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Identification, screening, and analysis of secondary metabolite content in methanol extracts of ant nests plant tubers from Aceh, Indonesia

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Abstract. *Roslizawaty R, Gholib G, Rahmi N, Khairan K, Idroes R, Syafruddin S, Abrar M. 2023. Identification, screening, and analysis of secondary metabolite content in methanol extracts of ant nests plant tubers from Aceh, Indonesia. Biodiversitas 24: 6934-6941.* This study aims to identify the chemical components obtained from extracting and partitioning methanol extracts from ant nest tubers (*Myrmecodia* sp.) originating from Aceh Jaya and Aceh Besar. These extracts were identified using phytochemical screening, Fourier Transform Infrared (FT-IR), and Gas Chromatography-Mass Spectroscopy (GC-MS) methods. The identification results showed that the ant nest plant from Aceh Jaya belongs to the species *Myrmecodia tuberosa* Jack. The ant nest plant from Aceh Besar belongs to the species *Hydnophytum formicarum* Jack. Phytochemical screening results showed that the methanol extract from Aceh Jaya's ant nest tubers contained flavonoids, tannins, saponins, and terpenoids, while Aceh Besar's ant nest tubers contained flavonoids, tannins, terpenoids. GC-MS analysis revealed that the major compound found in the methanol extract from Aceh Jaya's ant nest tubers was 2H-oxecin-2-one,3,4,7,8,9,10-hexahydro-4-hydroxy-10-methyl-,[4S-(4R*,5E,10S*)]- constituting 50.63% of the extract. In comparison, the primary compound in the methanol extract from Aceh Besar was resorcinol at 17.92%. FT-IR analysis indicated that the FT-IR spectra of the methanol extracts from Aceh Jaya's ant nest tubers contained O-H, C-H, C=O, C=C alkene, and C=C aromatic groups. At the same time, methanol extracts from Aceh Besar's ant nest tubers also contained O-H, C-H, C=O, C=C alkene, and C=C aromatic groups.

Keywords: Ant nest plant, GC-MS, medicinal plants, methanol extract, *Myrmecodia*, secondary metabolites

INTRODUCTION

Indonesia is considered the second most biodiverse country after Brazil, with an estimated 10% or around 25,000 plant species of flowering plants found within its borders, while there are approximately 28.000 plant species (Astirin 2000). Most of these Indonesian flowering plant species are known to have medicinal properties, which are deep-rooted in Indonesian society (von Rintelen et al. 2017). Plants serve as a source of chemical compounds, including primary and secondary metabolite compounds. Secondary metabolites are chemical compounds typically known for their bioactivity and role in defending against unfavorable environmental conditions such as temperature, climate, pests, and plant diseases. They can also treat various human illnesses (Ping et al. 2013). One method used to assess the content of secondary metabolites is phytochemical screening.

Phytochemical screening studies the active compound components in a sample, including their chemical structure, biosynthesis, natural distribution, biological functions, isolation, and chemical composition comparison among various plant species. The common compounds screened in this experiment are alkaloids, flavonoids, tannins, terpenoids/ steroids, and saponins. One plant known for its medicinal properties and believed to contain secondary metabolites is the ant nest plant. Ant nest plants are currently deemed suitable as raw materials for both modern and traditional medicine, with tubers being part of the plant used for medicinal purposes (Dirgantara et al. 2022).

Therefore, the fractions were tested through phytochemical screening and Gas Chromatography-Mass Spectroscopy (GC-MS) analysis to determine the compound content in the ant nest fractions. This was done to compare the results of qualitative phytochemical screening with the results of quantitative tests using GC-MS chromatography. In addition, GC-MS analysis is used to identify compound components present in plant cells that cannot be done using ordinary phytochemical screening (Baeshen et al. 2023). Other characterization analyses can also use Fourier Transform Infra-Red (FTIR). This characterization analysis is quite fast, accurate, and relatively sensitive. This type of analysis

can characterize samples in the form of liquids, solutions, pastes, powders, films, fibers, and gases. This analysis also makes it possible to analyze material on the surface of the substrate (Barnes et al. 2023). GCMS results report that the phenolic structure of *Myrmecodia* sp. includes kaempferol, luteolin, routine, quercetin, apigenin, rosmarinic acid, procyanidin B1, gallic acid, catechin, caffeic acid, p-coumaric acid, and ferulic acid (Dirgantara et al. 2022), while the FTIR test on M. pendens extracts from the n-butanol fraction has succeeded in isolating an isolated xanthine oxidase inhibitor with an IC_{50} of 79.77% (Simanjuntak et al. 2010).

