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Exploration of antioxidant metabolites in Minnie root (*Ruellia tuberosa* L.) leaves

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Abstract

Minnie root (*Ruellia tuberosa* L.) is a medicinal plant known for its various therapeutic uses. The leaf extract of Minnie root contains secondary metabolites like alkaloids, flavonoids, triterpenoids, steroids, and saponins, which exhibit antidiabetic, antibacterial, and antioxidant properties. This study aims to analyze the abundance of metabolite compounds, antioxidant levels, and antioxidant metabolites in Minnie root leaves using GC-MS analysis. Additionally, the study explores the antioxidant metabolites present in the ethanol extract of Minnie root leaves through various online databases. The results revealed that the ethanol extract of Minnie root leaves contained 17 compounds, with 9 of them identified as antioxidant metabolites, constituting 72.38% of the total compounds. Some of the antioxidant bioactive compounds identified include *D-limonene* and *5-hydroxymethylfurfural*. The antioxidant concentration in Minnie root leaves was measured at is 99.480 ± 8.224 ppm using ascorbic acid standards and 133.500 ± 7.912 ppm using quercetin standards. Further research is necessary to explore other potential bioactivities of Minnie root leaf metabolites, such as their antidiabetic properties, for developing herbal medicines.

Keywords: *Ruellia tuberosa* L.; Antioxidant metabolites; GC-MS; *5-Hydroxymethylfurfural*

1. Introduction

Antioxidants are substances that can halt the oxidation process and protect cells from free radicals originating from the body's metabolism and other external sources. Antioxidant compounds that are stable enough to provide electrons or hydrogen to free radical molecules neutralize them, reducing their ability to carry out free radical chain reactions. Natural antioxidants in plants and food mainly come from phenol and flavonoid derivatives, hydroxamic acid derivative compounds, coumarins, organic acids, and vitamin C [1]. Plant antioxidants are bioactive compounds consisting of phenolic diterpenes, tannins, alkaloids, phenolic compounds, sulfur-containing compounds, and vitamins [2]. Natural antioxidants can be found in various plant parts, including wood, seeds, leaves, fruits, roots, flowers, and pollen [3]. These antioxidants are commonly used in functional foods, drinks, and herbal medicine treatments.

Herbal medicine is a type of medicine made from plants that have been extracted and processed in various methods to create powders or pills without the use of chemicals. Herbal medicine can effectively treat diseases with minimal side effects. One widely used herbal medicine in the community is the Minnie root plant (*Ruellia tuberosa* L.), known for its antioxidant, antibacterial, and anticancer properties [4]. The Minnie root plant has beneficial effects on human health and can treat various diseases [5]. The Minnie root plant contains secondary metabolite compounds such as flavonoids, alkaloids, steroids, triterpenoids, and saponins. Extracts from the Minnie root plant leaves are rich in antioxidants [6].

Previous research [7] revealed that Minnie root plants contain a variety of phytochemicals, including flavonoids, triterpenoids, alkaloids, steroids, and saponins, exhibiting properties such as antidiabetic, antioxidant, antibacterial,

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antifungal, and anticancer effects. Observed [8] that male Wistar rats treated with Minnie root leaf extract showed reduced levels of MDA in their intestines, attributed to the antioxidant properties of the extract. identified [9] four active compounds in the ethanol extract of Minnie root leaves using Gas Chromatography-Mass Spectrometry (GC-MS): *9-Octadecenamide*, *Hexadecanamide*, *Octadecenamide*, and *1,2 Benzenedicarboxylic acid*. However, the bioactivity of these compounds remains unknown. Further research is necessary to explore the abundance and dominance of metabolite compounds in Minnie root leaves and analyze the antioxidant concentration through GC-MS analysis of ethanol extracts.

2. Material and methods

2.1. Sampling

Sampling of Minnie root leaves was carried out in Jambu Ilir Village, Tanjung Lubuk District, Ogan Komering Ilir Regency, South Sumatra Province, Indonesia. The altitude of the place is approximately 10 meters above sea level in a lowland area, with coordinates of 3°28'18.5" South Latitude and 104°46'02.8" East Longitude.

This research was conducted at the Physiology and Development Laboratory and the Genetics and Biotechnology Laboratory of the Biology Department, Sriwijaya University as well as the Analysis Laboratory of the Pharmacy Department, Padjadjaran University, Indonesia.

