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Antioxidant Potential, Quantification of Phenolics and Flavonoids, and Characterization of Secondary Metabolites of Stem Methanol Extract of *Mitrella kentii* (Blume) Miq. Using LC-MS/MS Analysis

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Abstract

Mitrella kentii (Blume) Miq., a wild plant from the Annonaceae family, naturally grows in the peat swamp forests of Kalimantan, Indonesia. Known as Black Bajakah among the Dayak Ngaju community in Central Kalimantan, its stems have traditionally been used to treat various ailments and restore stamina during forest work. This study aimed to identify secondary metabolites in the methanolic extract of M. kentii stems and to explore its potential for medicinal applications and drug discovery. LC-MS/MS analysis was conducted using a C18 column on the Waters Acquity UPLC system, with electrospray ionization in positive and negative ion modes. Compound identification employed the UNIFI data processor with the Waters Traditional Medical Scientific Library database. The analysis revealed five bioactive compounds: d-Lirioferine (lirioferine), isosinomenine, N-(1,7-Dimethoxy-phenanthren-2-yl)-acetamide, scoulerine, and yuanhunine, all with documented biological activities. Quantitative analysis showed the extract contained 36.23% total phenols, 0.44% total flavonoids, and exhibited very strong antioxidant activity with an IC50 value of 12.06 ppm. These results support the traditional medicinal use of M. kentii and highlight its potential as a source of bioactive compounds for future pharmaceutical development.

Keyword: Antioxidant activity, Biochemical compound. LC-MS/MS, *Mitrella kentii*, Secondary Metabolites

INTRODUCTION

Mitrella kentii is a climbing liana plant that grows wild in peat swamp forests in Central Kalimantan, Borneo, Indonesia. This species belonging to the Annonaceae family. *Mitrella kentii* is native to Peninsular Malaysia, several regions of Indonesia including the island of Sumatra, Kalimantan and New Guinea. The Dayak tribe has long drunk water from the stems and the stems are used as medicine. The water is drunk when they feel feverish. The stems which are dried and then crushed are generally applied to injuries that are difficult to recover from (Almeida *et al*., 2022). In Malaysia, *Mitrella*

kentii is traditionally used as a drink in the form of a decoction of stems to cure fever (Eswani *et al*, 2010). Sidahmed *et al* (2013) in their research explained that this plant is rich in isoquinoline alkaloids, terpenylated dihydrochalcones and benzoic acid and has been reported to have anti-inflammatory activities. Research carried out shows that the compound desmosdumotin C, a new bioactive compound isolated from *Mitrella kentii* has a gastroprotective effect in a rat model of gastric ulcer. The current study reveals that desmosdumotin C compound demonstrated effective gastroprotective effects and therapeutic agent for gastric ulcer which could be attributed to its antioxidant effect, activation of HSP-70 protein, intervention with COX-2 inflammatory pathway and potent anti-*Helicobacter pylori* effect.

Research by Azizan and Hadi (2014) explained that chalcones, desmosdumotin C and their tautomer, 2-cinnamoyl- 3-hydroxy-5-methoxy-4,6,6-trimethylcyclohexa-2,4-dienone, flavanone, 7-hydroxy-5,6-dimethoxy-2-phenylchroman- 4-one, oxoaporphine alkaloids, Liriodenine and Atherospermidine and also terpenoid, -Sitostenone have been isolated from this species. All these compounds were isolated for the first time from *Mitrella kentii*. The isolated compounds were elucidated using spectroscopic techniques such as UV, IR,1D and 2D NMR and mass spectroscopy and by comparison their spectral data with those previously reported in the literatures and the desmosdumotin C showed the anti-ulcer activity.

Experiment by Saadawi *et al* (2012), Benosman *et al* (1997), and Ellis *et al* (1972) showed that the hexane extract of this plant's bark displays anti-inflammatory activities. Previous chemistry research on *Mitrella kentii* isolated isoquinoline alkaloids, terpenylated dihydrochalcones and 4 other benzoic acids. The results of the same study were also carried out by Jalil *et al* (2021) who found that the chemical content and biological activity of the leaves also showed anti-inflammatory activity. This promising activity warrants the development of *Mitrella kentii* leaf oil as an anti-inflammatory agent. Hexane extract of *Mitrella kentii* leaf was identified to contain thirteen compounds, showing low inhibitory activity on prostaglandin production in human blood. The benzyl ester component of the leaf oil may contribute to radical scavenging activity, having the potential for further development as an anti-inflammatory and antioxidant agent.

Literature Review

Botanical aspects

Annonaceae is a group of plants with the appearance of trees, shrubs and lianas. The distribution center of the Annonaceae family is in tropical areas. The structure of Annonaceae flowers is highly variable. Generally, flower petals and petals are multiples of three. The stigma often secretes a thick fluid called compitum to aid pollination. The pistil and stamens do not form together, so flowers need pollinators to help pollinate them.

Members of the Annonaceae family are widely known as plants fruit producers, biopharmaceutical plants, bio-pesticides, sources wood, ornamental plants and spice producers (Handayani, 2016).

Scientific classification:
Kingdom : Plantae
Clade : Tracheophytes
Clade : Angiosperms

Clade : Magnoliids
Order : Magnoliales
Family : Annonaceae
Subfamily : Annonoideae
Genus : *Mitrella*
Species : *Mitrella kentii* (Blume) Miq.