Ant nest plants are widely found in the Aceh Province and are commonly utilized as an alternative traditional treatment by the local population. The phytochemical screening of Aceh's ant nest tubers (*Myrmecodia* sp.) has revealed the presence of secondary metabolites such as phenolic compounds, saponins, triterpenoids, and steroids (Frengki et al. 2014). Ant nest tubers have the potential to act as anti-hyperglycaemic agents, enhance immunologic responses, and exhibit antibacterial properties (Efimenko et al. 2020). However, research and scientific publications on Aceh's ant nest plants are currently limited, and reports on their medicinal properties are scarce. Therefore, this study aims to conduct phytochemical screening of methanol extracts from Aceh's ant nest plant, Aceh Besar (representing the lowland area) and Aceh Jaya (representing the coastal area). In this research, methanol extracts from Aceh's ant nest tubers will undergo partitioning based on differences in polarity, from non-polar to polar. This research is expected to serve as an initial step in understanding the active compound contents within ant nest tubers that play an active role in disease healing.

MATERIALS AND METHODS

The ant nest tubers used in this research had been identified (determination test) at Herbarium Bogoriense, Direktorat Pengelolaan Koleksi Ilmiah BRIN Cibinong. The research on the screening and analysis of secondary metabolite content in methanol extracts of ant nest tubers (*Myrmecodia* sp.) from Aceh was conducted at the Natural Products Pharmacy Laboratory, Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Syiah Kuala University. FT-IR (Fourier Transform-Infrared) and GC-MS (Gas Chromatography-Mass Spectroscopy) analyses were conducted at the Instrumentation Laboratory, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Syiah Kuala University. The research was conducted from July to December 2022.

Preparation of ant nest tubers and sampling method

The ant nest plant specimens used in this research were the tubers of ant nest plants native to Aceh, obtained from the tropical forests of Aceh Province, specifically from Aceh Besar (representing the lowland region) and Aceh Jaya (representing the coastal area). Lowland areas are defined as those with an altitude of <400 masl (meters above sea level, while the coastal Areas fall within the range of 401-800 masl (Lestari et al. 2021). The sampling of these two specimens was based on their respective altitudes. The sampling method involved observing fresh ant nest plants that met specific criteria, including the tubers being neither too young nor old, well-formed tuber shapes, brown coloration, non-wilted and non-wrinkled skin, and a firm texture. Details of the plant's habitat, tuber color, odor, taste, and other distinctive features were recorded.

Preparation of simplicia

Intact tubers of ant nest plants were cleaned, washed, drained, and then sliced thinly. Drying was carried out naturally, meaning they were dried indirectly under sunlight. The duration of drying varied but lasted approximately 10 days. Once the ant nest tubers had dried, they were cut into small pieces and ground into a fine powder using a blender. The finely blended result was sieved and placed into clean jars. Simplicia characterization was conducted following the guidelines of Sriyanti et al. (2022).

Extraction

Approximately 2,000 g of simplicia powder from Aceh Jaya's ant nest tubers and 1,500 g of simplicia powder from Aceh Besar's ant nest tubers were placed in maceration containers and dissolved in 75 ppm of methanol. The mixture was occasionally stirred in closed containers and left undisturbed for 5 days, shielded from light, with occasional stirring. After 5 days, the sample was filtered, and the filtered residue was subjected to maceration again with 25 parts of methanol. This mixture was left for 2 days. The macerate was evaporated using a rotary evaporator at 45°C until a concentrated extract was obtained. Extract characterization was conducted following the guidelines of Heinrich et al. (2022).

Phytochemical analysis of methanol extracts from tubers ant nest plant from Aceh Jaya and Aceh Besar

Phytochemical screening for identifying flavonoids, terpenoids, and steroids was conducted following the guidelines of Sriyanti et al. (2022). The identification of alkaloids and saponins was performed according to the guidelines of Heinrich et al. (2022), while the identification of tannins was carried out by Sriyanti et al. (2022).

