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Kuntze Metabolite Profile and Antioxidant Activity of *Vincetoxicum villosum* (Blume) Kuntze

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Abstract: *Vincetoxicum villosum* belongs to the Apocynaceae family. *V. villosum* is often used as a traditional medicine for treating jaundice, gallstones, kidney stones, hepatitis B, and liver diseases. This study aims to determine the metabolite profile and antioxidant activity of V. villosum to provide new information and serve as a reference for its safe use as a medicinal plant. The methods used to determine the metabolite profile involved GC-MS, and antioxidant activity was assessed using the DPPH method. GC-MS analysis showed that V. villosum leaf extract contains n-hexadecanoic acid, phytol, 9,12,15-octadecatrienoic acid (Z,Z,Z)-, and 2,4-di-tert-butylphenol which have potential antioxidant activity. The antioxidant activity in V. villosum leaves is classified as very weak, with IC_{50} ranging 0.6 – 1.6 mg/mL, with the best antioxidant activity observed in polar solvents (ethanol extract) with a maceration time of 72 hours. The highest total phenol content was obtained using ethanol solvent with a maceration time of 72 hours, amounting to 416.17 mg GAE/g, and the highest total flavonoid content was obtained using ethyl acetate solvent with a maceration time of 24 hours, amounting to 168.78 mg QE/g.

Keywords: antioxidant, GC-MS, total phenol, total flavonoid, *Vincetoxicum villosum*

Abstrak: *Vincetoxicum villosum merupakan tanaman yang termasuk dalam family Apocynaceae (Kamboja-kambojaan). V. villosum sering dimanfaatkan sebagai obat tradisional untuk pengobatan penyakit kuning, batu empedu, batu ginjal, hepatitis B, dan liver. Penelitian ini bertujuan untuk mengetahui profil metabolit dan aktivitas antioksidan V. villosum sehingga dapat memberikan informasi baru serta menjadi acuan sebagai tanaman obat yang aman digunakan. Metode yang digunakan untuk mengetahui profil metabolit menggunakan GC-MS dan aktivitas antioksidan menggunakan metode DPPH. Senyawa utama yang dihasilkan dari analisis CG-MS menunjukkan ekstrak daun V. villosum mengandung senyawa asam n-heksadekanoat, fitol, asam 9,12,15-oktadekatrienoat (Z,Z,Z)-, dan 2,4-di-tert-butilfenol yang berpotensi memiliki aktivitas sebagai antioksidan. Aktivitas antioksidan didalam daun V. villosum termasuk kedalam kategori yang sangat lemah dengan nilai berkisaran antara IC_{50} 0,6 – 1,6 mg/mL, dengan aktivitas antioksidan terbaik pada pelarut bersifat polar (ekstrak etanol) dengan waktu maserasi 72 jam. Kadar total fenol terbaik dihasilkan dengan menggunakan pelarut etanol dengan waktu maserasi 72 jam yaitu 416,17 mg GAE/g, dan kadar total flavonoid terbaik menggunakan pelarut etil asetat dengan waktu maserasi 24 jam yaitu 168,78 mg QE/g.*

Kata kunci: *antioksidan, GC-MS, fenol, total flavonoid, Vincetoxicum villosum*

INTRODUCTION

The genus *Vincetoxicum* belongs to the Apocynaceae family, consisting of nearly 100 species spread from Europe and the Mediterranean to East Asia (Kara & Oksuz 2023). *Vincetoxicum villosum* (Blume) Kuntze has a native habitat in tropical climates with high humidity. Sources indicate that this plant has been found in Mount Gede, Mount Gede Pangrango National Park (MGPNP), South Sumatra (Pujihastuti *et al*. 2020). *Vincetoxicum villosum* has three synonyms: (1) *Tylophora chlorantha* Miq., (2) *Tylophora villosa* Blume, and (3) *Vincetoxicum chloranthum* (Miq.) Kuntze (Plantamor 2024). The people of Lubuk Linggau, South Sumatra, may refer to *V. villosum* as Nangka Kuning or *T. villosum*. This plant is a type of vegetation found in forests, and apart from thriving in the forest, it grows wild and is used as a medicinal and ornamental plant by the local population. *Vincetoxicum villosum* has numerous benefits,

Figure 1. a). *Vincetoxicum villosum (*Nangka kuning) plant, b). *Vincetoxicum villosum* leaves

traditionally used for the treatment of jaundice, gallstones, kidney stones, hepatitis B, and liver diseases (Angelia 2019).