Chemical content and biological activity aspects

The chemical content and biological activities of *Mitrella kentii* have not been widely published. *Mitrella kentii* is rich in isoquinoline alkaloids, terpenylated dihydrochalcones and benzoic acid and has been reported to have anti-inflammatory activities. The compound desmosdumotin C isolated from *Mitrella kentii* has a gastroprotective effect and therapeutic agent for gastric ulcer which could be attributed to its antioxidant effect, and potent anti-Helicobacter pylori effect (Sidahmed *et al*, 2013).

Azizan and Hadi (2014) explained that *Mitrella kentii* contains chalcones, desmosdumotin C and their tautomer, 2-cinnamoyl- 3-hydroxy-5-methoxy-4,6,6-trimethylcyclohexa-2,4-dienone, flavanone, 7-hydroxy-5,6-dimethoxy-2-phenylchroman- 4-one, oxoaporphine alkaloids, Liriodenine and Atherospermidine and also terpenoid, -Sitostenone. The hexane extract of this plant's bark displays anti-inflammatory activities from isoquinoline alkaloids, terpenylated dihydrochalcones and 4 other benzoic acids. The results of the same study were also carried out by Jalil *et al* (2021) who found that the chemical content and biological activity of the leaves also showed anti- inflammatory activity. This promising activity warrants the development of *Mitrella kentii* leaf oil as an anti-inflammatory agent.

METHOD

Plant materials

The stems of *Mitrella kentii* (Figure 1) were collected from the peat swamp forests of Central Kalimantan and identified at Herbarium Bogoriense, Indonesian Institute of Biological Research, Cibinong, West Java, Indonesia. Once collected, the stems were cut into about 40 cm long and washed with clean water, and the bark was removed. Then the wood is air-dried in a place that is not directly exposed to sunlight.

Figure 1. ***Mitrella kentii*** **(Annonaceae)**

Methanolic extract of the stems of Mitrella kentii

The wood of *Mitrella kentii* was cut into pieces with a size of about 5 cm x 1 cm, then air-dried in a 50^0C oven and pulverized into powder using a grinder. A total of 500 grams of *Mitrella kentii* stem powder was then macerated for 3x24 hours using 4000 ml of methanol with an orbital shaker at 100 rpm. The filtrate is then concentrated using a rotary vacuum evaporator. The viscous extract was collected, weighed, and then stored in a refrigerator at 5°C until used for further analysis (Figure 2).

Figure 2. Methanolic extract of the stems of *Mitrella kentii*

LC-MS/MS Analysis

L C-MS/MS analysis was carried out as described in previous research (Trifani *et al*., 2022) with minor modifications. A total of 0.5 grams of UCSME was dissolved in 10 ml methanol, followed by sonication for 30 minutes. Then it was diluted further with methanol, homogenized to the right concentration, and passed through a syringe filter with 0.22 m GHP/PTFE membrane. The LC-MS analysis was carried out in the C18 column of the Waters Acquity UPLC system (2.1 mm × 100 mm, 1.7 mm) equipped with an auto-sampler, column manager, and adjustable MS detector. The mobile phase was 0.1% formic acid in acetonitrile (solvent A) and 0.1% formic acid in bidestilated water (solvent B). For gradient elution, the flow rate of the mobile phase was kept at 0.6 mL/min. The total chromatography run time was 2.0 min. The temperatures for the column and auto-sampler were maintained at 40°C and 15°C. The injection volume was 10 L. MS analysis was performed with an electrospray ionization source (ESI) in positive and negative ion modes. MS data is obtained in the m/z range of 50-1200 and MSE Tof mode operation. The compounds were identified using a UNIFI data processor with a mass spectrum library of natural active substances from the Waters Traditional Medical Scientific Library database based on UPLC/QTof MSE Data Acquisition, which is integrated with an automatic identification process.

Total phenol analysis

Determination of total phenolic was carried out using spectrophotometric analysis techniques using the Folin-Ciocalteu method. The 1% NaOH reagent is made by weighing 1 gram of NaOH. Dissolve with distilled water and sonicate. Then, it is measured to a volume of 100 ml, and 1% NaOH is ready to use. Folin Ciocalteu reagent 7.5% is made by dissolving Folin Ciocalteu reagent in distilled water to a concentration of 7.5%. Weighed 10 mg of the extract into a 25 ml volumetric flask and dissolved it in methanol. Then, pipette 1 ml of the solution and put it in a test tube. Then 5 ml of Folin Ciocalteu 7.5% reagent was added, vortexed and incubated in the dark for ± 8 minutes. After that, 4 ml of 1% NaOH was added, vortexed and incubated in the dark for 1 hour. The solution was then transferred into a cuvette and the absorbance was read using a spectrophotometer at a wavelength of 730 nm. The standard solution used is gallic acid. First, a stock solution of gallic acid was made with a concentration of 500 ppm, weighed 12.5 mg and dissolved with methanol in a 25 ml volumetric flask. Then create a standard concentration range of 0; 10; 30; 50; 70; and 100 ppm in a 25 ml flask. Pipette 1 ml of each gallic acid solution into a test tube. Then 5 ml of Folin Ciocalteu 7.5% reagent was

added, vortexed and incubated in the dark for ± 8 minutes. After that, 4 ml of 1% NaOH was added, vortexed and incubated in the dark for 1 hour. After that, the absorption was measured using a spectrophotometer at a wavelength of 730 nm.