FT-IR analysis (Fourier Transform Infrared) and GC-MS Analysis (Gas Chromatography-Mass Spectrometry)

FT-IR analysis followed Sriyanti et al. guidelines (Sriyanti et al. 2022). GC-MS analysis was performed using Gas Chromatography-Mass Spectrometry with the Thermo Scientific ISQ 7000 Single Quadrupole GC-MS instruments.

RESULTS AND DISCUSSION

Phytochemical screening

The results of identifying herbarium samples at Herbarium Bogoriense, Direktorat Pengelolaan Koleksi Ilmiah BRIN Cibinong, are presented in Table 1. This identification needs to be done to determine the differences in ant nest plant species originating from Aceh Jaya and Aceh Besar.

Table 1. Identification of ant-nest plant herbarium samples

Identification	Aceh Jaya	Aceh Besar
Regnum/Kingdom	Plantae	Plantae
Sub Regnum/Sub Kingdom	Tracheobionta	Tracheobionta
Super Divisio/Super Division	Spermatophyta	Spermatophyta
Divisio/Division	Magnoliophyta	Magnoliophyta
Classis/Class	Magnoliopsida	Magnoliopsida
Sub Classis/Sub Class	Asteridae	Asteridae
Ordo/Order	Rubiales	Rubiales
Familia/Family	Rubiaceace	Rubiaceace
Genus/Genus	*Myrmecodia*.	*Hydnophytum*
Species/Species	*Myrmecodia tuberosa* Jack.	*Hydnophytum formicarum* Jack.

Table 2. Phytochemical screening results of Aceh ant nest plant extracts

Secondary metabolite compounds	Ant nest plant origin	
	Aceh Jaya	Aceh Besar
Flavonoid	+	+
Terpenoid	+	+
Steroid	-	+
Tanin	+	+
Fenolik	+	+
Saponin	+	-
Alkaloid		
- Dragendroff	-	-
- Meyer	-	-
- Wagner	-	-

Phytochemical screening conducted on the methanol extract results included tests for alkaloids, flavonoids, tannins, saponins, and terpenoids/steroids using reagents. The phytochemical screening results obtained in this study are presented in Table 2.

Several internal and external factors influence the presence of secondary metabolites in a plant. Internal factors, such as genetic and external factors, including light, temperature, humidity, pH, soil nutrient content, and altitude, play a significant role. Surface air temperature on Earth is relative and represents a region's degree of heat and cold. Different altitudes result in different temperatures. Altitude is one of the factors that affect the growth of a plant. The series of metabolic processes in plants can be disrupted, leading to variations in compounds produced at different altitudes (Nataraj et al. 2022).

The phytochemical screening results indicate that altitude can affect the content of secondary metabolites in ant nest tubers. The difference observed is the presence of saponins in the tubers of the Aceh Jaya ant nest plant, representing coastal areas with an altitude of 401-800 m above sea level, while the tubers of the Aceh Besar ant nest plant, representing lowlands with an altitude <400 m above sea level, do not contain saponins. This difference is attributed to variations in physical factors such as temperature and humidity between lowlands and coastal areas. Higher altitudes are associated with lower temperatures (Nataraj et al. 2022). Another difference in phytochemical screening results is the presence of steroids in the tubers of the Aceh Besar ant nest plant. In contrast, the Aceh Jaya ant nest tubers do not contain steroids. However, GC-MS analysis results show that both extracts contain steroids.

