Antioxidant Activity, α-Glucosidase Inhibitory, Total Phenolic Content and Total Flavonoid Content of Ethanol and Methanol Extracts of *Aerva sangunolenta* Sampled from Solo, Indonesia

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Abstract. This study examined the effects of ethanol and methanol as solvents on the antioxidant properties, total phenolic content (TPC), total flavonoid content (TFC), and α-glucosidase inhibition in Aerva sanguinolenta samples from Solo, Indonesia. Antioxidant activity was evaluated using the DPPH assay, while the plant's chemical profile was determined through TPC and TFC measurements. The potential antidiabetic properties of both ethanol and methanol extracts and their fractions were assessed via α-glucosidase inhibition bioassay. Statistical analysis was conducted using IBM SPSS Statistics 22 for Windows. Results are presented as mean ± standard deviation, analyzed using the Pearson regression model. The ethanol extract of Aerva sanguinolenta showed insignificant antioxidant activity with an IC50 of 1460 mg/ml, whereas the methanol extract had an IC50 of 628 mg/ml. For comparison, synthetic Vitamin C achieved an IC50 of 5.31 mg/ml.The study revealed significant α-glucosidase inhibitory effects in the ethanol extract (14.8 ± 3.4%), but minimal inhibition in the methanol extract. Total phenolic content across extracts, expressed as gallic acid equivalents per gram of dry extract, ranged from 37.490 to 47.779 mg. Flavonoid levels in the plant extracts varied between 15.275 and 17.287 mg.

Keywords: Aerva sanguinolenta, Fenol, Flavonoid, Antioxidant, α-glucosidase, Sambang Colok.

1 Introduction

Aerva sanguinolenta, also known as Sambang colok, is a plant with traditional medicinal uses for various conditions, including menstrual irregularities, pain relief, urinary issues, anemia,

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and uterine inflammation[1,2]. The plant's leaves and flowers have shown anti-inflammatory and wound-healing properties, useful for treating rheumatism and muscle aches. The whole plant acts as a demulcent and diuretic, with its decoction used to expel intestinal worms[3,4]. Young shoots are employed to enhance lactation in nursing mothers, while a paste made from leaves and roots is applied topically for body pain relief. The plant extract has demonstrated significant wound-healing capabilities, and its bark is used to treat hematuria. Biochemical studies of Aerva species reveal a rich composition of bioactive compounds, including alkaloids, flavonoids, coumarins, terpenoids, steroids, and other phenolics[5,6]. However, specific phytochemical research on A. sanguinolenta is limited. Previous studies have isolated terpenoids like ameroterpene and bakuchiol from A. sanguinolenta leaf extracts, showing antimicrobial properties against certain bacterial strains[7,8]. The ethanolic extract has yielded an anti-inflammatory cerebroside (ASE-1), while various betacyanins have been identified in its inflorescence. Some Aerva species, particularly A. sanguinolenta, exhibit antioxidant properties, though research on this specific species is sparse[9].This study aimed to compare the physicochemical properties (Total Phenolic Content, Total Flavonoid Content, and antioxidant activity) of methanol and ethanol extracts from Aerva sanguinolenta samples collected in Solo, Indonesia, contributing to the limited body of research on this particular species.

2 Material and Methods

Herbal Specimen

Samples of Aerva sanguinolenta were obtained from a local area in Tawangmangu, situated in Central Java, Indonesia. The plant species was verified by a botanical expert from the School of Biological Sciences at the Bandung Institute of Technology. This identification was officially documented with the certificate number 7881/IT1.C11.2/TA.00/2023.

Sample Extraction

The extraction process was adapted from Yim et al. (2013)[9,18] with slight modifications. In brief, the plant material was dehydrated in an oven at 50°C for a period of three days. After drying, the samples were pulverized into a fine powder. A 50-gram portion of this powder was then subjected to extraction using 150 ml of either deionized ethanol or methanol (Fisher Chemical). This mixture was agitated on a hotplate at 300 to 500 rpm for 20 minutes, followed by filtration. The extraction procedure was repeated three times with the remaining plant material. Subsequently, the collected extracts were concentrated using a Buchi Rotary Evaporator RII. The resulting dried extracts were kept at -20°C for future use.

Total Flavonoid Content

The total flavonoid concentration (TFC) in Aerva sanguinolenta samples was determined using a slightly modified version of the method described by Sarker and Oba (2018)[10]. Briefly, extracts were prepared at 2 mg/mL and diluted with either methanol or ultrapure water. Each test tube contained 100 µL of sample solution (2 mg/mL), 300 µL of 3% aluminum chloride (AlCl3) (Merck), 300 µL of 3% sodium acetate (NaCH3), 8.4 mL of distilled water, and 4.5 mL of methanol. After a one-hour incubation at 25°C in darkness, the flavonoid-aluminum complex formation was measured spectrophotometrically at 725 nm.