2.2. Tools and Materials

The tools used include GC-MS instruments, rotary evaporators, UV-Vis spectrophotometers, and the materials used are 70% Ethanol and DPPH (*2,2-diphenyl-1-picrylhydrazyl*).

2.3. Procedure

- **Preparation and Maceration**
  - Minnie root leaves are selected from the 3rd to 5th leaves of the shoots or mature leaves. Leaf samples are dried away from direct sunlight. The dried leaves are then ground using a blender until they become simplicia. After obtaining the simplicia powder, maceration is carried out on 200gs of simplicia with 70% ethanol (1000 ml) for 3 days. The mixture is then filtered with filter paper, and the filtrate obtained is evaporated with an evaporator to produce a thick extract yield.
- **Metabolite Content Analysis Using GC-MS**
  - The yield of Minnie root leaves is analyzed using GC-MS. A 0.1 mM sample is added with 2 ml of MeOH and 2 ml of chloroform, sonicated for 10 minutes, and centrifuged for 5 minutes at 10,000 rpm. The supernatant is then injected into GC-MS following the protocol of GC-MS Agilent 7890A gas chromatograph.
- **Antioxidant Concentration**
  - The antioxidant concentration is determined using DPPH reagent. 0.5 ml of Minnie root leaf sample extract is mixed with 2.5 ml of 0.1 mM DPPH ethanol solution and incubated for 30 minutes at 25°C. The absorbance is measured at 517 nm using a UV-Vis spectrophotometer. Antioxidant Concentration are calculated using the provided formula.

$$\text{Antioxidant concentration of sample} = \frac{Sample\ Absorbance}{Standard\ Absorbance} \times \text{Antioxidant concentration of standard}$$

2.4. Data Analysis

GC-MS data are analyzed using Cromelon 5 software to identify chemical components, chemical structure, retention time, and area. The detected metabolites are further analyzed for biosynthesis pathways, properties, and bioactive properties using *PubChem, KEGG, ChEBI, PlantCyc*, and *SpectraBase* websites. Antioxidant levels data are analyzed by calculating the average and standard deviation.

3. Results and discussion

3.1. Metabolite profile the Minnie root Leaves

The results of the interpretation of chromatogram data from the ethanol extract of Minnie root leaves revealed metabolite compounds as shown in Table 1.

Based on Table 1, 17 types of compounds were identified with a total abundance of 100%. The compounds obtained were more than in previous studies [9]. The results of GC-MS analysis showed four active compounds from the ethanol extract of Minnie root leaves. The identified compounds have different abundances, as shown in Table 1. It is known that there are nine dominant compounds in Minnie root leaves, including *5-Hydroxymethylfurfural* (48.96%), *2,6-Octadien-1-ol, 3,7-dimethyl-, acetate* (11.89%), *Acetic acid* (11.58%), *4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl* (8.37%), *D-Limonene* (4.57%), *2-Furancarboxylic acid, hydrazide* (2.04%), *1,4-Cyclohexanedimethanol* (1.96%), *1-Octanol, 2-nitro* (1.28%), *2,3-Anhydro-d-mannosan* (1.02%).

Table 1 Metabolite profile of compounds and abundance of compounds from the ethanol extract leaves of Minnie root (*Ruellia tuberosa* L.) using GC-MS

Compounds	**Chemical Formula**	***Retention Time***	**Relative Abundance**
Acetic acid	$C_2H_4O_2$	2.74	11.58
Acetic anhydride	$C_4H_6O_3$	3.08	0.7
2-Propenoic acid, ethenyl ester	$C_5H_6O_2$	3.87	0.5
Furfural	$C_5H_4O_2$	4.74	2.16
Methylenecyclopropanecarboxylic acid	$C_5H_6O_3$	5.45	0.65
2-Furancarboxaldehyde, 5-methyl	$C_6H_6O_2$	7.84	0.71
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	8.42	0.51
D-Limonene	$C_{10}H_{16}$	9.59	4.57
2-Furancarboxylic acid, hydrazide	$C_5H_6N_2O_2$	11.26	2.04
Ethanamine, N-ethyl-N-nitroso	$C_4H_{10}N_2O$	12.88	0.85
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	13.09	8.37
1-Octanol, 2-nitro	$C_8H_{17}NO_3$	13.23	1.28
5-Hydroxymethylfurfural	$C_6H_6O_3$	15.70	48.96
5-Hydroxymethylfurfural	$C_6H_6O_3$	16.09	2.25
2,6-Octadien-1-ol, 3,7-dimethyl-, acetate	$C_{12}H_{20}O_2$	19.47	11.89
2,3-Anhydro-d-mannosan	$C_6H_8O_4$	32.66	1.02
1,4-Cyclohexanedimethanol	$C_8H_{16}O_2$	35.97	1.96
Total Relative Abundance	100%		

