Trop J Nat Prod Res, December 2024; 8(12): 9686 - 9694

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Bioactive Compound Profiling and Biological Potential of Walay Rhizome (*Zingiberaceae*) from Southeast Sulawesi: GC-MS and LC-MS Analysis

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ARTICLE INFO

Article history:
Received 04 November 2024
Revised 30 November 2024
Accepted 05 December 2024
Published online 01 January 2025

ABSTRACT

The Zingiberaceae family comprises various plant species distributed worldwide, including in Indonesia. Recent discoveries have added new genera, such as Cinnamomum, Meistera, and Wurfbainia. Walay (*Meistera chinensis*) is an endemic plant found in Southeast Sulawesi, though its chemical compounds and biological activities are largely unexplored. This study examines the chemical composition of walay rhizomes using GC-MS and LC-MS and investigates its biological activities, including toxicity, anti-inflammatory and antioxidant. The concentration of phenolic and flavonoid was determined using the Folin-Ciocalteu and aluminum chloride. Toxicity was evaluated through the Brine Shrimp Lethality Test (BSLT). The bioactivity of the extract is studied using DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) for antioxidant assays and protein denaturation inhibition for anti-inflammatory assay. GC-MS analysis identified compounds including caryophyllene, hydroquinone, *z*-(13,14-epoxy) tetradec-11-en-1-ol acetate, cis-vaccenic acid, and copaene. LC-MS analysis identified phillygenin, 2-methoxyanofinic acid, feroxidin, (*E*)-hexadecyl-ferulate, and spinasterol. The walay rhizome extract demonstrated a high LC_{50} value at 25.37 mg/L. Its antioxidant effect differs from vitamin C, with IC_{50} values of 10.44 mg/L (DPPH) and 2.44 mg/L (ABTS), compared to vitamin C IC_{50} of 8.29 mg/L and 8.61 mg/L, respectively. Anti-inflammatory activity showed an IC_{50} of 2.70 mg/mL, compared to diclofenac's IC_{50} of 4.41 mg/mL. The extract's TPC and TFC were 515.71 mg GAE/g and 79.56 mg QE/g, respectively. GC-MS and LC-MS analyses identified bioactive compounds various chemical categories, including fatty acids, terpenoids, phenolics, aromatics, steroids, phenylpropanoids, and quinones. Supported by previous studies, the identified compounds in walay rhizome are potential with its anticancer, anti-inflammatory, and antioxidant properties.

Keywords: Antioxidant, Anti-inflammatory, Toxicity, Zingiberaceae, *Meistera chinensis*

Introduction

Zingiberaceae is one of the important plant families with promising biological activities for various diseases[1,2,3] and has been widely studied globally.[4,5] Indonesia, which has the second-highest biodiversity of medicinal plants in the world,[6] possesses several native species of Zingiberaceae that have been studied as medicinal plants due to their clinical effectiveness. Medicinal plants, as traditional medicine, are preferred due to their fewer side effects compared to synthetic drugs[7] and are known to help overcome chronic diseases, including cancer, diabetes, cardiovascular disorders, and neurodegenerative diseases.[8] According to a current WHO report, about 40% of pharmaceutical products are now sourced from natural ingredients.

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Citation: Musdalipah, Sahidin I, Muhidin, Adryan Fristiohady A. Bioactive Compound Profiling and Biological Potential of Walay Rhizome (*Zingiberaceae*) from Southeast Sulawesi: GC-MS and LC-MS Analysis. Trop J Nat Prod Res. 2024; 8(12): 9686 – 9694 https://doi.org/10.26538/tjnpr/v8i12.49

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

This finding emphasizes the importance of biodiversity preservation and further exploration of potential bioactive compounds in traditional and local medicinal plants for modern medicines development.[9] Several new genera of Zingiberaceae have been discovered, including Alpinia, Cinnamomum, Meistera, and Wurfbainia.[10] Some endemic Zingiberaceae in Indonesia are found in Southeast Sulawesi, including *Alpinia monopleura*,[11] *Meistera chinensis*,[12,13,14,15,16,17] Polygonum,[18] and Etlingera.[4,9,19,20,21,22,23,24,25,26]. Several studies have examined the composition of parts of *M. chinensis*, yet further research is fundamental to strongly establish it as a potential herbal raw material for medicine development. The chemical content and concentration of compounds in plants parts are influenced by variations in climate, rainfall, and the geographical location of growth.[27,28,29,30]

Meistera chinensis, locally known as walay (Tolaki), is a species of the Zingiberaceae that resembles Etlingera and can be found in the districts of Konawe, South Konawe, East Kolaka, and Baubau. The local use of *M. chinensis* as a spice, pain reliever, and immunity booster is aligned with previous studies that report antioxidant and anticancer activities in its fruit.[12,17] Phytochemical screening results revealed that ethanol extracts of *M. chinensis* fruits contain various secondary metabolite compounds, such as phenolics, flavonoids, steroids, terpenoids, alkaloids, and saponins, though its fruit extract is very toxic as previous study found an IC_{50} value of 5.02 ± 1.11 mg/L using BSLT method toxicity.[17] Terpenoids are known to have potential as antibacterials and anticancer agents. Phenolic and flavonoid compounds, in particular, exhibit significant biological activities, including antitumor, antioxidant, and anti-inflammatory effects.[31,32,33]