According to Pasaribu et al. (2018), ant nest plants from the genera *Hydnophytum* and *Myrmecodia* contain various compounds, making them biologically active. *Hydnophytum formicarum* Folium contains terpenoids, alkaloids, tannins, glycosides, and saponins, while *H. formicarum* Cortex contains flavonoids, alkaloids, terpenoids, and glycosides. Terpenoids, alkaloids, and phenolics are also found in the ant nest plant *Myrmecodia pendens*. Phytochemical screening of ant nest plants *M. Beccarii, Myrmecodia* sp., and *Hydnophytum* sp. indicates that all three species contain flavonoids, triterpenoids/steroids, and saponins (Mardany et al. 2016; Pasaribu et al. 2018). Pasaribu et al. (2018) found that the ant nest plant *Myrmecodia rumphii* Becc. contains various secondary metabolites, including triterpenoids/ steroids, phenolics, flavonoids, alkaloids, and tannins, distributed in methanol, ethyl acetate, and *n*-hexane solvents. Mardany et al. (2016) reported that the ethanol extract of *Myrmecodia beccarii* Hook.f. ant nest tubers contain flavonoids, saponins, and tannins. In addition, based on the phytochemical screening results conducted in this study, it is evident that ant nest tubers are positively rich in various types of secondary metabolites with medicinal properties. Ant nest extract has many medicine activities, namely antioxidant, antibacterial, cytotoxic, and anticancer (Dirgantara et al. 2022).

GC-MS analysis

The results of GC-MS chromatogram of methanol extracts from tubers of Aceh Jaya and Aceh Besar ant nest plants are presented in Figure 1 and Figure 2. Meanwhile, the analysis results of chemical compounds in methanol extracts from the ant nest tubers of Aceh Jaya and Aceh Besar were presented in Tables 3 and 4, respectively.

Based on the GC-MS analysis of methanol extracts from Aceh Jaya ant nest tubers, 22 compounds were identified. The most abundant compound group in the methanol extract from Aceh Jaya ant nest tubers in this study was coumarins, especially 2H-oxecin-2-one,3,4,7,8,9,10-hexahydro-4hydroxy- 10methyl-[4S-(4R*,5E,10S*)] representing 50.63% of the total compounds. This result is consistent with the FT-IR analysis, which indicated the presence of coumarin compounds characterized by O-H and C=C aromatic groups. Organic coumarin compounds are known for their anti-microbial activities (Widelski et al. 2018).

Based on the GC-MS analysis of methanol extracts from Aceh Besar ant nest tubers, 31 compounds were identified. The most abundant compound group in the methanol extract from Aceh Besar ant nest tubers in this study was simple phenolics, specifically resorcinol, accounting for 17.92% of the total compounds. This result is consistent with the FT-IR analysis, which indicates the presence of resorcinol compounds characterized by O-H, C=O, and C=C aromatic groups. Phenolic compounds represent the largest group of compounds that act as natural antioxidants in plants (Hamsar and Mizaton 2012). Phenolic compounds

from plants have the ability to act as antioxidants, anti-inflammatory agents, antiproliferative agents, antimutagenic agents, and anti-microbial agents. Those phenolic compounds also play a role in preventing and treating degenerative diseases, cognitive disorders, cancer, premature aging, and immune system disorders (Lin et al. 2016).

The differences in compounds obtained in the GC-MS analysis are due to the choice of solvents; polar compounds will dissolve in polar solvents such as methanol, while non-polar solvents like N-hexane (Nwabueze and Okocha 2008). In this study, compounds such as 2(5H)-furanone at retention time of 10.84, and hexadecenoic acid at retention time of 20.317 were identified, whereas in this study, the methanol extract from Aceh Besar ant nest tubers yielded 2 (5H)-furanone at retention time of 6.946 and 2(5H)-furanone at retention time of 7.017.

Based on the phytochemical screening results, Aceh Jaya ant nest tubers tested negative for alkaloids using three reagents: Meyer, Borchardt, and Dragendorf. This result was confirmed by FT-IR analysis and GC-MS analysis, indicating that the methanol extract from Aceh Jaya ant nests does not contain alkaloids. The phytochemical screening for flavonoid compounds in the methanol extract from Aceh Jaya ant nest tubers showed positive results. This is in line with the FT-IR analysis, which indicated the presence of flavonoids characterized by the C=O group, a distinctive feature of flavonoids. However, GC-MS analysis of the methanol extract from Aceh Jaya ant nest tubers did not identify any compounds belonging to the flavonoid group.