TFC was expressed as quercetin equivalent (QE) per gram of dry weight (DW) of the extract (mg QE/g DW). A quercetin standard curve was generated using seven concentrations from 50 to 300 ppm, with each experiment performed once. Methanol served as the negative control, while water was used as the blank. This method allowed for the quantification of flavonoids in the Aerva sanguinolenta samples, providing insight into their potential bioactive properties.

Total Phenolic Content

The total phenolic concentration of the extract was determined using a slightly modified version of the method developed by Ainsworth and Gillespie (2007)[11]. In summary, the sample was prepared at a concentration of 2 mg/mL in either distilled water or methanol. A 200 µL aliquot of this sample solution was then mixed with 1.5 mL of 10% (v/v) Folin-Ciocalteu reagent (Merck), followed by the addition of 1.5 mL of sodium carbonate (Na2CO3). After homogenization by vortexing, the mixture was left to incubate at room temperature for two hours. The absorbance was then measured spectrophotometrically at 765 nm[10]. The total phenolic content was quantified as gallic acid equivalent (GAE) per gram of dried extract (mg GAE/g DW). To facilitate this quantification, a standard curve was established using eight sequential concentrations of gallic acid, spanning from 25 ppm to 500 ppm. This method allowed for the accurate assessment of phenolic compounds in the extract, providing valuable information about its potential antioxidant properties.

α-Glucosidase Inhibitory test

Samples, a blank, and acarbose were prepared at concentrations of 100, 10, and 1 µg/mL. The assay began by adding 50 µL of phosphate buffer (pH 7) to a cuvette, followed by 100 µL of each sample dilution[13,15]. Then, 150 µL of rat intestinal enzyme was added, and the mixture was incubated for 30 minutes at 37°C. Post-incubation, 300 µL of p-nitrophenyl-α-D-glucopyranoside (pNPG) substrate was introduced, followed by another 20-minute incubation at 37°C. The reaction was stopped by adding 600 µL of sodium carbonate (Na2CO3).The reaction products were measured spectrophotometrically at 405 nm, as per Lankatillake et al. (2021)[17]. Acarbose served as a positive control in an identical reaction system. The experiment was performed in triplicate, with percentage inhibition calculated to determine the IC50 value. A blank, serving as a negative control, included all components except the alpha-glucosidase enzyme, which was added last. Table 1 outlines the details of the reaction system. This method allowed for the assessment of α-glucosidase inhibition, providing insights into the potential antidiabetic properties of the samples

Table. 1 α-Glucosidase Inhibition reaction system

Reactor	Volume (µl)	
	Sample	**Blank**
Posphate Buffer	50	50
Sample/Acarbose/DMSO 5%	100	100
Enzyme	150	-
10 minutes incubation at 37°C		
PNP Substrate	300	300
20 minutes incubation at 37°C		
Na_2CO_3 termination	600	600
Enzyme	-	150

Total	**1200**	**1200**

Antioxidant activity: DPPH free radical scavenging assay

The antioxidant activity of Aerva sanguinolenta extract was assessed by its ability to scavenge the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, using a modified version of Shahidi and Zong's (2015)[15] method. A stock solution was prepared by mixing 500 mg of sample with 5 mL of methanol, then vortexing and sonicating. From this, 16 µL was combined with 1584 µL of methanol and 400 µL of DPPH.Test samples were prepared in triplicate at five concentrations from 62.5 to 1000 ppm, with the highest being 1000 ppm (80 µL extract in 7920 µL methanol). After 30 minutes of incubation at room temperature, absorbance was measured at 517 nm using a FLUOstar Omega microplate reader.The IC50 value, representing the extract concentration needed to inhibit 50% of DPPH radicals, was determined by testing various extract concentrations. Each assay was performed in triplicate, with aquades as controls and a blank of methanol with DPPH.The radical scavenging activity percentage was calculated using a formula where AB is the sample absorbance and AB is the blank absorbance. This method allowed for quantification of the extract's antioxidant potential through its ability to neutralize free radicals.

Radical trapping activity (%) = [(AB - AS) / AB] x 100[12,14].

Statistical Analyis

All statistical analyses were performed using GraphPad Prism version 10. Data are presented as means, and one-way ANOVA with Tukey's post hoc test was utilized to assess the significance of the parameters.