Inflammation is a normal, complex physiological process initiated by the body to protect itself from stimuli such as infection or tissue injury caused by physical, chemical, or biological factors. Nevertheless, destructive inflammation may occur due to disrupted regulatory mechanisms, potentially worsening major diseases symptoms and leading to serious pathological complications.[34] Investigating plants based on their traditional uses for antioxidant activity and inflammatory responses represents a promising and practical approach in the search for new anti-inflammatory drugs.[35] The antioxidant and anti-inflammatory properties of the Zingiberaceae family have been extensively studied and demonstrate great potential[36,37] Antioxidants are compounds that can slow or inhibit oxidation, a chemical reaction that produces free radicals. Antioxidants protect the body from free radicals, preventing damage to biological molecules.

While walay fruit is more commonly utilized by the local community, its rhizome has yet to be explored and further studied for its potential as the most economically valuable part of the plant, being a richer source of potent phytochemicals with various biological activities. Therefore, this study aims to identify and profile the bioactive compounds in walay rhizome using GC-MS, LC-MS, and discuss the potential activities of the compounds found.

Materials and Methods

Material

Walay (*M. chinensis*) rhizomes were collected from South Konawe district, Andoolo sub-district, Southeast Sulawesi, Indonesia at coordinates Lat -4.30780° and Long 122.272036° as part of the collection of Indonesian Institute of Sciences (LIPI) at Herbarium Bogoriense, Biology Research Center, Cibinong Science Center, voucher sample number (601). The fresh rhizomes (Figure 1) were then cleaned, dried at 40°C for four days without exposure to sunlight, and ground using a mechanical tool, resulting in a *simplisia* (Indonesian term for unprocessed natural material used as medicine).

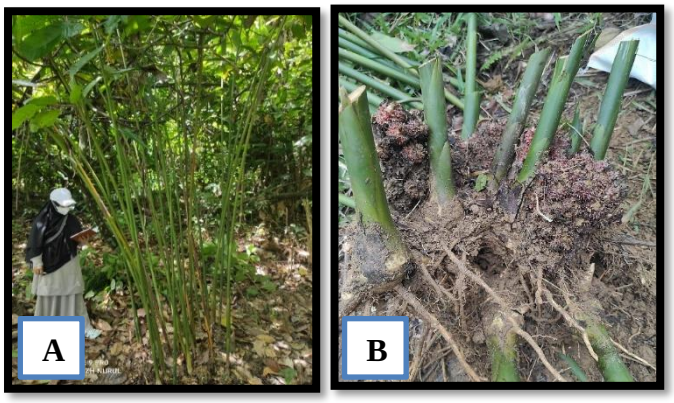

Figure 1: Plant of walay (*Meistera chinensis*); (A) plant; (B) fruit and rhizome

Essential oil

The *simplisia* were ground to a particle size of no more than one centimeter. A total of 500 g was hydrodistilled using clevenger apparatus for 5 hours. The essential oil obtained was dried with additional of anhydrous sodium sulfate and stored in tightly closed bottles at -18°C before being used for GC-MS testing.[36]

Extraction

A total of 1,000 g of dried *simplisia* was macerated with methanol for 3×24 hours. The filtrate obtained was separated and evaporated using a vacuum rotary evaporator (Stuart RE300, USA) at 45°C at a speed of 80 rpm to produce a thick extract.[17] The thick extract of *M. chinensis* rhizome obtained was tested for its antioxidant, anti-inflammatory, and toxicity activities

GC-MS/MS analysis

The volatile compounds in the extract samples of *M. chinensis* rhizome were analyzed using tandem gas chromatography on an Agilent 6890N GC coupled to 5973N quadrupole MS. A 1 µL sample was injected in 250°C with splitless mode, where the ion-source temperature is set at 230°C, and the scan range from 40 to 700 Daltons. The automatic oven temperature was set from 40°C to 300°C with increment of 4°C/min. Helium as the carrier gas set at 0.5 mL/min flowrate. The Wiley NBS mass spectrum database is used for identification of compounds from interpreted fragmentation mass spectra resulted, where it presented as the relative percentage of the peak area in the chromatogram.[32]

LC-MS/MS analysis

The phenolic and flavonoid compounds in the rhizome extract were identified using LC-MS/MS using a Xevo G2-XS QTOF (Waters Corporation, Milford, USA) equipped with an Electrospray Ionization (ESI) source and coupled to a Waters Acquity UPLC system. A mix of Solution A (0.1% formic acid in water) and B (acetonitrile with 0.1% formic acid) is used as the eluent set at a flow rate of 0.3 mL/min with 1 µL/injection. The elution gradient was set with ratio of Solution B in 5% from 0–8 min, 40% B from 8–11 min, until reaching 100% B from 11–16 min. The mass detection range was 50–1,200 m/z with source in 120°C, using a desolvation setting for gas of 1,000 L/h in 500°C. After the LC-MS data were obtained and processed, the UNIFI platform is used for peak-pick and analyzing.[5]

Quantitative of total phenolic content (TPC)