Total flavonoid analysis

Determination of total flavonoid was carried out using spectrophotometric analysis techniques using the aluminum chloride method. The first to be prepared was a 0.5% w/v HMT (hexamethylenetetramine) reagent solution, 25% HCl solution, 5% v/v glacial acetic acid solution in methanol, and 2% AlCl3 solution in glacial acetic acid solution. After that, a stock solution was made, 200 mg of the extract was put into a round bottom flask, added with 1 ml of HMT solution, 20 ml of acetone, and 2 ml of HCl solution, hydrolyzed by refluxing for 30 minutes. The mixture was filtered using cotton wool, then the filtrate was put into a 100 ml volumetric flask. The residue was refluxed again with 20 ml of acetone for 30 minutes, filtered and the filtrate was mixed into a 100 ml volumetric flask. The filtrate mixture in the volumetric flask was added with acetone to 100 ml. Take 20 ml of the filtrate into a separating funnel, add 20 ml of water and extract 3 times each with 15 ml of ethyl acetate. The ethyl acetate fraction was collected and added with ethyl acetate to 50 ml in a volumetric flask. Then a blank solution was made, 10 ml of the stock solution was taken, added with glacial acetic acid solution to 25 ml in a volumetric flask. Continue by making a sample solution, take 10 ml of the stock solution, add 1 ml of AlCl3 solution and glacial acetic acid solution to 25 ml in a volumetric flask. Next, measurements were carried out 30 minutes after adding AlCl3 using a spectrophotometer at a wavelength of 425 nm with a quercetin comparator.

Calculation method:

$$\% = \frac{Cp\ (As\text{-}Abs)}{(Ap\text{-}Abp)} \times 1{,}25 \times \frac{100}{\text{Sample Weight}}$$

Cp = Comparison concentration
As = Sample absorption
Abs = Absorption of sample blank
Ap = Comparative absorption
Abp = Absorption of the comparison blank

Antioxidant IC50 - DPPH analysis

Antioxidant power measurements were carried out using spectrophotometric analysis techniques using the DPPH method. The first thing to do is make a stock of 125 µM DPPH, that is, weigh 2.5 mg of DPPH, then dissolve it with ethanol in a volumetric flask, measure it to a volume of 50 ml, then coat the measuring flask with aluminum foil, and the DPPH is ready to be used. The next step is sample and vitamin C preparation, weighing 10 mg of the sample and vitamin C each, dissolving 1 ml of DMSO, sonicating until dissolved then vortexing, the sample and vitamin C are ready for use. In the next procedure, 100 µL of sample was inserted into the microplate. For replicate samples 1 and 2, 100 µL of DPPH was added, while for the negative control only 100 µL of ethanol

was added. After that, it was incubated at room temperature in the dark for 30 minutes. Then the absorption was measured using an Elisa instrument at a wavelength of 517 nm. For the blank solution, replications 1 and 2 only contained 100 µL of ethanol and 100 µL of DPPH was added, while the negative control only contained 200 µL of ethanol.

The inhibition percentage is calculated using the following formula:

$$\% \text{ Inhibition} = \frac{\text{(Blank absorbency - Sample absorbance)}}{\text{Blank absorbency}} \times 100\%$$

Percent inhibition is the free radical inhibition value. To calculate the IC50 value, namely the concentration of extract (µg/ml) required to reduce DPPH by 50%, it is obtained from the intersection of the line between the concentration axis and the percent inhibition axis, then entered into the equation y = a + bx, with y = 50 and the value x indicates IC50.

RESULT

LC-MS Analysis

LC-MS chromatogram of the methanolic extract of *Mitrella kentii* stems showed five peaks (Figure 3) which indicates the presence of five phytochemical compounds, namely d-Lirioferine (lirioferine), isosinomenine, N-(1,7-Dimethoxy-phenan-thren-2-yl)-acetamide, scoulerine, and yuanhunine. The five compounds were characterized and identified as shown in Table 1, and their chemical structures and bioactivities were presented in Table 1.

Table 1. Five phytochemicals identified by LC-MS/MS analysis in the methanolic extract of *Mitrella kentii* stems

Retention Time (min)	Name of Compound	Observed (m/z)	Neutral Mass (Da)	Detector Counts	Response	Formula
4.21	d-Lirioferine (lirioferine)	342.1694	341.16271	3725213	2667660	C20H23NO4
4.09	Isosinomenine	330.1695	329.16271	2317422	2317422	C19H23NO4
5.76	N-(1,7-Dimethoxy-phenan-thren-2-yl)-acetamide	296.1281	295.12084	840892	683270	C18H17NO3
7.12	Scoulerine	328.1539	327.14706	1309443	1046247	C19H21NO4
4.76	Yuanhunine	356.1848	355.17836	2149261	1684214	C21H25NO4