Vincetoxicum villosum has a morphological form that climbs and creeps on the surrounding tree trunks. The morphology of *V. villosum* leaves (Figure (a) *Vincetoxicum villosum* (Nangka kuning) Plant; (b) *Vincetoxicum villosum* leaves) is heart-shaped, oval, pointed at the tip, growing alternately, petioled, with a somewhat rough and fine hairy texture. The stem is greenish-brown, round, and wrinkled. This plant is often used in traditional medicine, but research on *V. villosum* leaves is still very limited, thus it holds great potential for the development of herbal medicines. Several species from this genus have traditionally been used to treat neurosis, malaria, ruptre, fever, scrofula, scabies, injuries, cancer, and ssdwounds. Various biological activities such as antibacterial, antifungal, antioxidant, antidiarrheal, antispasmodic, antileishmanial, antimalarial, and cytotoxic activities have been reported in the literature for this genus (Kara & Oksuz 2023).

Natural antioxidants can be obtained from plants containing secondary metabolite compounds such as flavonoids and phenols, which are useful for counteracting free radicals. Flavonoids are phenolic compounds widely isolated from plants. The total flavonoid and phenol content in a sample is related to its antioxidant activity. The higher the total flavonoid and phenol content, the higher its antioxidant ability to suppress free radicals (Nur *et al*. 2019). Exploration of antioxidant sources from plants continues to be developed as an effort to prevent the onset of diseases. One plant that has the potential as a source of natural antioxidants is *V. villosum*. Flavonoids are a group of phenolic compounds that are part of secondary metabolites, with their benzene structure substituted with OH groups (Ningsih *et al*. 2023). Almost all forms of flavonoids have antioxidant activity. The mechanisms of flavonoids as antioxidants are: 1) inhibiting enzyme activity or binding elements involved in the formation of free radicals, thereby suppressing the formation of Reactive Oxygen Species (ROS); 2) directly binding to ROS; and 3) enhancing antioxidant activity (Widiasriani *et al*. 2024).

One technique that can be used for analyzing the compound content in *V. villosum* is by using Gas Chromatography-Mass Spectrometry (GC-MS). This instrument can show fragmentation patterns and the relative abundance of each chemical compound (Prasetya & Ngadiwiyana 2006). By using this analytical method, it is expected to identify various bioactive compounds that have the potential to be used as traditional medicine.

Studies on the leaves of *V. villosum* have shown hepatoprotective effects, which are suspected to be due to its alkaloid and flavonoid content (Ruyani *et al*. 2019). This plant also exhibits antibacterial effects in the ethanol extract of *V. villosum* at a 25% concentration, with the most optimal inhibition against *Enterococcus faecalis* bacteria (Oktoviani *et al*. 2021). The genus *Vincetoxicum* has potential as a traditional medicinal plant, with several studies finding beneficial compounds for medicinal purposes. *Vincetoxicum pumilium* and *Vincetoxicum nigrum* are good sources of antioxidants and can be used as effective food preservatives. The antioxidant content of *V. pumilium* is higher than that of *V. nigrum* in counteracting free radicals (Noorian 2015). Given the significant benefits of *V. villosum* as an herbal medicine, research on the extract content in various solvents is needed to determine the metabolite profile and antioxidant activity. This will provide new information and serve as a reference for the safe use of *V. villosum* as a medicinal plant.

MATERIALS AND METHOD

The materials used in this study are yellow jackfruit leaves (*Vincetoxicum villosum*), ethanol solution, ethyl acetate, chloroform, distilled water, DPPH stock solution, 5.25% sodium carbonate stock solution, Folin-Ciocalteu reagent, 1M sodium acetate (CH_3COON_2) solution, and 10% Aluminum Chloride ($AlCl_3$) solution. The equipment includes a grinder, plastic ziplock bags, an analytical balance, measuring glasses, glass bottles, aluminum foil, filter paper, an orbital shaker, 1.5 ml microtubes, an oven, funnels, a mortar, a UV-Vilabtop spectrophotometer/UV-Vis

spectrophotometer with a microplate reader, a mix met, microplates, a vortex, gas chromatography/mass spectrometry (GC-MS), a camera, paper, and Microsoft Word.