The extract at concentration of 1 mg/mL was used and mixed 1:1 (v/v) with 68 µL of 50% Folin-Ciocalteu reagent, then vortexed for 1 minute. Following this, 1,364 µL of 2% sodium carbonate (Na_2CO_3) was added, and the mixture was incubated for 30 minutes. Gallic acid is used as standard solution is prepared at concentrations ranging from 0.031 to 0.250 mg/mL. The absorbance of all solutions was measured at 750 nm using a UV-Vis spectrophotometer to calculate the concentration or TPC in the unit of mg of gallic acid equivalent per gram of extract (mg GAE/g).[33]

Quantitative of total flavonoid content (TFC)

The total flavonoid content was determined using the aluminum chloride ($AlCl_3$) colorimetric method. The test solution consisted mixture of 0.3 mL of 5% sodium nitrite ($NaNO_2$), 0.5 mL of distilled water, and 0.5 mL of the sample, which was incubated for 5 minutes at 25°C. After incubation, 10% $AlCl_3$ was added in 3 mL, followed by 2 mL of 1 M NaOH and shaken to mix before measuring its absorbance at 432 nm using UV-Vis spectrophotometer. As the quercetin used as the standard, the TFC calculation were expressed as milligrams of quercetin equivalent per gram of extract (mgQE/g).[38]

Determination of antioxidant capacity

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay

A total of 2 mL of aqueous extract was prepared at varying concentrations (10–50 mg/mL) and combined with 1 mL of 0.1 mM DPPH solution (HIMEDIA). The mixture was incubated in 30 minutes with the absence of light at room temperature. A 1 mL of DPPH solution in distilled water is used as the control and ascorbic acid as the standard. The standard, control, and samples absorbance were measured at 517 nm using UV-Vis spectrophotometer and replicated triple. The DPPH radical inhibition (% inhibition) for the extract will then calculated using equation below.[5,11]

$$I\% = \frac{A0 - A1}{A0} \times 100$$

Where; A_1= absorbance of the tested extract solution; Ao = absorbance of the control

ABTS (2,2'-azino bis (3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging assay

A mixture of ABTS (7 mM) and potassium persulfate (2.45 mM) is kept in dark place at room temperature for 12 hours, and diluted with

methanol in a 1:50 ratio before using as the reagent for the test. The solution preparation is aimed to achieve an absorbance of 0.706 ± 0.01 at 734 nm. Aliquots of 300 µL of the extract sample in different concentrations (5–500 µg/mL) and the negative control (absolute methanol) were added to a 3 mL of the ABTS solution per test tubeand let the mixture for 6 minutes in a dark incubator. The absorbance of all samples were measured at 734 nm to obtain the result of radical scavenging inhibition, using the formula below:[39]

$$(I\%) = \frac{A0-A1}{A0}\ x\ 100$$

Where; A_1=Extract sample's absorbance; Ao = Control's absorbance

Bovine Albumin Denaturation for Anti-Inflammatory Activity Assay
The aqueous solution is prepared by mixing 2 ml of 1% bovine albumin, 2.8 ml of phosphate-buffered saline solution (PBS in pH 6.4), and varying volume of extract sample to achieve final concentrations of 2,000, 1,000, and 500 µg/ml, where no extract added to make the control. The mixture was heated for 10 –15 minutes at 37.5°C, and the temperature was increased to 65°C after which it was incubated for 5 minutes. The absorbance of the sample was measured at 650 nm which had been cooled. The same concentration of positive control was made using diclofenac sodium (2,000, 1,000, and 500 µg/ml) and the absorbance was measured.[40]

$$\%\ \text{inhibition of denaturation} = \frac{\text{Control's absorbance} - \text{Sample's absorbance}}{\text{Control's absorbance}}\ x\ 100$$

Toxicity: Brine Shrimp Lethality Test (BSLT)
Seawater and 10 shrimp larvae were prepared and added into test tubes containing 1 ml of extract solution until the volume reached 5 ml. A control test is made similarly without the extract. the test tubes were incubated for 24 hours and then observed by counting the number of *A. salina* larvae that moved and died across three repetitions. LC_{50} value was determined by looking at the percentage of death of shrimp larvae. The percentage of mortality was calculated using the formula:[41,42]

$$\%\ \text{death larvae} = \frac{\text{number of death larvae}}{\text{total number of initial larvae}}$$

Data Analysis
IC_{50} values for DPPH and ABTS antioxidant activity, and LC_{50}, were determined using the following steps: (a) plotting the inhibitory activity (y-axes) against the concentration (x-axes) at six different concentrations (100, 50, 25, 15.5, 6.3, and 3.3 mg/L), (b) determining the regression line equation (y = ax + b), and then (c) substituting y = 50 into the regression equation (b) to find the sample concentration (x). Anti-inflammatory activity was analyzed using SPSS software version 25 for all treatment groups.