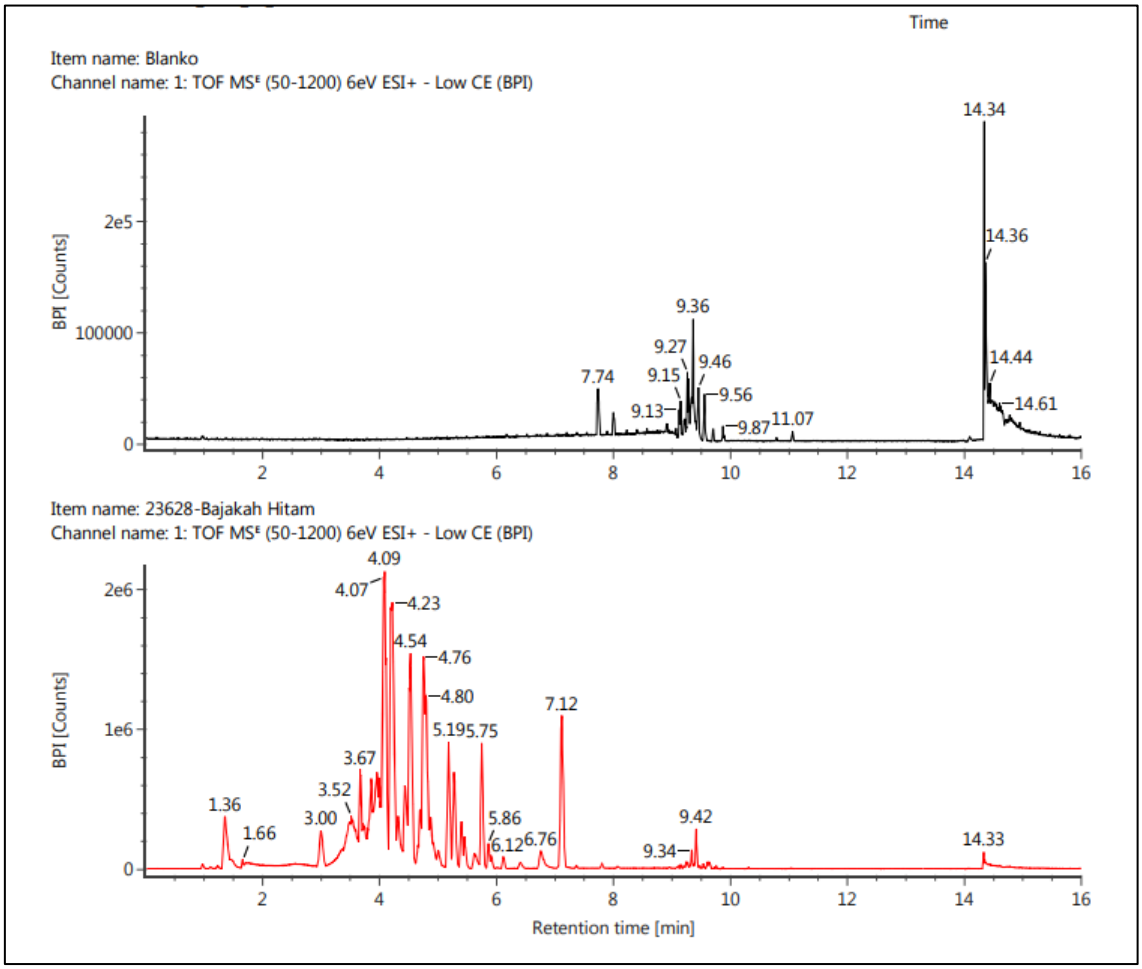
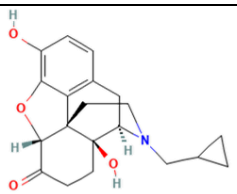
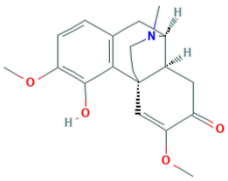
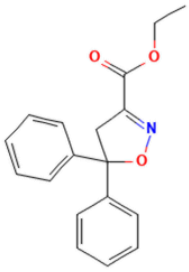


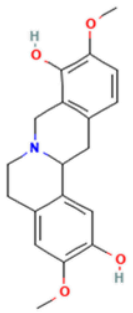
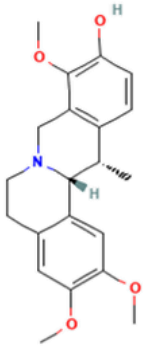
Figure 3. LC-MS chromatogram of *Mitrella kentii* stem methanolic extract

Description of bioactivity of compounds identified in the methanolic extract of *Mitrella kentii* by LC-MS based on PASS Software's prediction database were presented in Table 2.

Table 2. Bioactivity of compounds identified in the methanolic extract of *Mitrella kentii* by LC-MS (Based on PASS Software's prediction database)

Name of Compound	Formula	Chemical Structure	Bioactivity
d-Lirioferine (lirioferine)	C20H23NO4		- Respiratory analeptic (Pa 0.981) - Antitussive (Pa 0.968) - UGT1A substrate (0.969) - UGT1A1 substrate (Pa 0.966) - UGT2B1 substrate (Pa 0.965) - Antinociceptive (0.955) - Laxative (Pa 0.947) - UGT1A3 substrate (0.947)

			- Opioid dependency treatment (0.944) - Opioid kappa 3 receptor antagonist (0.932) - Antialcoholic (0.931) - 5 Hydroxytryptamine release stimulant (Pa 0.970) - Antitussive (Pa 0.850) - Neurotransmitter antagonist (Pa 0.788) - Sigma receptor agonist (Pa 0.745) - Polarisation stimulant (Pa 0.742) - CYP2D2 inhibitor (Pa 0.737) - Nicotinic alpha4beta4 receptor agonist (Pa 0.715) - Histamine release stimulant (Pa 0.700) - Antinociceptive (Pa 0.689) - Membrane permeability inhibitor (Pa 0.681)
Isosinomenine	C19H23NO4		
N-(1,7-Dimethoxy-phenan-thren-2-yl)-acetamide	C18H17NO3		- Mycothiol-S-conjugate amidase inhibitor (Pa 0.941) - Neurodegenerative diseases treatment (Pa 0.941) - 5-O-(4-coumaroyl)-D-quinate 3'-monooxygenase inhibitor (0.829) - Aspulvinone dimethylallyltransferase inhibitor (Pa 834) - 3-Hydroxybenzoate 6-monooxygenase inhibitor (Pa 0.787) - Creatininase inhibitor (Pa 0.780) - Phobic disorders treatment (Pa 0.792) - tRNA-pseudouridine synthase I inhibitor (Pa 0.760) - Antiparkinsonian (Pa 0.755) - 2-Hydroxymuconate-semialdehyde hydrolase inhibitor (Pa 0.748)