This study employs laboratory experimental methods using Gas Chromatography-Mass Spectrometry (GC-MS) techniques and the analysis of antioxidant data, total phenol, and total flavonoid using SPSS with a Completely Randomized Design (CRD), followed by Duncan's post-hoc test. The research was conducted in the Scientific Collection Area of Cibodas Botanical Garden for sample extract preparation. The *Vincentoxicum villosum* plant was brought from SBIH Ruyani, Bengkulu. Leaf sample of *V. villosum* (http://www.theplantlist.org/tpl/record/tro-2602757; plant identification was verified with the help of Research of Biology, Indonesian Institute of Sciences; http://lipi.go.id/), and the testing of metabolite profiles and antioxidant activity was carried out using Elsa-BRIN Analysis Services. The research took place from August to December 2024.

Preparation of *V. villosum* Extract Material

The collection of materials for extraction from *V. villosum* leaves involves several important steps. The initial stage is the selection of *V. villosum* leaf samples, ensuring that the leaves are healthy and free from diseases and pests. The quality of the selected leaves directly affects the phytochemical content in the leaf extract. The chosen leaf samples are then air-dried until fully dried and subsequently ground using a grinder to proceed to the extraction process, with a weight of 10 grams for each type of solvent.

Extraction of *V. villosum* Leaves

The preparation of *V. villosum* leaf extract uses the maceration method, a traditional technique for extracting natural materials. The extraction of V. villosum simplicia leaves is carried out using three solvents: a polar solvent (ethanol), a semi-polar solvent (ethyl acetate), and a non-polar solvent (chloroform), with a ratio of 1:5 and an extract weight of 10 grams for each solvent. The mixture is then shaken using a shaker for varying durations of 1x24 hours, 2x24 hours, and 3x24 hours at room temperature. The maceration results are filtered using filter paper to separate the residue from the solution. The filtered results are placed in 1.5 ml microtubes and dried until they become concentrated extracts using an oven at 40°C, ready to be used as research test materials.

Gas Chromatography-Mass Spectrometry (GC-MS)

The analysis was conducted at the Advanced Characterization Laboratory in Serpong – BRIN. Using gas chromatography equipped with mass spectrometry (GC-MS-2010 Plus-Shimadzu), the bioactive compounds of methanol and n-hexane plant crude extracts were identified by GC-MS-2010 Plus-Shimadzu, Column type 19091S-433: 93.92873 DB-5MS UI 5% phenyl methyl silox, dimension: 30 m x 250 µm x 0.25 µm. Initial temperature: 40°C; hold time: 1 min. Post run, 300 °C with retention time (Rt) total for 30 min. The sample chromatogram was identified by comparing the mass spectra with library data (NIST 11 Library and Wiley Library) and the GC retention time against known standards.

DPPH Antioxidant Test

The procedure for the DPPH test can be conducted by following these steps: First, dilute the *V. villosum* sample extract with ethanol at a ratio of 10 mg in 1 ml ethanol. For this test, use a ratio of 40:160 µL, consisting of 10 µL stock solution and 39.2 µL ethanol, making a total of 40 µL solution. Add 40 µL of the solution into a plate, then add 160 µL DPPH to the plate, homogenize, and incubate for 30 minutes in the dark. Then, measure the absorbance at a wavelength of 517 nm using a UV-Vis spectrophotometer. The presence of antioxidant activity is indicated by a decrease in absorbance and a visible color change from purple to yellow, due to the reduction of DPPH radicals to non-radical compounds.

Total Phenol Analysis

The initial step involves diluting the *V. villosum* sample extract with ethanol at a ratio of 10 mg in 1 ml ethanol. The standard dilution solutions range from 200 – 100 – 80 – 60 – 40 – 20 – 0 mg/mL. The sample extract is prepared at 10 mg/ml with ethanol. Add 20 µL of the diluted *V. villosum* sample extract to the plate, then add 20 µL of Folin-Ciocalteu reagent, homogenize with a plate shaker using a mix meter, and let it stand/incubate for 5 minutes in the dark. After that, add 160 µL of 5.25% Sodium Carbonate, homogenize, and let it stand/incubate again for 30 minutes in the dark. Then, measure the absorbance at a wavelength of 750 nm using a UV-Vis spectrophotometer.