Results and Discussion

Identification of the chemical composition of walay rhizome (M. chinensis) by GC-MS
The volatile composition of the rhizome was analyzed using GC-MS, as shown in Table 1, which includes retention time (RT), formula, compound, metabolite group, and biological activity. The analysis identified fifteen compounds with diverse phytochemical profiles, potentially exhibit biological activities such as anticancer, antioxidant, antimicrobial, and anti-inflammatory effects. Research into their derivatives is ongoing, with the aim of exploring new therapeutic prospects for the pharmaceutical industry.[43]
Figure 2 presents expanded views of the peaks from Table 1, displaying the spectrogram of the identified compounds. A total of fifteen compounds were identified in the extract of *M. chinensis* rhizome, including: cis-vaccenic acid, hexadecanoic acid methyl ester, 9-hexadecenoic acid, octanal, 2-(phenylmethylene)-, *z*-(13,14-epoxy) tetradec-11-en-1-ol acetate, (*E*)-tetradec-2-enal, cholestan-3-ol, 2-methylene-, (3ß,5a)-, 2-butanone, 4-(4-hydroxyphenyl)-, alloaromadendrene, cis-a-bisabolene, caryophyllene, copaen, hydroquinone, α-Pinene, and 2-myristynoyl pantetheine.

Table 1: Chemical composition of *Meistera chinensis* rhizome based on GC-MS

Retention Time (min)	Formula	Compounds Name	Group Metabolite	Biological Activity
1.592	$C_{25}H_{44}N_2O_5S$	2-Myristynoyl pantetheine	Fatty acid	Anti-inflammatory[44]
3.741	$C_{10}H_{16}$	α-Pinene	Terpenoid	Anti-inflammatory, antioxidant, anticancer[45,46]
9.309	C_6H_6O	Hydroquinone	Phenolic	Antimicrobial, anti-inflammatory, antiplatelet[47,48]
11.271	$C_{15}H_{24}$	Copaene	Terpenoid	Antioxidant[49]
12.043	$C_{15}H_{24}$	Caryophyllene	Terpenoid	Anti-inflammatory[50]
12.611	$C_{15}H_{24}$	cis-a-Bisabolene	Terpenoid	Anti-inflammatory[51]
12.737	$C_{15}H_{24}$	Alloaromadendrene	Terpenoid	Antioxidant[52]
14.048	$C_{10}H_{12}O$	2-Butanone, 4-(4-hydroxyphenyl)-	Aromatic	Cardiovascular[53]
14.842	$C_{28}H_{48}O$	Cholestan-3-ol, 2-methylene-, (3ß,5a)-	Steroid	Anti-inflammatory, anticancer[54]
15.863	$C_{14}H_{26}O$	(*E*)-Tetradec-2-enal	Fatty acid	-
16.968	$C_{16}H_{28}O_3$	*z*-(13,14-Epoxy) tetradec-11-en-1-ol acetate	Terpenoid	Antioxidant[55]
17.033	$C_{15}H_{20}O$	Octanal, 2-(phenylmethylene)-	Aromatic	-
18.907	$C_{16}H_{30}O$	9-Hexadecenoic acid	Fatty acid	Anti-inflammatory[56]
19.376	$C_{17}H_{34}O_2$	Hexadecanoic acid methyl ester	Fatty acid	Antibacterial[57]
20.944	$C_{18}H_{34}O_2$	cis-vaccenic acid	Fatty acid	Anticancer[58]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

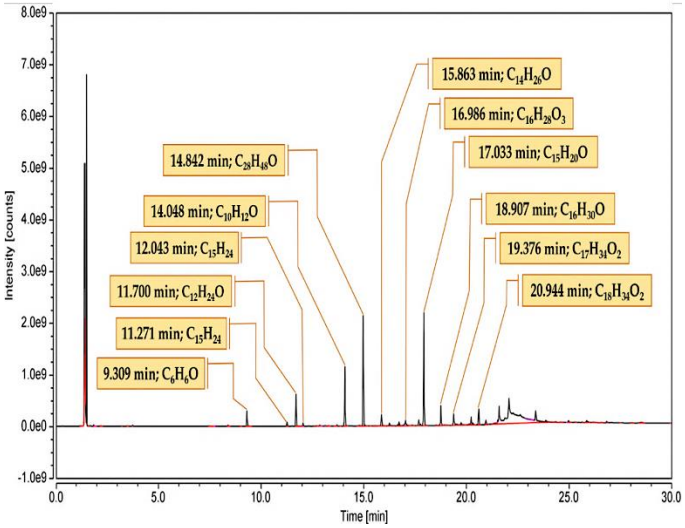

Figure 2: GC-MS chromatogram of the chemical constituents of walay rhizome (*M. chinensis*) extract

Identification chemical composition of walay rhizome (M. chinensis) by LC-MS

The LC-MS effectively screened and identified multiple compounds, selected based on the similarity in their retention time (RT) and molecular mass percentages (Table 2). The presence of a high percentage of phenolic and flavonoid compounds such as phillygenin, 2-methoxyanofinic acid, and (*E*)-hexadecyl-ferulate, identified in the LC-MS analysis, support the high TPC and TFC values observed in this rhizome extract.