The most dominant compound is the *5-Hydroxymethylfurfural* (*5HMF*) compound with a total abundance of 48%. This compound can be produced in plants due to biotic or abiotic stress. The abundance of the *5-HMF* compound in plant metabolites can vary depending on the type of plant, growth conditions, and environmental factors. According to [10],, the phytochemical content of a plant is influenced by several factors such as genes, light, temperature, humidity, pH, differences in altitude, which can produce different compounds, and the type of solvent used.

3.2. Antioxidant Levels of Minnie root Leaves

The following Table 2. results of antioxidant levels with ascorbic acid and quercetin standards.

Table 2 Antioxidant Levels of Minnie root (*Ruellia Tuberosa* L.) Leaves with Ascorbic Acid and Quercetin Standards

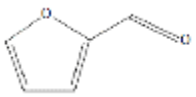
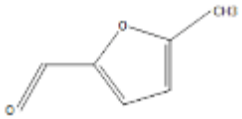
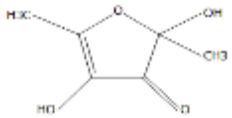
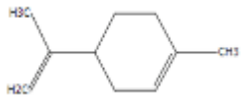
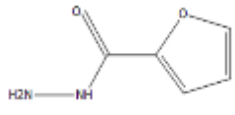
Antioxidant Standard	**Antioxidant concentration (*x* mean ± sd) ppm**
Ascorbic acid	99.480 ± 8.224
Quercetin	133.500 ± 7.912

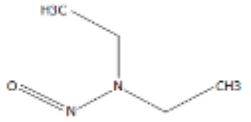
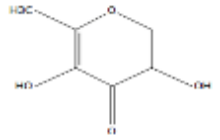
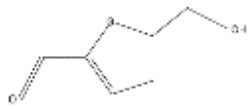
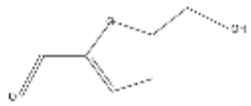
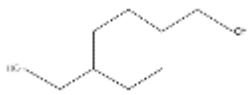
The antioxidant levels of Minnie root leaves are 99.480 ± 8.224 ppm with ascorbic acid standards and 133,500 ± 7.912 ppm with quercetin standards. According to [11], the ethanol extract of *Ruellia tuberosa* L. leaves can inhibit free radicals as an antioxidant agent with an IC50 value of 28.6 µg/ml, classified as a compound with strong antioxidant properties in reducing and preventing the formation of free radicals. In addition to the antioxidant concentration, Minnie root leaf extract also exhibits antibacterial and antidiabetic properties classified as flavonoids. Reported [12], [13] that *Ruellia* leaves contain flavonoids, which function as antioxidants, antibacterials, and antidiabetics based on phytochemical tests. Furthermore, [9] found that Minnie root leaf extract contains active compounds in the polyphenol group that stimulate the repair of beta cells, leading to increased insulin production.

3.3. Antioxidant Exploration of Minnie root Leaves

The search for metabolite compounds in Minnie root leaves yielded active compounds that function as antioxidants. These compounds are synthesized through the shikimate pathway, mevalonate pathway, and glucose pathway, and are classified into various classes such as alkanoic acid, shikimate acid, furan, flavonoids, monoterpenes, amino acids, ketones, glucose, hydrocarbons. Based on the active compounds identified, the bioactivity of these antioxidants and their pharmacological effects were further investigated using *the PubChem, KEGG*, and *ChEBI* websites. Table 3 explains the bioactivity of the metabolite compounds, their total abundance, pharmacological functions, and compound structures found in Minnie root leaves.

Table 3 Antioxidant Metabolite Compounds, Pharmacological Effects, Compound Structures, and Abundance (%) in Minnie root (*Ruellia tuberosa* L.) Leaves