Corelation analysis

Pearson Correlation analysis was performed to analyze the correlation between TPC, TFC and α-glucosidase inhibitory with IC50. Aprobability (p) Value ≤0.05 indicated statistical signficance.

3. Results and Discussion

Total Phenolic content

The total phenolic and flavonoid contents, along with the in vitro antioxidant activities (assessed via DPPH and ABTS assays), were evaluated for both ethanol and methanol extracts of *Aerva sanguinolenta*. The results concerning the total phenolic content in the examined plant extracts are displayed in Table 1. The phenolic levels across various extracts, expressed as gallic acid equivalents (GAEs) per gram of dried material, ranged from 37.490 to 47.779 milligrams GAE/gram. Notably, the methanol extracts demonstrated the highest phenolic content at a concentration of 2 milligrams per milliliter.

Flavonoid content

The summarized information about the quantities of flavonoids identified in the analyzed extract is provided in Table 1 and illustrated in Figure 1. The flavonoid concentrations in the methanolic and ethanolic extracts of *Aerva sanguinolenta* were determined through a spectrophotometric technique employing aluminum nitrate nonahydrate. The flavonoid levels in the plant extracts

spanned from 15.275 to 17.287 milligrams quercetin equivalent per gram, with the highest concentration observed in the methanol extracts at a concentration of 2 milligrams per milliliter. Flavonoids, which are frequently present in plants, play a vital role in the diets of both humans and animals. Nonetheless, the diverse biological activities of plant secondary metabolites indicate that their consistent intake could have substantial health effects, ranging from advantageous to adverse outcomes.

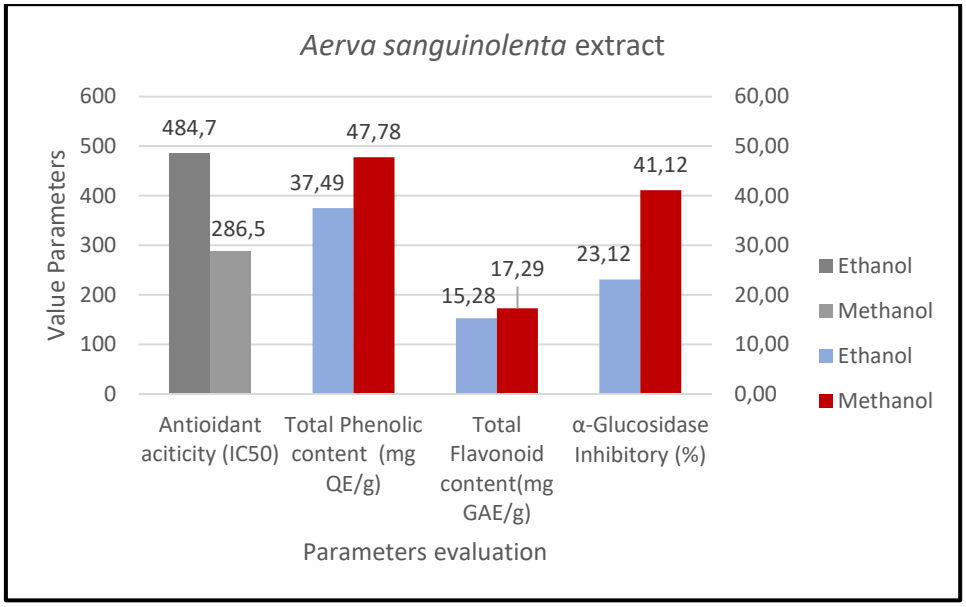
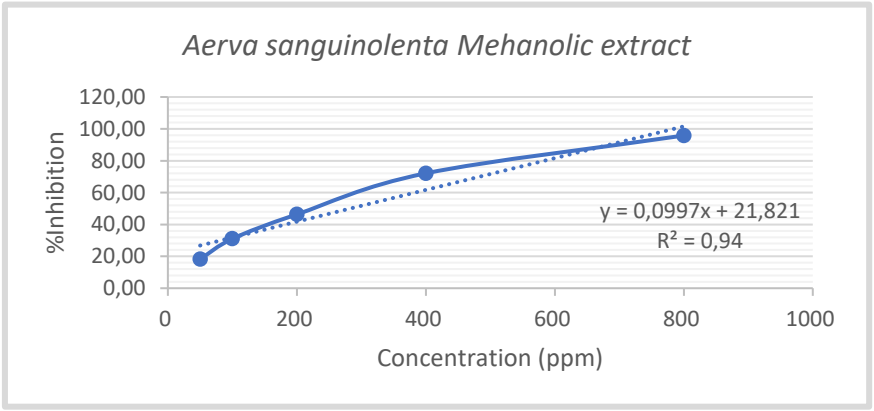