The LC-MS/MS analysis revealed a variety of compounds present in the ethanol extract of walay (*M. chinensis*) rhizome. Figure 4 provides the molecular structures of identified compounds which include phenylpropanoids (phillygenin and (*E*)-hexadecyl-ferulate that appeared at m/z 373.1645 (7.04 min), m/z 419.3151 (9.69 min)), phenolic (2-Methoxyanofinic acid that appeared at m/z 235.0963 (8.35 min)), quinone (feroxidin that appeared at m/z 195.1011 (8.66 min)), and steroid (spinasterol that appeared m/z 413.3778 (10.42 min)) respectively (Figure 3). Spinasterol, a steroid metabolite, exhibited the longest retention time and the highest observed molecular mass. According to previous literature, the chemical composition of walay rhizome has drug activities such as anti-inflammatory, antioxidant, antitumor,[59,60,61] antibacterial,[62] anti-malarial,[63] anti-diabetes mellitus, hypolipidemic, anti-ulcer, neuroprotection, and anti-pain.[65,66]

Table 2: Chemical composition of walay rhizome *(M. chinensis)*

No	RT (min)	Formula	Compound Name	Group Metabolite	Biological Activity
1	7.04	$C_{21}H_{24}O_6$	Phillygenin	Phenylpropanoids	Anti-inflammatory, antioxidant, antitumor, and antibacterial agent[59,60,61]
2	8.35	$C_{13}H_{14}O_4$	2-Methoxyanofinic acid	Phenolic	Antibacterial[62]
3	8.66	$C_{11}H_{14}O_3$	Feroxidin	Quinones	Anti-inflammatory, antioxidant, anticancer, antibacterial, anti-malarial[63]
4	9.69	$C_{26}H_{42}O_4$	(*E*)-Hexadecyl-ferulate	Phenylpropanoids	Antioxidant, antiviral[64]
5	10.42	$C_{29}H_{48}O$	Spinasterol	Steroid	Anti-diabetes mellitus, Anti-inflammatory, hypolipidemic, anti-ulcer, neuroprotection, anti-pain and antitumor[65,66]

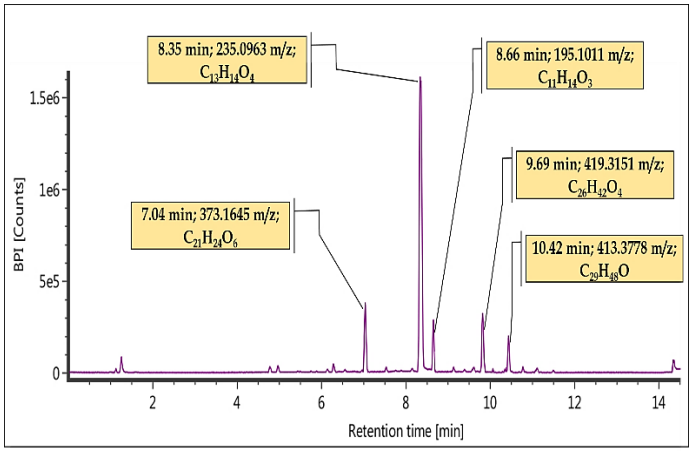

Figure 3: LC-MS chromatogram of the chemical constituents of walay rhizome (*M. chinensis*) extract

Determination of total phenolic and flavonoid content walay rhizome (M. chinensis)

Phenolic and flavonoid compounds, as key representatives of secondary metabolites in plants, possess natural antioxidant, anti-inflammatory, and anticancer properties.[67] The phenolic and flavonoid content was determined using the Folin-Ciocalteu with gallic acid standard phenolic and the $AlCl_3$ colorimetric method with quercetin standard, resulting in TPC and TFC values, respectively. The results of these tests are shown in Table 3. The gallic acid standard calibration curve resulting in a linear regression equation of ($y = 0.0861x + 0.2769$), $R^2 = 0.958$, as shown in Figure 5. In other hand, the quercetin standard curve results in ($y= 0.0577x + 0.0335$) with $R^2 = 0.997$ (Figure 5). Based on these equation, the average TPC and TFC of walay rhizome extract was calculated to be 515.71 mgGAE/g and 79.56 mgQE/g extract.

Determination of antioxidant capacity: DPPH and ABTS radical scavenging activity

An antioxidant activity is indicated by the decrease in DPPH absorbance in presence of the extract solution, indicating the active ingredient's capacity as a radical scavenger, as shown previously in Figure 3. The antioxidant activity of the tested compounds is measured by comparing the absorbance of the solution before and after treatment with antioxidants (Figure 6) and the result is presented as IC_{50} value. In the DPPH test, vitamin C was used as an antioxidant standard, serving as a reference for the free radical scavenging ability.[68] The antioxidants in the ethanol extract of walay rhizome can donate electrons to neutralize DPPH free radicals, leading to a color change in the DPPH solution from purple to yellow or clear.38,67,69 In the ABTS method, a decrease in absorbance shows the ability of antioxidant contents to react directly with ABTS cation radicals thus neutralize colored ABTS cations. When reduced by antioxidants, the nitrogen-centered ABTS radical changes from a blue-green color to a colorless non-radical form.[38,70]

The IC_{50} values of the DPPH and ABTS assays are shown in Figure 7. Antioxidant activity (ABTS and DPPH) on the ethanol extract of *M. chinensis* showed results of 2.44 mg/L and 10.44 mg/L, respectively, while Vitamin C as a positive control had a value of 8.29 mg/L and 8.61 mg/L. According to the classification of antioxidant potential proposed by Molyneux (2004), antioxidant activity is divided into four categories: IC_{50} >200 mg/L is considered no activity, >150-200 mg/L is having weak activity, >100-150 mg/mL is moderately strong, >50-100 mg/L is strong, and <50 mg/mL is indicating a very strong activity. Based on the IC_{50} result, the antioxidant activity in the sample is very