Total Flavonoid Analysis

The initial step involves diluting the *V. villosum* sample extract with ethanol at a ratio of 10 mg in 1 ml ethanol. The standard dilution solutions range from 200 – 100 – 80 – 60 – 40 – 20 – 0 mg/mL. After dilution, prepare a 50 µg/mL sample extract consisting of 5 µL extract and 45 µL ethanol, then add 100 µg/mL ethanol. Proceed by adding 20 µg/mL of 10% AlCl3, and let it stand/incubate for 3 minutes in the dark. Then, add 20 µL of 1M CH3COON2 and 60 µL ethanol, and let it stand/incubate for 40 minutes in the dark. Finally, measure the absorbance at a wavelength of 430 nm using a UV-Vis spectrophotometer.

RESULT AND DISSCUSION

The extraction of *Vincetoxicum villosum* leaves was carried out in stages using three types of

solvents: polar, semi-polar, and non-polar, with 1.5 ml microtubes allocated for each type of solvent with the extraction method being maceration. The extraction was performed using maceration with three different solvents varying in polarity because different solvents affect the compounds that will dissolve during the extraction process based on their polarity. The maceration method was also conducted in stages with different maceration times of 24, 48, and 72 hours to determine the most effective time for binding the compounds.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The leaf extract of *Vincetoxicum villosum* has potential as an antioxidant, so a test was conducted to identify the compounds present in the V. villosum leaf extract. The results from the test using gas chromatography equipped with mass spectrometry (GC-MS) can be seen in Table 1 for ethanol solvent, Table 2 for ethyl acetate solvent, and Table 3 for chloroform solvent, showing similar major compounds with varying solvent polarities.

Table 1. The main compounds of *V. villosum* leaf extract Identified by GC-MS in a ethanol solvent

No	Compound Name	Class of Compounds	Maceration 24	Maceration 48	Maceration 72	Similarity index
			Area %	Area %	Area %	%
1	n-Hexadecanoic acid	Fatty acid	15.76	18.06	17.69	99
2	Phytol	Diterpenoid	15.52	13.66	14.54	99
3	9,12,15-Octadecatrienoic acid,(Z,Z,Z)-	Polyunsaturated fatty acids	8.20	8.79	7.82	99
4	Squalene	Triterpenoid	6.20	4.87	3.92	99
5	Linoleic acid ethyl ester	Fatty acid esters	3.75	4.47	4.17	99
6	Hexadecanoic acid, ethyl ester	Fatty acid esters	3.21	4.57	4.44	99
7	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	Fatty acid esters	3.02	4.70	3.46	99
8	Loliolide	Benzofuran	3.27	2.46	2.93	99
9	10E,12Z-Octadecadienoic acid	Polyunsaturated fatty acids	3.23	3.95		99
10	Octadecanoic acid, ethyl ester	Fatty acid esters	1.51			99

Table 2. The main compounds of V. villosum leaf extract identified by GC-MS in a ethyl acetate solvent

No	Compound Name	Class of Compounds	Maceration 24	Maceration 48	Maceration 72	Similarity index
			Area %	Area %	Area %	%
1	n-Hexadecanoic acid	Fatty acid	17.69	21.94	12.47	99
2	Phytol	Diterpenoid	14.54	12.13	9.60	99
3	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	Polyunsaturated fatty acids	7.82	8.90	5.57	99
4	2,4-Di-tert-butylphenol	Fenol	2.86	6.65	15.91	99
5	Squalene	Triterpenoid	5.20	4.99	2.17	99
6	Octadecanoic acid	Fatty acid	4.11	3.79	3.67	99
7	9,12-Octadecadienoic acid (Z,Z)-	Polyunsaturated fatty acids	3.15	3.60	2.58	99
8	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	Benzofuran	1.77	1.70	1.76	98
9	Loliolide	Benzofuran	2.98	2.46		98
10	2-Pentadecanone, 6,10,14-trimethyl	Keton	1.44			99

Table 3. The main Compounds of *V. villosum* leaf extract identified by GC-MS in a Chloroform solvent

