indonesian Ministry of Health. 2000. General Standard Parameters of Medicinal Plant Extracts. First Printing. [Indonesian]

Indriani D, Revianti S, Prananingrum W. 2020. Identification of compounds isolated from a methanolic extract of *Acanthus ilicifolius* leaves and evaluation of their antifungal and antioxidant activity. Biodiversitas 21 (6): 2531-2525. DOI: 10.13057/biodiv/d210625.

Khadeeja S, Johney J, Ragunathan R. 2023. Evaluation of antioxidant, antimicrobial, anticancer, and wound healing properties of leaf extracts of *Acanthus Ilicifolius* L. Intl J Curr Pharm Res 15 (1). DOI: 10.22159/ijcpr.2023v15i1.2066.

Kokpol U, Chittawong V, Miles DH. 1986. Chemical constituents of the roots of *Acanthus illicifolius*. J Nat Prod 49 (2): 355-6. DOI: 10.1021/np50044a033.

Koksal M, Gökhan N, Erdogan H, Özalp M, Ekizoglu M. 2002. Synthesis of 3-(4- substituted benzoylmethyl)-2- benzoxazolinones and screening antimicrobial activities. Il Farmaco 57: 535-538. DOI: 10.1016/S0014-827X(02)01244-2.

Kusmana C. 2018. Mangrove plant utilization by local coastal community in Indonesia. IOP Conf Ser: Earth Environ Sci 196 (1): 012028. DOI: 10.1088/1755-1315/196/1/012028.

Le L, Nguyen NA, Thi LTU, Nguyen TTL. 2022. The effects of mangrove plant leaves (*Acanthus ilicifolius*) collected in can Gio district on white mice (*Mus musculus* var. *albino*) with diabetes. J Etmol Zool Stud 10 (6): 54-59. DOI: 10.22271/j.ento.2022.v10.i6a.9127.

Long S, Wei X, Chen Y, Zheng G, Li, X, Peng X, Li S. 2009. Preparation and application of *Acanthus ilicifolius* alkaloid A and its derivative with anti-inflammatory and analgesic activities. Faming Zhuanli Shenqing Gongkai Shuomingshu.

Minocha PK, Tiwari KP. 1981. A triterpenoidal saponin from roots of *Acanthus illicifolius*. Phytochemistry 20 (1): 135-7. DOI: 10.1016/0031-9422(81)85232-6.

Murty MS, Solimabi S, Kamat SY. 1984. Isolation of 2-benzoxazolinone from *Acanthus ilicifolius*. Indian J Pharm Sci 46: 218-9. DOI: 10.5555/19850332630.

Naher K, Moniruzzaman M, Islam S, Hasan A, Paul GK, Jabin T, Biswas S, Zaman S, Saleh MA, Uddin MS. 2022. Evaluation of biological activity and in silico molecular docking studies of *Acanthus ilicifolius* leaf extract against four multidrug-resistant bacteria. Informatics Med Unlocked 33 (1): 1-10. DOI: 10.1016/j.imu.2022.101092.

Natarajan S, Rameshbabu V, Aridharasudhan S, Hayavadana J, Rajpurohit T, Prakash C. 2023. Antimicrobial properties of Acanthus ilicifolius and determination of phytochemical composition. Biomass Conversion Biorefinery 14 (23): 29719-29726. DOI: 10.1007/s13399-023-04723-7.

Nugraha AS, Firli LN, Rani DM. 2023. Indonesian marine and its medicinal contribution. Nat Prod Bioprospecting 13 (38): 1-27. DOI: 10.1007/s13659-023-00403-1.

Nurdiani R, Prihanto A, Firdaus M, Aini FA, Nabilah FA, Talib RA, Huda N. 2023. Physicochemical characteristics of Pangasius sp. skin-gelatin-based-edible film enriched with silver nanoparticles. F1000Research 12: 160. DOI: 10.12688/f1000research.129024.1.

Ojo CA, Dziadek K, Sadowska U, Skoczylas J, Kopec A. 2024. Analytical assessment of the antioxidant properties of the coneflower (*Echinacea purpurea* L. Moench) grown with various mulch materials. Moleculs 29: 971. DOI: 10.3390/molecules29050971.

Paramashivappa R, Kumar PP, Subbarao PV, Srinivasarao A. 2003. Design, synthesis and biological evaluation of benzimidazole/benzothiazole and benzoxazole derivatives as cyclooxygenase inhibitors. Bioorg Med Chem Lett 13: 657-660. DOI: 10.1016/S0960-894X(02)01006-5.

Parthiban A, Sivasankar R, Sachithanandam V, Khan SA, Jayshree A, Murugan K, Sridhar R. 2021. An integrative review on bioactive compounds from Indian mangroves for future drug discovery. South Afr J Bot 149: 899-915. DOI: 10.1016/j.sajb.2021.10.004.

Paul T, Ramasubbu S. 2017. The antioxidant, anticancer and anticoagulant activities of *Acanthus ilicifolius* L. roots and *Lumnitzera racemosa* Willd. leaves, from southeast coast of India. J Appl Pharm Sci 7 (3): 81-87. DOI: 10.7324/JAPS.2017.70313.