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

strong. The strength of activity is thought to be affected by the flavonoids due to the presence of phenol groups.[17]
Phenol groups can donate hydrogen atoms, neutralize free radicals, and inhibit oxidation.[67] These compounds serve as major antioxidants, especially in plants, and play a role in protecting against oxidative stress caused by various environmental factors.71 The active compounds containing phenol groups, identified through GC-MS and LC-MS analysis of *M. chinensis* including 2-Myristynoyl pantetheine, Hydroquinone, and 2-methoxyanofinic acid. These compounds have been shown to have antioxidant, anti-inflammatory, antibacterial, and anticancer activities.[47,48]

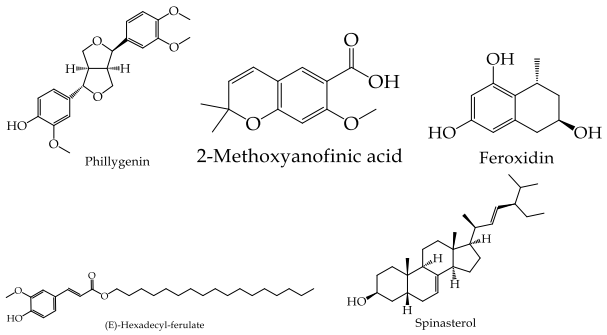

Figure 4: Molecular structure of identified compounds from walay rhizome (*M. chinensis)*

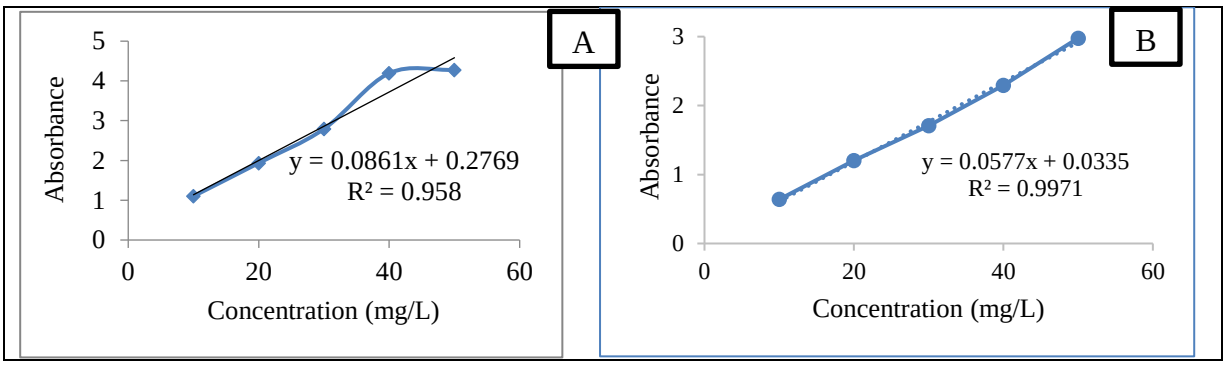

Figure 5: Standard curve for determination of total flavonoid content: Gallic acid (A), Quercetin (B)

Table 3: Total phenolic and flavonoid content of walay rhizome (*M. chinensis*)

Replicate	**Abs**	**Total Phenolic Content (mgGAE/g)**	**Phenolic Content (mgGAE/g extract)**
I	1.57	502.44	515.71
II	1.63	523.71	
III	1.62	521.00	
Replicate	Abs	Total Flavonoid Content (mgQE/g)	Flavonoid Content (mgQE/g extract)
I	0.517	83.78	79.56
II	0.466	74.94	
III	0.495	79.97	