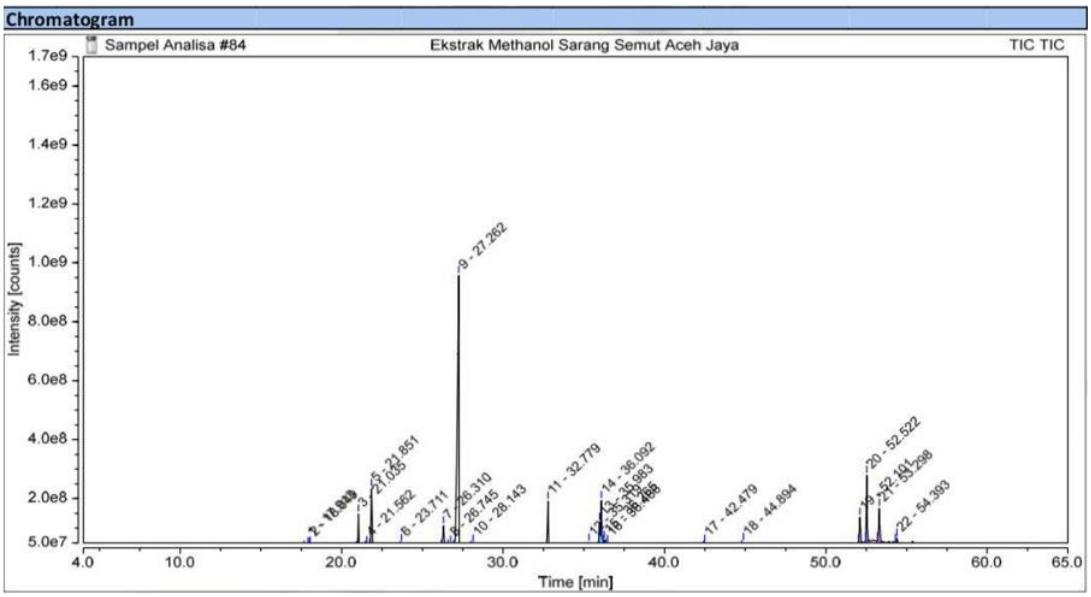

Figure 1. GC-MS analysis results of methanol extract from Aceh Jaya ant nest tubers

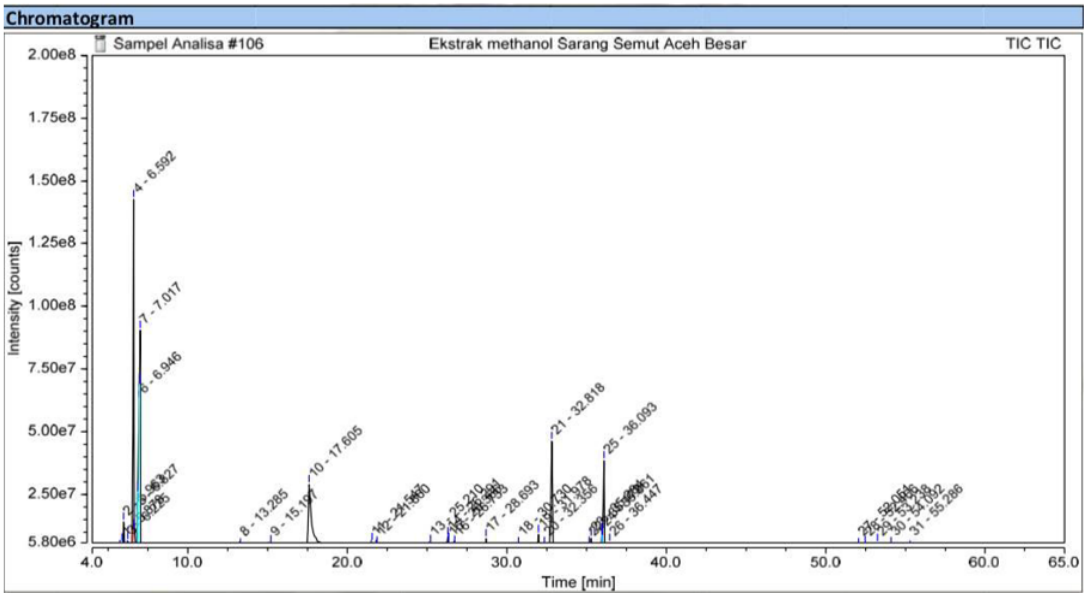

Figure 2. GC-MS analysis results of methanol extract from Aceh Besar ant nest tubers