Fig 1. Antioxidant acitivity, Total phenolic content, Total flavonoid content and α-glucosidase inhibitory of Ethanolic and methanolic extract of *Aerva sanguinolenta*



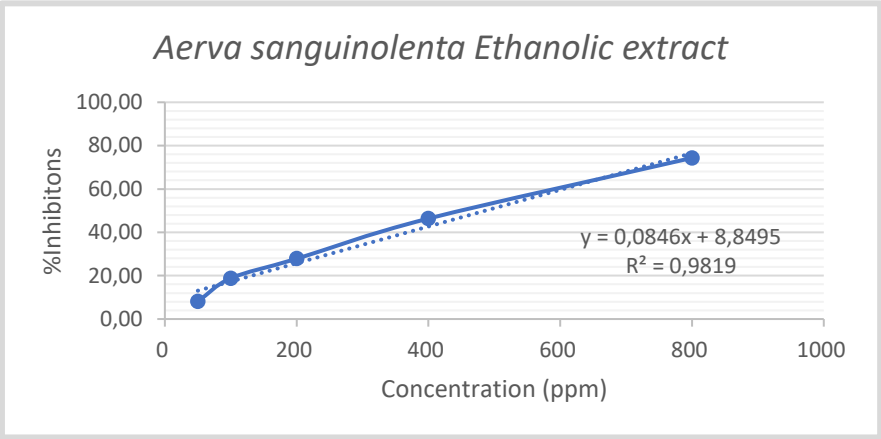


Fig 2. The data represent the percentage inhibition of antioxidant activity of Methanolic and Ethanolic Extract of *Aerva sanguinolenta*

Table 2. Extracts of *Aerva sanguinolenta* species were compared for their total phenolic, total flavonoid, α-glucosidase inhibitory, and antioxidant content**.**

Aerva sanguinolenta	**Antioidant aciticity (IC_{50})**	**Total Phenolic content** (mg QE/g)	**Total Flavonoid content**(mg GAE/g)	**α-Glucosidase Inhibitory (%)**	**Sig (<0.005)**	**Correlations**
Ethanol	484,70	37.490	15.275	23.12		
Methanol	286,50	47.779	17.287	41.12	0.048*	0.976**

p Value (0.05) One Way ANOVA Post Hoc Tukey
****p Value (0.05)Pearson correlations***

To evaluate the relationship between these biocompounds and the antioxidant activity of the plants, we performed a Pearson correlation analysis that included total phenolic content (TPC), total flavonoid content (TFC), and the antioxidant activity of the tested plants (Table 2). A very strong positive correlation was found between TPC and antioxidant activity for each of the test plants. This positive association indicates that the enhanced antioxidant activity in the methanolic extract may be due to its higher TPC content. Our findings are consistent with previous studies that showed a significant correlation between TPC and antioxidant activity.

DPPH free radical scavenging assay

Antioxidants' capability to donate hydrogen atoms or participate in radical scavenging is thought to be the mechanism behind their ability to neutralize DPPH radicals. When a substance capable of donating a hydrogen atom interacts with a DPPH solution, the violet color dissipates, resulting in reduced diphenylpicrylhydrazine (Baliyan et al., 2022)[16]. The DPPH assay was used

to assess the extract's free radical scavenging capacity, and the results are presented in Table 1. The methanol extract of *Aerva sanguinolenta* exhibited an IC50 value of 286.50 milligrams per milliliter, while the ethanol extract demonstrated inactivity with an IC50 value of 484.70 milligrams per milliliter. In comparison, synthesized vitamin C had an IC50 value of 5.31 milligrams per milliliter. The overall IC50 values indicate a lack of antioxidant activity. However, the methanol extract of *Aerva sanguinolenta* showed superior antioxidant activity compared to the ethanol extract. Statistical analysis revealed significant differences among the examined parameters (0.048). Given the limited research on antioxidant activity, total phenolic content, total flavonoid content, and α-glucosidase activity, these findings can serve as a basis for further comprehensive studies on *Aerva sanguinolenta.*

4. Conclusion

The methanol and water extracts from *Aerva sanguinolenta* have been assessed for their potential as protective agents against oxidative stress caused by DPPH and hydroxyl radicals. However, their antioxidant activity was found to be relatively low compared to synthetic Vitamin C, leading to the conclusion that they exhibit somewhat limited efficacy. Nonetheless, the total phenolic content (TPC), total flavonoid content (TFC), and glucose inhibitory properties show promising potential.

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