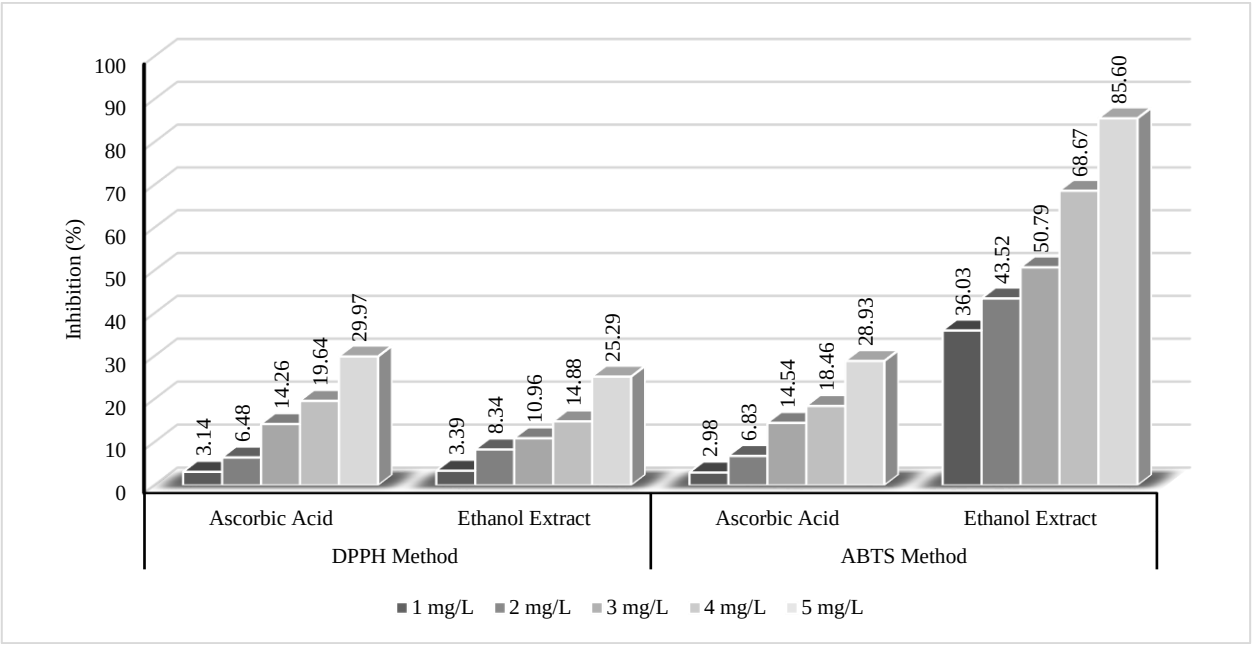

Figure 6: Antioxidant activity of *M. chinensis* rhizome based on ABTS and DPPH radical scavenging assay

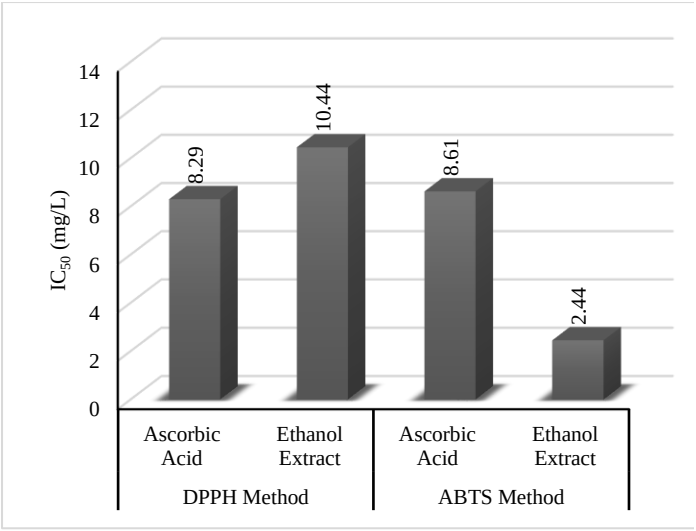

Figure 7: The IC_{50} values of DPPH and ABTS radical scavenging activity of *M. chinensis* rhizome

Anti-Inflammatory Activity: Bovine Albumin Denaturation Assay

The bovine serum albumin (BSA) denaturation test is used to assess plant extracts' anti-inflammatory activity. This assay evaluates a compound's ability to inhibit the denaturation of BSA, serving as a model to assess protein stability under inflammatory conditions. Heat-induced BSA denaturation is considered to simulate an inflammatory condition, and compounds that can prevent this process are deemed to have potential anti-inflammatory properties.[72] As the concentration of methanol extract from walay rhizome and diclofenac sodium increases, the recorded absorbance value decreases, which is accompanied by an increase in the percentage inhibition (Figure 8).

In chronic inflammation such as rheumatoid arthritis, denatured proteins act as autoantigens that is significant to trigger autoimmune disease. Nonsteroidal anti-inflammatory drugs (NSAIDs) in general works by inhibiting the protein denaturation. Therefore, testing the protein denaturation inhibitory activity of a compound can be delivered by comparing it with diclofenac, a general NSAID drug, as a standard. The anti-inflammatory test results showed that the IC_{50} value of walay rhizome was 2.70 mg/L and diclofenac sodium was 4.41 mg/L. A compound has a very strong anti-inflammatory when IC_{50} < 10 mg/L, strong IC_{50} 10-30 mg/L, moderate IC_{50} 31-50 mg/l, weak IC_{50} 51-100 mg/l and very weak IC_{50} > 100 mg/L.

Based on these results, walay rhizome has very strong anti-inflammatory activity. Based on GC-MS and LC-MS analysis in previous studies, several compounds have identified to exhibit anti-inflammatory effects, including: α-Pinene, caryophyllene, copaene, cis-vaccenic acid, 9-hexadecenoic acid, cis-a-bisabolene, phillygenin, feroxidin, and spinasterol.[45,50,65,66]

Toxicity: Brine Shrimp Lethality Test (BSLT)

BSLT is a preliminary test used to screen bioactive compounds that have potential as anticancer drugs. The toxicity test aims to evaluate the toxic effect of a compound within 24 hours.[42] The test results showed that the highest larval mortality rate was achieved at concentrations of 1,000 mg/L, 500 mg/L, 250 mg/L, 125 mg/L followed by 62.5, 31.25, 15.625, and 7.8125 (Figure 9). Thus, various concentration levels of extract were used to examine the relationship between the test solution and the larval mortality rate of shrimp.