No	Compound Name	Class of Compounds	Maceration 24	Maceration 48	Maceration 72	Similarity index
			Area %	Area %	Area %	%
1	2,4-Di-tert-butylphenol	Fenol	8.14	12.37	15.64	96
2	Phytol	Diterpenoid	11.42	12.11	7.22	95
3	9,12,15-Octadecatrienoic acid, (Z, Z,Z)-	Polyunsaturated fatty acid	6.22	5.82	3.84	99
4	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	Benzofuran	2.0	2.24	1.78	
5	n-Hexadecanoic acid	Fatty acid	15.16	13.12		98
6	Octadecanoic acid	Fatty acid	3.24	3.52		99
7	Squalene	Triterpenoid	3.81	3.64		98
8	10E,12Z-Octadecadienoic acid	Polyunsaturated fatty acids	2.85	2.54		
9	2-Pentadecanone, 6,10,14-trimethyl	Keton	1.18	1.14		98
10	9,12-Octadecadienoic acid (Z,Z)-	Polyunsaturated fatty acids	0.93		1.64	99

The GC-MS analysis results of *V. villosum* leaves with ethanol solvent can be seen in Table 1. The ethanol extract of *V. villosum* leaves contains 10 compounds, with 3 main compounds having the largest area and present in all three different solvents, namely n-Hexadecanoic acid, Phytol, and 9,12,15-Octadecatrienoic acid (Z,Z,Z)-. The compound n-Hexadecanoic acid has the largest area at a maceration time of 48 hours with an area value of 18.06%. The compound Phytol has the largest area at a maceration time of 24 hours with an area value of 15.52%. The compound 9,12,15-Octadecatrienoic acid (Z,Z,Z)- has the largest area at a maceration time of 48 hours with an area value of 8.79%.

The GC-MS analysis results of *V. villosum* leaves with ethyl acetate solvent can be seen in Table 2. The ethyl acetate extract of *V. villosum* leaves contains 10 compounds, with 3 main compounds having the largest area and present in all three different solvents, namely n-Hexadecanoic acid, Phytol, and 9,12,15-Octadecatrienoic acid (Z,Z,Z)-. The compound n-Hexadecanoic acid has the largest area at a maceration time of 48 hours with an area value of 21.94%. The compound Phytol has the largest area at a maceration time of 24 hours with an area value of 14.54%. The compound 9,12,15-Octadecatrienoic acid (Z,Z,Z)- has the largest area at a maceration time of 48 hours with an area value of 8.90%.

The GC-MS analysis results of *V. villosum* leaves with chloroform solvent can be seen in Table 3. The chloroform extract of *V. villosum* leaves contains 10 compounds, with 3 main compounds having the largest area and present in all three different solvents, namely 2,4-Di-tert-butylphenol, Phytol, and 9,12,15-Octadecatrienoic acid (Z,Z,Z)-. The compound n-Hexadecanoic acid has the largest area at a maceration time of 72 hours with an area value of 15.64%. The compound Phytol has the largest area at a maceration time of 24 hours with an area value of 12.11%. The compound 9,12,15-Octadecatrienoic acid (Z,Z,Z)- has the largest area at a maceration time of 24 hours with an area value of 6.22%.

Based on the GC-MS analysis results of *V. villosum* leaf extract with solvents of different polarities, namely ethanol, ethyl acetate, and chloroform, and with different maceration times, it was found that n-Hexadecanoic acid is one of the main compounds in ethanol and ethyl acetate solvents. This compound has the largest area in ethyl acetate solvent with a maceration time of 48 hours. It belongs to the fatty acid group and has potential biological properties as an antioxidant (Mohan *et al*. 2012) antimicrobial (Dewatisari *et al*. 2022), and antibacterial (Dewatisari & Hariyadi 2024; Shaaban *et al*. 2021). Phytol is one of the main compounds in all solvents used, with the largest area in ethanol solvent with a maceration time of 24 hours. It belongs to the diterpenoid compound group and has potential biological properties as an antioxidant, anticancer, antiviral, and diuretic (Mohan *et al*. 2012). Additionally, it can be used as a schistosomicide drug and a precursor for the synthesis of vitamin E and vitamin K1 (PubChem 2024). 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- is one of the main compounds in all solvents used, with the largest area in ethyl acetate solvent with a maceration time of 48 hours. It belongs to the polyunsaturated fatty acids group and has potential biological properties such as reducing the risk of coronary heart disease (CHD) by lowering LDL and total cholesterol levels and increasing HDL levels by 6% (Hasbi *et al*. 2020). 2,4-Di-tert-butylphenol is one of the main compounds in the solvents used, with the largest area in chloroform solvent with a maceration time of 24