Pothiraj C, Balaji R, Shanthi M, Gobinath R, Babu A, Al-Dosary M, Ashrafe KR, Kumar V, Veeramanikandan, Arumugam. 2021. Evaluating antimicrobial activities of *Acanthus ilicifolius* L. and *Heliotropium curassavicum* L against bacterial pathogens: An in-vitro study. J Infect Public Health 14: 1927-1934. DOI: 10.1016/j.jiph.2021.10.013.

Prananingrum W, Rahmitasari F, Andriani D, Razak A. 2022. The effect of effervescent *Acanthus ilicifolius* L. extract on antifungal properties and flexural strength acrylic. Dental J 9 (2): 310-318. DOI: 10.30659/odj.9.2.310-318.

Purnomo D, Putri AY, Hisyam HM, Rimatunnisa R, Indriani DR, Adinda PR, Hasanah YS, Hamad A. 2023. Effect of drying method on antioxidant activity and total flavonoid content of java tea crude drug *(Orthosiphon aristatus)*. Res Chem Eng 2 (1): 29-33. DOI: 10.30595/rice.v2i1.87.

Rahmaniah A, Salni, Widjajanti H. 2019. Antioxidant activity of the secondary metabolites produced by endophytic fungi isolated from Jeruju *(Acanthus ilicifolius L.)* plant. Biol Res J 5 (2): 14-19. DOI: 10.24233/BIOV.5.2.2019.153.

Rahmiyani I, Yuliana A, Rukmana DH, Tuslinah L, Nurviana V. 2023. α-mangostin content of mangosteen leaves (*Garcinia mangostana* L.) based on different growing conditions. Planta Tropika 11 (2): 160-167. DOI: 10.18196/pt.v11i2.16848.

Rizeki E, Safrida S, Supriatno. 2019. Ehanol extract from *Achantus Ilicifolius* L. leaves as antiinflammatory ulcers in *Mus musculus* L. J Phys: Conf Ser 1460 (1): 012056. DOI: 10.1088/1742-6596/1460/1/012056.

Sandrawati N, Ningsih W, Layla R, Putra AE, Ismed F, Tallei TE, Handayani D. 2023. Endophytic fungi from mangrove plant *Acanthus ilicifolius* L.: Antimicrobial, anticancer, and species determinatio. Trends Sci 20 (7): 1-9. DOI: 10.48048/tis.2023.5089.

Santoso P, Yuda PESK, Dewi NLKAA. 2020. Analgesic activity test of ethyl acetate, n-hexane, water fractions of cemcem leaves extract (*Spondias pinnata* (Linn.f.) Kurz.) as part of Usadha Bali. Intl J Health Med Sci 3(1): 48-53. DOI: 10.31295/ijhms.v3n1.134.

Saranya A, Ramanathan T, Kesavanarayanan KS, Adam A. 2015. Traditional medicinal uses, chemical constituents and biological activities of a mangrove plant, *Acanthus ilicifolius* Linn.: A brief review. Am-Eurasian J Agric Environ Sci 15 (2): 243-250. DOI: 10.5829/idosi.aejaes.2015.15.2.12529.

Sardar PKS, Dev MA, AlBari S, Paul MS, Yeasmin AK, Das, Biswas. 2018. Antiallergic, anthelmintic and cytotoxic potentials of dried aerial parts of *Acanthus ilicifolius* L. Clin Phytosci 4 (1): 34. DOI: 10.1186/s40816-018-0094-7.

Sumanti DM, Hanidah II, Abdullatif MA. 2022. Physical, chemical, and functional characteristics of composite flours from banana corm and tempeh. Industrial Jurnal Teknologi dan Manajemen Agroindustri 11 (2): 139-150. DOI: 10.21776/ub.industria.2022.011.02.5.

Tillman AD, Hartadi H, Reksohadiprodjo S, Prawirokusumo S, Lebdosoekojo S. 2005. Ilmu Makanan Ternak Dasar. Gadjah Mada University Press, Yogyakarta. [Indonesian]

Ucar H, Van derpoorten K, Cacciaguerra S, Spampinato S, Stables JP, Depovere P, Isa M, Masereel B, Delarge J, Poupaert JH. 2003. Synthesis and anticonvulsant activity of 2(3H)-benzoxazolone and 2(3H)-benzothiazolone derivatives. J Med Chem 41 (7): 1138-1145. DOI: 10.1021/jm970682+.

World Health Organization [WHO]. 2011. Quality Control Methods for Medicinal Plant Material. Switherland.

Zhang TY. Tian B, Jiang M, Miao W, Mu. 2014. Purification, preliminary structural characterization and in vitro antioxidant activity of polysaccharides from *Acanthus ilicifolius*. LWT- Food Sci Technol 56: 9-14. DOI: 10.1016/j.lwt.2013.11.010.

Zhang Y, Shen J, Ma X, Yao M, Zhang Y, Cao D. 2022. Anti-infammatory and antioxidant activities of acteoside isolated from *Acanthus ilicifolius* var. *Xiamenensis*. Appl Biol Chem 65 (1): 29. DOI: 10.1186/s13765-022-00695-w.