The higher the concentration used, the greater the content of active compounds contained in the extract, thus increasing the toxic effect and resulting in increased larval mortality. In this study, the LC_{50} value was calculated based on the mortality of *Artemia salina* larvae caused by exposure to walay rhizome extract. The LC_{50} value was obtained through probit analysis to determine the concentration of the extract that caused 50% mortality of the tested larvae. Based on the calculation of the LC_{50} value, it is known that walay rhizome extract has an LC_{50} value of 25.37 mg/L and potassium dichromate (positive control) of 6.22 mg/L. Meyer et al classified the toxicity level of extracts as follows: extracts with LC_{50} values ≤ 30 mg/L is highly toxic; LC_{50} ≤ 1,000 mg/L is toxic; while LC_{50} > 1,000 mg/L is non-toxic.[41] Therefore, walay rhizome is categorized as highly toxic. Based on a literature review of GC-MS and LC-MS compounds including anticancer properties such as α-pinene, cholestan-3-ol, 2-methylene-, (3ß,5a)-, cis-vaccenic acid, phillygenin, feroxidin, and spinasterol.

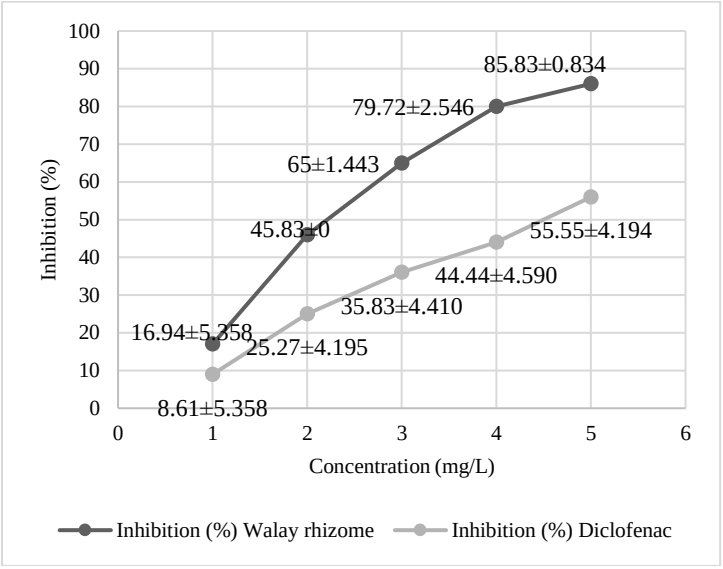

Figure 8: Inhibition of walay rhizome (*M. chinensis*) and diclofenac

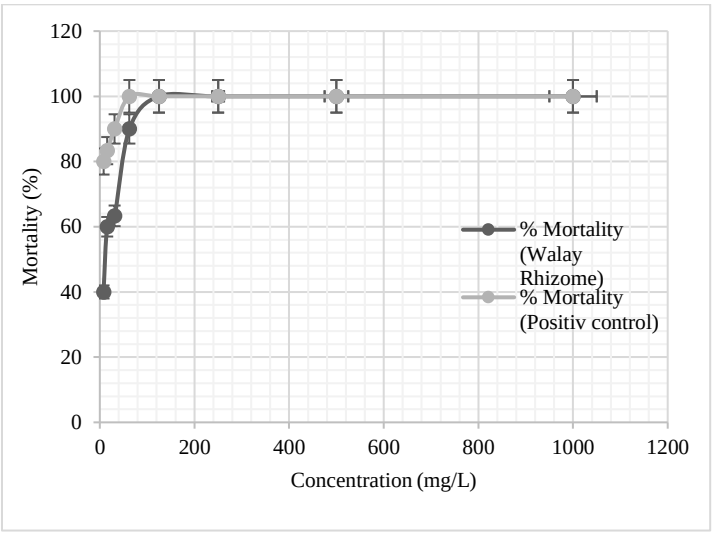

Figure 9: Toxicity of walay rhizome and positive control on shrimp larval mortality

Conclusion

In the present study, the rhizome of walay (*M. chinensis*) was shown to contain various secondary metabolites with diverse pharmacological properties. Through GC-MS analysis, metabolite groups were identified, including fatty acids (2-myristynoyl pantetheine, 9-hexadecenoic acid, cis-vaccenic acid), terpenoids (α-pinene, copaene, caryophyllene, cis-α-bisabolene, alloaromadendrene, *z*-(13,14-epoxy) tetradec-11-en-1-ol acetate), phenolics (hydroquinone), steroids (cholestan-3-ol, 2-methylene-, (3ß,5α)-), and aromatics (2-butanone, 4-(4-hydroxyphenyl)-). LC-MS analysis identified metabolite groups including phenylpropanoids (phillygenin and (*E*)-hexadecyl ferulate), phenolics (2-methoxyanofinic acid), quinones (feroxidin), and steroids (spinasterol). These compounds are proven to show various activities

such as antioxidant, anti-inflammatory, toxicity, and other biological activities with therapeutic effects. Further research is needed to isolate, characterize, and determine the biological activities of the compounds in *M. chinensis* rhizome and test their biological activity.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The authors would like to thank Politeknik Bina Husada Kendari for supporting this research. We also thank the head of the Integrated Chemistry Laboratory at Politeknik Bina Husada Kendari for laboratory access.

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