Table 3. Analysis results of chemical compounds in methanol extracts from Aceh Jaya and Aceh Besar (Indonesia) ant nest using the GC-MS method

Sample origin	Peak name	Retention time (min)	SI (%)	% area	Compound
Aceh Jaya	1	17.913	70.4	1.22	1-Acetoxy-p-menth-3-one
	2	18.049	68.6	1.30	1-Acetoxy-p-menth-3-one
	3	21.035	76.1	3.22	2(3H)-Furanone, 4,5-dihydro-4-(2-methyl 3-methylene-1-buten-4-yl)-
	4	21.562	79.2	0.61	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester
	5	21.851	88.1	4.43	(4S,4aR,7S,7aR)-4,7-Dimethylhexahydrocyclopenta[c]pyran1(3H)-one
	6	23.711	72.1	0.73	6-Nonenal, 3,7-dimethyl-
	7	26.310	76.9	3.49	Geranyl vinyl ether
	8	26.745	80.7	1.09	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester
	9	27.262	73.2	50.63	2H-Oxecin-2-one, 3,4,7,8,9,10-hexahydro-4-hydroxy-10-methyl-, [4S(4R*,5E,10S*)]-
	10	28.143	75.6	0.90	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester
	11	32.779	89.9	4.96	n-Hexadecanoic acid
	12	35.319	87.1	0.65	11-Octadecenoic acid, methyl ester
	13	35.983	88.7	3.57	Linoelaidic acid
	14	36.092	90.3	6.91	trans-13-Octadecenoic acid
	15	36.265	84.1	2.05	17-Octadecynoic acid
	16	36.466	78.7	0.61	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester
	17	42.479	81.4	0.55	Ethanol, 2-(9-octadecenyloxy)-, (Z)
	18	44.894	82.5	1.48	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester
	19	52.101	84.7	2.57	Campesterol
	20	52.522	89.6	5.36	Stigmasterol
	21	53.298	85.8	3.09	Sitosterol
	22	54.393	75.6	0.59	Ethyl iso-allocholate
Aceh Besar	1	5.878	81.1	0.87	Ethanol, 2-butoxy-
	2	5.963	87.7	1.19	Ethanol, 2-butoxy-
	3	6.225	77.3	0.36	Cyclopentanone, 2-methyl-
	4	6.592	91.8	17.17	Ethanol, 2-butoxy-
	5	6.827	89.2	2.14	2(5H)-Furanone
	6	6.946	91.7	14.13	2(5H)-Furanone
	7	7.017	92.8	13.84	2(5H)-Furanone
	8	13.285	74.2	0.36	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy- 6-methyl-
	9	15.197	71.5	1.32	Catechol
	10	17.605	88.5	17.92	Resorcinol
	11	21.547	78.2	0.47	E-7-Tetradecenol
	12	21.860	74.5	0.27	2-Butyl-3-methylcyclopent-2-en-1-one
	13	25.210	80.1	0.54	Phenol, 2,6-dimethoxy-4-(2-propenyl)-
	14	26.291	74.6	1.25	7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(2- methyloxiranyl)-
	15	26.366	72.2	0.66	4-Chloro-3-n-hexyltetrahydropyran
	16	26.733	75.2	0.43	R-Limonene
	17	28.693	81.7	0.83	Tetradecanoic acid
	18	30.730	69.8	0.59	Pentadecanoic acid
	19	31.978	83.1	0.95	Pentadecanoic acid, 14-methyl-, methyl ester
	20	32.356	74.6	0.35	Benzenepropanoic acid, 3,5-bis(1,1- dimethylethyl)-4-hydroxy-, methyl ester
	21	32.818	89.2	10.58	n-Hexadecanoic acid
	22	35.172	83.4	0.33	11,14-Eicosadienoic acid, methyl ester
	23	35.284	85.5	0.73	11-Octadecenoic acid, methyl ester
	24	35.961	87.9	1.84	Linoelaidic acid
	25	36.093	92.3	8.11	cis-Vaccenic acid
	26	36.447	78.1	0.76	Octadecanoic acid
	27	52.051	68.4	0.36	Ethyl iso-allocholate
	28	52.466	71.7	0.44	Cholesta-22,24-dien-5-ol, 4,4-dimethyl-
	29	53.238	72.3	0.54	ß-Sitosterol
	30	54.092	69.4	0.46	1-Heptatriacotanol
	31	55.286	63.0	0.24	Ethyl iso-allocholate