Scoulerine	C19H21NO4		- 5 Hydroxytryptamine release stimulant (Pa 0.962) - MAP kinase stimulant (Pa 0.949) - Antidyskinetic (Pa 0.905) - UGT2B12 substrate (0.821) - Membrane permeability inhibitor (0.773) - JAK2 expression inhibitor (Pa 0.719) - General pump inhibitor (Pa 0.702) - Aspulvinone dimethylallyltransferase inhibitor (Pa 0.739) - Gluconate 2-dehydrogenase (acceptor) inhibitor (Pa 0.727) - CYP2D2 inhibitor (0.657)
Yuanhunine	C21H25NO4		- MAP kinase stimulant (0.920) - Antidyskinetic (0.890) - 5 Hydroxytryptamine release stimulant (Pa 0.863) - Membrane permeability inhibitor (Pa 0.709) - UGT2B12 substrate (Pa 0.702) - Sigma receptor agonist (Pa 0.662) - Gluconate 2-dehydrogenase (acceptor) inhibitor (Pa 0.699) - General pump inhibitor (Pa 0.642) - Cardiovascular analeptic (Pa 0.636) - HIF1A expression inhibitor (Pa 0.621)

Total phenolic content of the methanol extract of Mitrella kentii stems

Based on the results of spectrophotometric analysis, the total phenolic content in the methanol extract of *Mitrella kentii* stems was 36.23%. The total phenolic content was determined using Folin Ciocalteau reagent and then analyzed using a spectrophotometer. The phenolic compounds in the methanol extract of *Mitrella kentii* stems will react with the reagent and form a blue complex compound with the color intensity depending on the phenolic compound content present. The standard solution used is gallic acid which is a

stable and simple phenolic compound derived from hydroxybenzoic acid. From the measurement results of the gallic acid standard solution, a calibration curve was obtained with the regression equation y = 0.0052x + 0.0066 with an R2 value of 0.9986. The total phenolic content in *Mitrella kentii* stem extract was calculated using a linear regression equation. The total phenolic content is expressed as gallic acid equivalent (GAE). From the results of the analysis, it is known that the level of total phenolic compounds in *Mitrella kentii* stem extract is 36.23% (Table 3).

Table 3. Results of measurement of total phenolic content of methanol extract of *Mitrella kentii* stems

	Sample Name	
	***Mitrella kentii* stem extract**	**Blank**
U1	0.24	0.05
U2	0.24	0.05
U3	0.24	0.04
K-	0.03	0.03
Abs average	0.240	0.045
Abs-(K-)	0.206	0.011
Abs corrected	0.195	
Weight (mg)	10	
Volume (ml)	100	
Slope	0.0052	
Intercept	0.0066	
Concentration (ppm)	36.2308	
Sample Concentration	36.231%	

Total flavonoid content of the methanol extract of Mitrella kentii stems

The total flavonoid content of the methanol extract of *Mitrella kentii* stems was determined by the method of forming a complex between aluminum chloride with the keto group on the C-4 atom and the hydroxy group on the neighboring C-3 or C-5 atoms from the flavon and flavonol groups. Quercetin is used as a standard for determining flavonoid levels because quercetin belongs to the flavonol group which has a keto group on the C-4 atom and also a hydroxyl group on the neighboring C-3 and C-5 atoms (Kamtekar *et al*., 2014). From the calculation results, an intercept value of 0.1104 and a slope value of 0.0819 are obtained so that the standard curve equation y = 0.0819x - 0.1104 is obtained. This equation was used to determine the flavonoid content as quercetin in the methanol extract of *Mitrella kentii* stems. The results of spectrophotometric analysis showed that the total flavonoid content of the methanol extract of *Mitrella kentii* stems was 0.44% (w/w) (Table 4).

Table 4. Results of measurement of total flavonoid content of methanol extract of *Mitrella kentii* stems

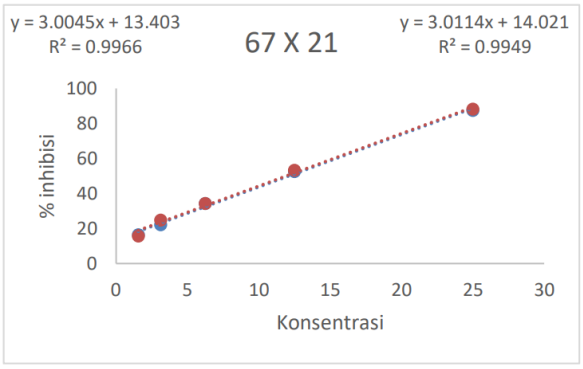
Sample name	***Mitrella kentii* stem extract**
Sample code	66/X/21
Sample weight	0.3096
	0.3096
Abs	0.067
	0.067
a	0.0819
	0.0819
b	-0.1104
	-0.1104
Flavonoid	2.1661
	2.1661
FP	6.25
	6.25
Vol (ml)	100
	100
Flavonoid content (%w/w)	0.4373
	0.4373
Average	0.4373

Antioxidant activity of methanol extract of Mitrella kentii stems

The results of this research show that the antioxidant activity of the methanol extract of *Mitrella kentii* stems is very strong, with an IC50 of 12.06 ppm (IC50 value < 50 ppm). The antioxidant activity test results of the methanol extract of *Mitrella kentii* stems were shown as a percentage of DPPH free radical inhibition and compared with the antioxidant activity of ascorbic acid (Table 5).