hours. It belongs to the phenol compound group and has potential biological properties as an antioxidant (PubChem 2024), anti-inflammatory, and antimicrobial (Zhao *et al*. 2020).

The main compounds with antioxidant functions in *V. villosum* leaf extract are n-Hexadecanoic acid, phytol, and 2,4-Di-tert-butylphenol. According to Mohan *et al*. 2012, the plant *Aristolochia krysagathra* has 9 dominant compounds in the ethanol extract analyzed by GC-MS, including n-Hexadecanoic acid (10.25%) and Phytol (4.46%), which exhibit antioxidant activity. Similarly, Sogandi *et al*. (2019) identified secondary metabolite compounds from the ethyl acetate fraction of licorice root (*Glycyrrhiza glabra* L) using GC-MS, showing that the main group of compounds is fatty acids, with n-Hexadecanoic acid being the most abundant, having a peak area of 14.14%, and acting as an antioxidant and antibacterial. According to Siregar *et al*. (2018), GC-MS analysis of date seed extract with n-hexane solvent revealed that 2,4-Di-tert-butylphenol has antioxidant activity, presumably due to the presence of free hydroxyl groups that can donate protons or hydrogen atoms to neutralize free radicals.

Research by Ruyani *et al*. (2019) found that the ethanol extract of *Vincetoxicum villosum* (Blume) Kuntze contains secondary metabolites, including alkaloids, flavonoids, and triterpenoids. This plant can be used as a medicinal plant due to its compound content, making it a potential new innovation in the medical field for development as a component in medicine. The use of different solvents and maceration times still produces the same main compounds because GC-MS analysis has the capability to detect compounds with a high level of specificity. However, differences in the compound area obtained can be attributed to the variations in the polarity properties of the solvents used. Solvents with suitable polarity, such as ethanol, are more efficient in extracting specific compounds, resulting in a larger compound area in the GC-MS analysis. This aligns with Fahmi (2020), who stated that solvent variations affect the compound area produced by GC-MS analysis based on their polarity. In this case, ethanol, as a polar solvent, combined with an optimal maceration time of 24 hours, provides the best results for binding the active compound content in the *V. villosum* leaf extract. Therefore, selecting the right solvent based on the chemical properties of the target compounds is a key factor in ensuring extraction and analysis efficiency.

DPPH Antioxidant Test and Quantitative Analysis

The antioxidant test, total phenol, and total flavonoid analyses (Table 4) are conducted to determine the secondary metabolite content of *V. villosum* leaf extracts that can be utilized as antioxidants.

DPPH Antioxidant Test

Antioxidant activity is indicated by the IC_{50} value, which represents the concentration of the sample solution (extract or vitamin C) needed to reduce 50% of DPPH free radicals. A substance with high antioxidant activity will have a low IC_{50} value. According to Susana *et al*. (2018), the strength category of antioxidant activity based on IC_{50} values is as follows very strong: IC_{50} less than 0.05 mg/mL (<50 µg/mL), strong: IC_{50} between 0.05 – 0.1 mg/mL (50-100 µg/mL), moderate: IC_{50} between 0.1 – 0.15 mg/mL (100-150 µg/mL), Weak: IC50 between 0.15 – 0.2 mg/mL (150-200 µg/mL), Very weak: IC_{50} greater than 0.2 mg/mL (>200 µg/mL).