FT-IR Analysis

The phytochemical screening for tannin compounds in the methanol extract from Aceh Jaya ant nest tubers yielded positive results. This result is consistent with the FT-IR analysis, which indicates the presence of tannins characterized by the aromatic C=C and O-H groups (Table 4). However, GC-MS analysis of the methanol extract from Aceh Jaya ant nest tubers did not identify any compounds belonging to the tannin group. This may be due to the presence of volatile compounds that are not well-suited for GC-MS analysis. For other compounds like flavonoids and saponins, it may be due to limitations in standard compounds available in the GC-MS spectrophotometer library (Parastar and Weller 2023).

Phytochemical screening for terpenoid/steroid compounds in the methanol extract from Aceh Jaya ant nest tubers yielded positive results for terpenoids (Table 2). This result aligns with the FT-IR analysis, which indicates the presence of terpenoids characterized by O-H, C-H, C=O, and C=C functional groups, suggesting the presence of terpenoids, but also contains O-H, alkane C-H, and C=C, indicating the presence of steroids. The positive terpenoid results in phytochemical screening and the functional group analysis in FT-IR align with the GC-MS analysis results, which show the presence of monoterpenoids such as geranyl vinyl ether at 3.49%, 1-acetoxy-P-menth-3-one at 1.30%, and 6-nonenal, 3,7-dimethyl- at 0.73%. Additionally, the functional group analysis in FT-IR indicating the presence of steroids aligns with the GC-MS analysis results, which show the presence of steroids such as stigmasterol at 5.36%, sitosterol at 3.09%, campesterol at 2.57%, and ethyl iso-alcoholate at 0.59%.

In this study, there were differences in the results of the content of secondary metabolite compounds tested by phytochemical screening and GC-MS analysis. This can be attributed to the qualitative nature of phytochemical screening, while GC-MS analysis is quantitative. The results analyzed by GC-MS are more accurate than those tested by phytochemical screening. Furthermore, GC-MS analysis has been widely used to identify hundreds of compound components present in plant cells, which cannot be achieved with conventional phytochemical screening, as it is limited to identifying compound groups (Al-Rubaye et al. 2017).

Based on the phytochemical screening of Aceh Besar ant nest tubers, the results showed negative results for alkaloid. This is consistent with the FT-IR and GC-MC analyses, indicating that the methanol extract from Aceh Besar ant nest tubers does not contain alkaloids. Phytochemical screening for flavonoid compounds in the methanol extract from Aceh Besar ant nest tubers yielded positive results. This aligns with the FT-IR analysis, which indicates the presence of flavonoids characterized by the C=O group, a distinctive feature of flavonoids.

The phytochemical screening for tannin compounds in the methanol extract from Aceh Besar ant nest tubers also yielded positive results. This result is consistent with the FT-IR analysis, which indicates the presence of tannins characterized by the O-H and C=C aromatic groups. However, phytochemical screening for saponin compounds in the methanol extract from Aceh Besar ant nest tubers resulted in a negative outcome consistent with FT-IR and GC-MS analysis. The absence of certain secondary metabolite groups, such as tannins, could be attributed to the presence of volatile compounds that are not well-suited for GC-MS analysis. Compounds like flavonoids and saponins may be due to limitations in the standard compounds available in the GC-MS spectrophotometer library (Parastar and Weller 2023).

The phytochemical screening for terpenoid/steroid compounds in the methanol extract from Aceh Besar ant nest tubers yielded positive results for terpenoids. This result aligns with the FT-IR Analysis, which indicates the presence of terpenoids characterized by O-H, C-H, C=O, and C=C functional groups, suggesting the presence of terpenoids. It also contains O-H, alkane C-Hm, and C=C, indicating the presence of steroids. The positive terpenoid results in phytochemical screening and the functional group analysis in FT-IR align with the GC-MS analysis results, which show the presence of a monoterpenoid, R-limonene, at 0.43%. The functional group analysis in FT-IR indicating the presence of steroids aligns with the GC-MS analysis results, which show the presence of steroids such as β-sitosterol at 0.54%, cholesta-22,24-dien-5-Ol, 4,4-dimethyl- at 0.44%, and ethyl iso-alcoholate at 0.36%.