Table 5. Results of measurement the antioxidant activity of methanol extract of *Mitrella kentii* stems

67 x 21 *Mitrella kentii*							
	Abs			Abs corrected		% Inhibition	
ppm	U1	U2	K-	U1	U2	U1	U2
100	0.061	0.065	0.047	0.014	0.018	94.6768	93.1559
50	0.08	0.077	0.051	0.029	0.026	88.9734	90.1141
25	0.081	0.079	0.048	0.033	0.031	87.4525	88.2129
12.5	0.168	0.166	0.043	0.125	0.123	52.4715	53.2319
6.25	0.222	0.222	0.049	0.173	0.173	34.2205	34.2205
3.125	0.254	0.247	0.049	0.205	0.198	22.0532	24.7148
1.5625	0.262	0.264	0.042	0.22	0.222	16.3498	15.5894
0	0.305	0.305	0.042	0.263	0.263		



y	a	b	x
50	3.0045	13.403	12.18073
50	3.0114	14.021	11.9476
IC_{50} 67 X 21			**12.064**

DISCUSSION

The LC-MS chromatogram of the methanolic extract from *Mitrella kentii* stems revealed five distinct peaks (Figure 3), each corresponding to a specific phytochemical compound. These identified compounds include d-lirioferine (lirioferine), isosinomenine, N-(1,7-dimethoxy-phenanthren-2-yl)-acetamide, scoulerine, and yuanhunine. The characterization and identification of these five compounds are detailed in Table 1, which also outlines their chemical structures and associated biological activities.
Research by Lima *et al* (2015) found that several of the natural products isolated from *Xylopia amazonica* including lirioferine exhibit in vitro antiplasmodial activity. Based on PASS Software's prediction database, d-Lirioferine (lirioferine) has many biological activities, including respiratory analeptic, antitussive, UGT1A substrate, UGT1A1 substrate, UGT2B1 substrate, antinociceptive, laxative, UGT1A3 substrate, opioid dependency treatment, opioid kappa 3 receptor antagonist, and antialcoholic.

Morphine alkaloids such as isosinomenine isolated from Fangchi species have been reported to have anti-angiogenic, anti-inflammatory and anti-rheumatic effects. Fangchi is one of the most commonly used traditional herbal medicines derived from the rhizoma of Sinomenium acutum and the radix of Stephania tetrandra (Menispermaceae). Sinomenium acutum and Stephania tetrandra have been widely used for the treatment of rheumatic arthritis. The main bioactive components in Sinomenium acutum are alkaloids and lignans such as isosinomenine (Sim *et al*, 2013). According to PASS Software's prediction database, isosinomenine has many biological activities, including 5 Hydroxytryptamine release stimulant, antitussive, neurotransmitter antagonist, sigma receptor agonist, polarisation stimulant, CYP2D2 inhibitor, nicotinic alpha4beta4 receptor agonist, histamine release stimulant, antinociceptive, and membrane permeability inhibitor.

Based on PASS Software's prediction database, N-(1,7-Dimethoxy-phenan-thren-2-yl)-acetamide (C18H17NO3) has many biological activities, including mycothiol-S-conjugate amidase inhibitor, neurodegenerative diseases treatment, 5-O-(4-coumaroyl)-D-quinate 3'-monooxygenase inhibitor, aspulvinone dimethylallyltransferase inhibitor, 3-Hydroxybenzoate 6-monooxygenase inhibitor, creatininase inhibitor, phobic disorders treatment, tRNA-pseudouridine synthase I inhibitor, antiparkinsonian, and 2-Hydroxymuconate-semialdehyde hydrolase inhibitor.

The results of research conducted by Habartova *et al* (2018) show that isoquinoline alkaloid such as scoulerine indicated promising suppression of cancer cells growth. Scoulerine reduces the mitochondrial dehydrogenases activity of the evaluated leukemic cells. Scoulerine exerted potent antiproliferative activity in lung, ovarian and breast carcinoma cell lines. Leukemic cells treated with scoulerine were decreased in proliferation and viability. The potent antiproliferative and proapoptotic function of scoulerine in cancer cells caused by its ability to interfere with the microtubule elements of the cytoskeleton, checkpoint kinase signaling and p53 proteins. This is the first study of the mechanism of scoulerine at cellular and molecular level. Scoulerine is a potent antimitotic compound and that it merits further investigation as an anticancer drug. According to PASS Software's prediction database, scoulerine has many biological activities including 5 hydroxytryptamine release stimulant, MAP kinase stimulant, antidyskinetic, UGT2B12 substrate, membrane permeability inhibitor, JAK2 expression inhibitor, general pump inhibitor, aspulvinone dimethylallyltransferase inhibitor, gluconate 2-dehydrogenase (acceptor) inhibitor, and CYP2D2 inhibitor.