Antioxidant Metabolites	Relative Abundance	Pharmacological Effects	Compound Structure
Furfural	2.16	The *Furfural* compounds can act as antioxidant agents by binding to free radicals, particularly hydroxyl radicals (OH), which can cause oxidative damage to biological molecules. By binding to these free radicals, furfural compounds can help protect cells and tissues from oxidative stress.	
2-Furancarboxaldehyde, 5-methyl	0.71	This compound has a reactive role in reacting with free radicals, such as hydroxyl radicals (OH), converting them into stable compounds to prevent oxidative damage to cells. Additionally, this compound can inhibit the chain reaction process initiated by free radicals.	
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	0.51	The mechanism of antioxidant action of this compound involves capturing free radicals and inhibiting reactions in the region. It works by protecting cellular structures such as cell membranes.	
D-Limonene	4.57	The *D-limonene* compound can stimulate the production of antioxidant enzymes in cells, such as superoxide dismutase (SOD) and glutathione peroxidase enzymes, which help neutralize free radicals.	
2-Furancarboxylic acid, hydrazide	2.04	The antioxidants in this compound work directly by changing superoxide anions (O2) and hydroxyl radicals (OH) by donating hydrogen atoms, thereby	

		stopping the chain reaction of lipid peroxidation.	
Ethanamine, N-ethyl-N-nitroso	0.85	This antioxidant can interact with heavy metals by forming complexes that accelerate the oxidation reaction process, thereby reducing the negative effects of these metals on cells.	
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	8.37	The mechanism of action of antioxidants in this compound involves several processes, such as capturing free radicals, inhibiting free radical chain reactions, and protecting sensitive molecules like proteins and nucleic acids.	
5-Hydroxymethylfurfural	48.96	*5-Hydroxymethylfurfural*, abbreviated as *5-HMF,* has an antioxidant mechanism that involves a combination of scavenging reactive oxygen species, inhibiting lipid peroxidation by reacting with lipid hyperoxidation and breaking the chain reaction, and protecting against apoptosis due to oxidative stress.	
5-Hydroxymethylfurfural	2.25	The *5-HMF* compound possesses antioxidant properties that can capture and inhibit free radicals, the increase in enzyme activities of SOD, CAT, and GPx	
1,4-Cyclohexanedimethanol	1.96	This compound is an organic compound with potential as an antioxidant, specifically by neutralizing superoxide anions to reduce the effects of free radicals.	
Total relative abundance 72,38 %			

Based on Table 3, 72.38% of the total abundance of active compounds detected in the extract of Minnie root leaves are potential antioxidants. The compounds that function as antioxidants include: *Furfural* (2.16%), *2-Furancarboxaldehyde, 5-methyl* (0.71%), *2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one* (0.51%), *D-Limonene* (4.57%), *2-Furancarboxylic acid hydrazide* (2.04%), *Ethanamine, N-ethyl-N-nitrosoI* (0.85%), *4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl* (8.37%), *5-Hydroxymethylfurfural* (2.25%), and *1,4-Cyclohexanedimethanol* (1.96%). The dominant antioxidant metabolite compound is 5-*Hydroxymethylfurfural* (5-HMF) at 48.86%. The results of the study [14] [15] [16], showed that 5-HMF has new antioxidant activity by cleaning free radicals. In addition, 5-HMF inhibits the proliferation of cancer cells and has higher antiproliferative activity.

The active compounds identified in Minnie root leaf extract can act as antioxidants by preventing cell damage from free radicals. These compounds also exhibit bioactivity as antimicrobial, anti-inflammatory, antidiabetic, and antiproliferative agents. According to [17], Minnie root leaf extract has antifungal, anti-inflammatory, and antidiabetic properties [18], antimicrobial [19], antipyretic, analgesic, antihypertensive, and antidotal [20]. The genus *Ruellia* has been traditionally claimed to be used for the treatment of flu, asthma, fever, bronchitis, high blood pressure, eczema, and diabetes [21].

4. Conclusion

The exploration of antioxidants in Minnie root leaves (*Ruellia tuberosa* L.) can be summarized as follows: The ethanol extract of Minnie root leaves identified 17 compounds, with 9 of them being antioxidants, accounting for 72.38% abundance. The compounds with antioxidant bioactivity include *Furfural*; *2-Furancarboxaldehyde, 5-methyl*; *2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one*; *D-Limonene*; *2-Furancarboxylic acid, hydrazide*; *Ethanamine, N-ethyl-N-*

nitroso; *4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl*; *5-Hydroxymethylfurfural*; *5-Hydroxymethylfurfural*; dan *1,4-Cyclohexanedimethanol.* The antioxidant concentration of metabolite compounds in Minnie root leaves is 99.480 ± 8.224 ppm with ascorbic acid standards and 133.500 ± 7.912 ppm with quercetin standard.

Further exploration of Minnie root leaf metabolites is recommended to investigate other bioactivities such as antidiabetic properties, which have the potential to be developed into herbal medicines.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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