According to Hertiani et al. (2013), GC-MS analysis of ant nest isolates (subfractions obtained from ethyl acetate-methanol) revealed 78.80% purity of isolates occurring at 10.8 minutes. Stable ion fragments at 110 m/z with 100% abundance were identified as 1,3 benzediol (Similarity Index: 81). 1,3 benzediol (resorcinol) belongs to the group of simple phenolics. This correlates with the findings in this study regarding the methanol extract from Aceh Besar ant nest tubers, where the most abundant compound is resorcinol at 17.92%, which is a simple phenolic compound. The differences in the results of GC-MS analysis for some compounds may be due to species, growing locations, and various solvents with different polarities.

Table 4. FT-IR analysis results of methanol extracts from ant nest tubers of Aceh Jaya and Aceh Besar

Sample origin	Functional groups	Obtained wavenumber (cm^{-1})	Literature wavenumber (cm^{-1})	References
Aceh Jaya	O-H	3424.76	3200-3600	Skoog et al. (2016)
	C-H	2931.93	3000-2850	Pavia et al. (1979)
	C=O	1704.18	1725-1700	Pavia et al. (1979)
	C=C	1623.17	1680-1600	Pavia et al. (1979)
	C=C aromatics rings	1519.32	1500-1600	Skoog et al. (2016)
Aceh Besar	O-H	3385.22	3400-3200	Pavia et al. (1979)
	O-H	3385.22	3400-2400	Pavia et al. (1979)
	C-H stretching	2931.93	3000-2850	Pavia et al. (1979)
	C-H stretching	2867.31	3000-2850	Pavia et al. (1979)
	O-H	2733.25	3400-2400	Pavia et al. (1979)
	C=O	1713.83	1725-1705	Pavia et al. (1979)
	C=O	1713.83	1725-1700	Pavia et al. (1979)
	C=C	1610.63	1680-1600	Pavia et al. (1979)
	C=C aromatic rings	1520.94	1500-1600	Skoog et al. (2016)

The polarity of each solvent type will affect the selectivity of the compound types that will be extracted. The solubility of compounds follows the principle that solvents with the same polarity will dissolve compounds with similar polarity. Chemical compounds in plant organs have varying polarities depending on the location of hydroxyl groups and the chemical element arrangements. Areas with an altitude of below 400 m asl. are categorized as lowlands. These soil types are relatively fertile with high soil fertility indexes (Mujiyo et al. 2022). Environmental conditions play a crucial role in plant growth and development. Differences in altitude can result in significant differences in environmental conditions.

Altitude can affect media, nutrient retention, water viability, and erosion (Rahmawaty et al. 2023). Lowlands have higher light intensity and environmental temperatures than medium and highlands. Sunlight intensity significantly affects the plants' photosynthesis, which correlates with the content of both primary and secondary metabolites (Zhou et al. 2022). Adequate light intensity enhance the synthesis of organic compounds in ant nest tubers through photosynthesis (Farji-Brener and Werenkraut 2017).

In conclusion, the methanol extract from Aceh Jaya ant nest tubers contains flavonoids, tannins, saponins, and terpenoids. The ethyl acetate extract contains flavonoids, tannins, and terpenoids, while the methanol extract from Aceh Besar ant nest tubers contains flavonoids, tannins, and terpenoids. The main compound in the methanol extract from Aceh Jaya ant nest tubers is 2H-oxecin-2-one, 3,4,5,8,9,10-hexahydro-4-hydroxy-10-methyl-, [4S-(4R*,5E,10S*)]-, constituting 50.63% of the extract. Meanwhile, the primary compound in the methanol extract from Aceh Besar ant nest tubers is resorcinol, constituting 17.92%. FT-IR spectra of the methanol extracts from Aceh Jaya and Aceh Besar ant nest tubers indicate the presence of O-H, C-H, C=O, C=C alkenes, and C=C aromatics.

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