Yuanhunine derived from medicinal plants like *Corydalis rhizoma*, has been studied for its potential in traditional medicine. Research on the traditional knowledge of medicinal plants used by the Yuan in Thailand has documented and analyzed traditional medical practices, including the use of plants like *Corydalis rhizoma*. This research highlights the importance of preserving traditional medicinal knowledge, which can lead to the discovery of new medicines (Inta *et al*, 2013). Yuanhunine has been identified as having pharmacological relevance. A study exploring exogenous ligands for the orphan receptor BRS-3 found that yuanhunine from *Corydalis rhizoma* can antagonize BRS-3 to varying degrees. This finding is significant for the study of BRS-3's function and could lead to new drug discoveries (Qiu *et al*, 2022). Yuanhunine's potential in treating depression was explored in a study involving Corydalis Rhizoma, known as YuanHu in China. The study investigated the antidepressive effect of total alkaloids from YuanHu and identified specific biomarkers related to depression. The findings suggest the therapeutic efficacy of these alkaloids, including yuanhunine, against depression, demonstrating the utility of NMR-based metabolomics in evaluating herbal therapeutics (Wu *et al*, 2015). Based on PASS Software's prediction database, Yuanhunine has many biological activities, including MAP kinase stimulant, antidyskinetic, 5 hydroxytryptamine release stimulant, membrane permeability inhibitor, UGT2B12 substrate, sigma receptor agonist, gluconate 2-dehydrogenase (acceptor) inhibitor, general pump inhibitor, cardiovascular analeptic, and HIF1A expression inhibitor.

Spectrophotometric analysis revealed that the methanolic extract of *Mitrella kentii* stems contained a total phenolic content of 36.23%. This value was obtained using the Folin–Ciocalteu reagent, which reacts with phenolic compounds to produce a blue-colored complex, the intensity of which correlates with the concentration of phenolics present. Gallic acid, a simple and stable phenolic compound derived from hydroxybenzoic acid, was used as the standard for quantification. The calibration curve generated from gallic acid measurements yielded a regression equation of $y = 0.0052x + 0.0066$ with a high correlation coefficient ($R^2 = 0.9986$). Using this linear regression, the phenolic content in the extract was calculated and expressed as gallic acid equivalent

(GAE). The final analysis confirmed that the total phenolic content of the extract was 36.23%, as shown in Table 3.

This study measured the total content of phenolic compounds in methanol extracts from *Mitrella kentii* stems using a spectrophotometric method with Folin–Ciocalteu reagent. Phenolic compounds are bioactive compounds that are widely found in plants and are known to have high antioxidant activity.

In the process, Folin–Ciocalteu reagent is used because it can react with phenolic compounds. When reacting, this reagent forms a blue complex. The more phenolic compounds, the stronger the blue color. The intensity of this blue color is then measured with a spectrophotometer, a tool that can read how much light is absorbed by the colored solution. The standard used to determine the levels of phenolic compounds in the extract, the researchers compared it with a standard solution of gallic acid, a pure phenolic compound that is stable and commonly used as a comparison. From the measurement of various concentrations of gallic acid, a calibration curve was made that produced a straight-line equation: $y = 0.0052x + 0.0066$, where: x is the concentration (ppm); y is the absorbance (blue color level), and $R^2 = 0.9986$ indicates that the relationship between concentration and absorbance is very strong and linear.

The final result, using this equation, the researcher calculated that the methanol extract of *Mitrella kentii* stems contained 36.23% phenolic compounds, and this result was expressed in units of gallic acid equivalents (GAE). This means that the amount of phenolic compounds present is equivalent to 36.23% of the weight of the extract when compared to gallic acid. The conclusion is that the methanol extract of *Mitrella kentii* stems is rich in phenolic compounds, which have the potential to provide high antioxidant effects. This is important in pharmacological research and the development of herbal medicines or natural supplements.

The total flavonoid content in the methanolic extract of *Mitrella kentii* stems was measured using a colorimetric method based on the formation of a complex between aluminum chloride and the keto group at the C-4 position, as well as the hydroxyl groups at the C-3 or C-5 positions of flavones and flavonols. Quercetin, a member of the flavonol group containing these functional groups, was used as the reference standard (Kamtekar et al., 2014). Based on the standard curve generated from quercetin, a regression equation of $y = 0.0819x - 0.1104$ was obtained, with an intercept of 0.1104 and a slope of 0.0819. This equation was applied to quantify the flavonoid content in the extract, expressed as quercetin equivalent. Spectrophotometric analysis indicated that the methanol extract contained a total flavonoid content of 0.44% (w/w), as shown in Table 4.

Flavonoids are a group of secondary metabolite compounds known to have many biological activities, including as antioxidants, anti-inflammatories, and antimicrobials. Measurement of total flavonoid content in methanol extract of *Mitrella kentii* stems was carried out using the colorimetric method, which is a method that utilizes color changes as an indicator of the amount of substance being measured. In this case, flavonoids in the extract will react with aluminum chloride ($AlCl_3$). The reaction occurs between $AlCl_3$ and the keto group at position C-4, as well as the hydroxyl group (–OH) at position C-3 or C-