Based on the research conducted on *V. villosum* leaf extract using the DPPH method by UV-Vis spectrophotometry, the results are shown in Table 4. From the antioxidant activity test, it was found that the ethanol extract of *V. villosum* leaves with maceration times of 24, 48, and 72 hours produced 0.6495, 0.6322, and 0.6108 mg/mL, respectively. The ethyl acetate extract with maceration times of 24, 48, and 72 hours produced 1.0102, 1.0305, and 0.9439 mg/mL. The chloroform extract with maceration times of 24, 48, and 72 hours produced 1.3620, 1.5416, and 1.6128 mg/mL. Based on these results, the antioxidant activity of *V. villosum* leaf extract with ethanol, ethyl acetate, and chloroform solvents and maceration time variations of 24 hours, 48 hours, and 72 hours showed very weak antioxidant activity with absorption values reaching IC_{50} 0.6 – 1.6 mg/mL.

A comparison of IC_{50} values across the three different solvents, according to their polarity, indicates that ethanol solvent has the highest antioxidant activity because it has the lowest IC_{50} value compared to ethyl acetate and chloroform solvents. This is likely due to the high content of polar bioactive compounds in *V. villosum* leaves compared to semi-polar and non-polar bioactive compounds. Therefore, the polar solvent (ethanol) extracts more bioactive components from the *V. villosum* leaf extract. According to Yang *et al*. (2011), antioxidants based on solubility consist of nonpolar (lipophilic) and polar (hydrophilic) antioxidants. Thus, the *V. villosum* leaf extract is categorized as a hydrophilic antioxidant or soluble in polar solvents. The antioxidant activity of the ethanol solvent with a maceration time of 72 hours binds more compounds and produces the highest antioxidant activity with an IC_{50} value of 0.6108 mg/ml compared to maceration times of 24 and 48 hours.

Total Phenol Analysis

Based on Table 4, the total phenol content in *V. villosum* leaf extract with ethanol solvent and maceration times of 24, 48, and 72 hours was 182.78, 216.34, and 416.17 mg GAE/g, respectively. With

Table 4. Total phenol content, total flavonoid content, and antioxidant analysis

Types of Extracts	Solvent / Maceration Time	TPC mg GAE/g	SD	TFC mg QE/g	SD	Antioxidant (IC_{50}) (ppm) (mg/ml)	SD
Vincetoxicum villosum leaves	Ethanol 24	182.78 e	2.65	134 e	3.66	0,6495 a	12.5
	Ethanol 48	216. 34 f	3.96	121. 07 d	4.27	0,6322 a	27.40
	Ethanol 72	416.17 g	21.21	128. 76 de	2.47	0,6108 a	19.59
	Ethyl Acetate 24	158.45 d	7.32	168.78 g	6.40	1,0102 ab	17.44
	Ethyl Acetate 48	120.69 c	5.53	123. 86 d	5.72	1,1030 ab	98.62
	Ethyl Acetate 72	153.11 d	13.50	146.42 f	5.31	0,9439 ab	23.46
	Chloroform 24	112.69 c	7.13	107.44 a	6.46	1,3620 bc	155.16
	Chloroform 48	46.77 a	4.55	54.36 a	3.55	1,5416 c	134.61
	Chloroform 72	67.16 b	1.48	63.84 b	1.79	1,6128 c	120.30

ethyl acetate solvent and maceration times of 24, 48, and 72 hours, the values were 158.45, 120.69, and 153.11 mg GAE/g. Lastly, using chloroform solvent with maceration times of 24, 48, and 72 hours, the values were 112.69, 46.77, and 67.16 mg GAE/g. The highest total phenol content was produced by *V. villosum* leaf extract with ethanol solvent and a maceration time of 72 hours, which was 416.17 mg GAE/g. The lowest total phenol content was produced by *V. villosum* leaf extract with chloroform solvent and a maceration time of 48 hours, which was 46.77 mg GAE/g. This aligns with the research of Suhendra *et al*. (2019) which stated that ethanol is a solvent capable of dissolving semi-polar to polar compounds, one of which is phenolic compounds. Ethanol can dissolve phenolic compounds because it can degrade cell walls. This finding is also consistent with the research of Handayani *et al*. (2024), which noted that phenols are polar compounds due to having a number of unsubstituted hydroxyl groups or a sugar. Phenolic compounds can dissolve in polar solvents such as methanol, ethanol, butanol, and acetone. According to Hapsari *et al*. (2018), 80% ethanol is a solvent with high polarity, thus being able to dissolve phenols better, resulting in higher concentrations in the extract. The use of polar solvents yields a higher total phenol content compared to non-polar solvents.