5 in the flavonoid ring structure. The results of this reaction form a colored complex, the color intensity of which reflects the concentration of flavonoids in the sample. Researchers use quercetin as a standard or comparison solution, because this compound belongs to the flavonol group which has a typical structure (keto at C-4 and –OH at C-3 and C-5), so it can react optimally with $AlCl_3$. Quercetin is used to create a standard curve, which is a graph of the relationship between quercetin concentration and its color intensity (absorbance). The result obtained a linear regression equation: $y = 0.0819x – 0.1104$ with x = quercetin concentration (ppm); y = absorbance (spectrophotometer reading); 0.0819 = slope/ability of the solution to absorb light, –0.1104 = intercept value (initial value at zero concentration). The results of measurements using this equation, the researcher calculated that the methanol extract of *Mitrella kentii* stems contained 0.44% flavonoids in weight per weight (w/w). This value is expressed as quercetin equivalent, meaning that the flavonoid content is measured based on how many compounds have the same activity and structure as quercetin. In conclusion, this method allows researchers to quantitatively measure how many flavonoid compounds are in a plant extract. The result of 0.44% shows that although the amount of flavonoids is not as high as the phenolic compounds in this extract, its content is still significant and contributes to the antioxidant activity detected in this study.

The findings of this study indicate that the methanolic extract of *Mitrella kentii* stems exhibits very strong antioxidant activity, as evidenced by an IC_{50} value of 12.06 ppm (categorized as strong when $IC_{50} < 50$ ppm). The antioxidant potential was assessed through the percentage of DPPH free radical inhibition and benchmarked against the activity of ascorbic acid, as presented in Table 5. This study measured the antioxidant activity of *Mitrella kentii* stem extract using the DPPH (2,2-diphenyl-1-picrylhydrazyl) test. The DPPH test is one of the most common methods to assess the antioxidant ability of a substance to neutralize free radicals, which are reactive molecules that can damage body cells and cause various degenerative diseases. DPPH is a stable free radical that is dark purple in color. When antioxidants react with DPPH, there is a process of donating electrons or hydrogen atoms from the antioxidant compound to the DPPH radical. This reaction changes the color of the DPPH solution to pale yellow, and the decrease in the intensity of the purple color is measured by a spectrophotometer. The greater the decrease in color, the stronger the antioxidant's ability to neutralize free radicals.

IC_{50} (Inhibitory Concentration 50%) is the concentration of extract needed to inhibit 50% of DPPH free radicals. This value is an indicator of how strong the antioxidant activity of a sample is. Based on the general classification, IC_{50} <50 ppm → very strong antioxidant activity; 50–100 ppm → strong; 100–150 ppm → moderate; and 150 ppm → weak. In this study, the IC_{50} of *Mitrella kentii* stem extract was 12.06 ppm, which is included in the very strong category. To ensure the reliability of the results, the antioxidant activity of this extract was compared with ascorbic acid (vitamin C) as a standard. Ascorbic acid is a very strong pure antioxidant and is often used as a comparison in similar studies. The results are displayed as a percentage of DPPH inhibition, which indicates how many percent of free radicals were successfully neutralized by the extract compared to the standard. In conclusion, the methanol extract of *Mitrella kentii* stem has a very high antioxidant potential, even approaching the antioxidant activity of ascorbic

acid. This indicates that this extract contains bioactive compounds (such as phenolics and flavonoids) that play an important role in protecting body cells from oxidative damage.

The results showed that the methanol extract of *Mitrella kentii* stems contains phenolic and flavonoid compounds that contribute to very strong antioxidant activity. The total phenolic content obtained was 36.23% (equivalent to gallic acid), indicating that this extract is rich in phenolic compounds that have a high ability to capture free radicals. Phenolic compounds are widely known as natural antioxidants that can prevent cell damage due to oxidative stress through the mechanism of electron donation. In addition, the total flavonoid content in the extract was also detected although in a lower amount, namely 0.44% (equivalent to quercetin). Flavonoids, as part of secondary plant metabolites, play an important role in protecting biological tissues from oxidative damage and inflammation through scavenging activity against various types of free radicals.

Antioxidant activity test using the DPPH method showed that the methanol extract of *Mitrella kentii* stems had an IC_{50} value of 12.06 ppm. Based on general criteria, this value is included in the category of very strong antioxidant activity (IC_{50} <50 ppm). These results indicate that although the flavonoid content is relatively small, the presence of phenolic compounds in high concentrations can provide a major contribution to the overall antioxidant activity of the extract. Comparison with ascorbic acid as a reference standard shows that the extract activity is close to pure antioxidant activity, which strengthens the potential of *Mitrella kentii* as a source of natural antioxidants. Overall, these results strengthen the evidence that *Mitrella kentii* has good prospects as a natural ingredient with pharmacological potential, especially in preventing or reducing the impact of oxidative stress associated with various degenerative diseases. These findings are in line with previous studies on tropical plant species that are rich in secondary metabolites and show promising biological activities.

CONCLUSION

Based on the results of this study, it can be concluded that the methanol extract of *Mitrella kentii* stems contains phenolic and flavonoid compounds and shows very strong antioxidant activity, with an IC_{50} value of 12.06 ppm. The total phenolic content was recorded at 36.23% (GAE), while flavonoids were detected at 0.44% (QE), contributing to the extract's ability to neutralize free radicals. In addition, analysis of metabolite compounds showed the presence of various chemical compounds that have potential pharmacological activity. Thus, the methanol extract of *Mitrella kentii* stems has the potential as a source of natural antioxidants that can be utilized in the development of pharmaceutical and phytotherapy products to counteract oxidative stress, including its prospects as a source of traditional medicinal ingredients based on natural bioactive compounds. Further research is needed to further identify the main active compounds and evaluate their biological activity through in vitro and in vivo tests.

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