Total Flavonoid Analysis

Based on Table 4, the total flavonoid content of *V. villosum* leaf extract in ethanol solvent with maceration times of 24, 48, and 72 hours was 134, 121.07, and 168.78 mg QE/g, respectively. With ethyl acetate solvent and maceration times of 24, 48, and 72 hours, the values were 168.78, 123.86, and 146.42 mg QE/g, respectively. Using chloroform solvent with maceration times of 24, 48, and 72 hours, the values were 107.44, 54.36, and 63.84 mg QE/g, respectively. The highest total flavonoid content was found in *V. villosum* leaf extract using ethyl acetate solvent with a maceration time of 24 hours, producing 168.78 mg QE/g of flavonoid compounds. Conversely, the lowest total flavonoid content in *V. villosum* leaf extract was obtained using chloroform solvent with a maceration time of 48 hours, yielding 54.36 mg QE/g.

This is consistent with the research by Rahmati *et al*. (2020), which determined the total flavonoid content of saliara leaves (*Lantana camara* L.) using 70% ethanol, ethyl acetate, and n-hexane solvents, showing that ethyl acetate solvent had the highest flavonoid content. Ethyl acetate is a semi-polar solvent that can extract both polar and non-polar flavonoid compounds (Yani *et al*. 2023). In plants, several free flavonoid compounds are easily soluble in semi-polar solvents, such as flavone, flavanone, and flavonol (Rahmati *et al*. 2020). Therefore, these flavonoid compounds are likely to dissolve in the semi-polar ethyl acetate solvent. A compound will dissolve in a solvent with the same polarity. The lowest total flavonoid content was found in the non-polar chloroform solvent, which can only dissolve non-polar compounds. Some types of flavonoids that can dissolve in non-polar solvents include polymethoxyflavone aglycones or isoflavones whose sugar groups or glycoside forms have been removed, making them soluble only in non-polar solvents (Rahmati *et al*. 2020). The research by Ruyani *et al*. (2019) stated that the phytochemical test results showed that *V. villosum* contains alkaloids, flavonoids, and triterpenoids through qualitative analysis. This is consistent with the findings that the *V. villosum* leaf extract has the highest total phenol content in ethanol solvent with a maceration time of 72 hours, which is 416.17 mg GAE/g. Similarly, the highest total flavonoid content is found in ethyl acetate solvent with a maceration time of 24 hours, which is 168.78 mg QE/g.

Based on the research results, each test conducted showed that *V. villosum* possesses total phenolics, total flavonoids, and antioxidant activity, indicating its potential as a natural antioxidant source. Similarly, the study by Nur *et al*. (2019) stated that natural antioxidants can be derived from plants containing secondary metabolites such as flavonoids and

phenols, which are useful in countering free radicals. The total flavonoid and phenol content in a sample is associated with its antioxidant activity. The higher the total flavonoid and phenol content, the greater the antioxidant capability to suppress free radicals. Thus, the determination of TPC (Total Phenolic Content) and TFC (Total Flavonoid Content) becomes part of the sample analysis related to antioxidant activity. Samples with high antioxidant activity usually contain secondary metabolite compounds of the phenolic group at relatively high levels. In line with the GC-MS analysis results, the main compounds identified in the *V. villosum* leaf extract include n-hexadecanoic acid, phytol, 9,12,15-octadecatrienoic acid (Z,Z,Z)-, and 2,4-di-tert-butylphenol, which possess potential antioxidant activity.

CONCLUSION

Based on the results of GC-MS analysis, the main compounds found in the leaf extract of *V. villosum* are n-hexadecanoic acid, phytol, 9,12,15-octadecatrienoic acid (Z,Z,Z)-, and 2,4-di-tert-butylphenol, which have potential antioxidant activity. The highest total phenolic content was obtained using ethanol as a solvent with a maceration time of 72 hours, reaching 416.17 mg GAE/g. Meanwhile, the highest total flavonoid content was achieved using ethyl acetate as a solvent with a maceration time of 24 hours, reaching 168.78 mg QE/g. These compounds are effective in counteracting free radicals, making the *V. villosum* leaf extract a potential natural source of antioxidant activity with an absorption value of IC_{50} ranging from 0.6 to 1.6 mg/mL.

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