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The Phytochemical and Biological Activities of Two *Phyllanthus* Species: Insights into Metabolit, Antioxidant and Antibacterial Activity

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Abstract. *Phylanthus* species, including *P. niruri* and *P. urinaria* have pharmacological potential due to their rich phytochemical composition. People usually used this plant for medicinal treatments. This study aimed to compare the phytochemical and antioxidant and antibacterial activities of two *Phyllanthus* species. The whole of plants was extracted using maceration method with ethanol as solvent. Phytochemical content analyzed using spectrophotometer. The reagent used for each compounds that were Folin-ciocalteu for phenolics, $AlCl_3$ for flavonoid, dimethyl sulfoxide for chlorophyll and carotenoid. Bioactivity analysis using 1,1-diphenyl-2-picrylhydrazyl method for antioxidant activity and Kirby-Bauer method for antibacterial activity. The highest flavonoid content (12.22 mg QE/gram extract) and total chlorophyll (43.2 µg/ml extract) in *P. niruri* while phenolic content (80.8 mg GAE/gram extract) in *P. urinaria*. The carotene of both *Phyllanthus* were similar (11.9 µg/ml extract. The IC_{50} values of *P. urinaria* (6.16 ± 0.42 µg/ml) and *P. niruri (*17.72 ± 0.80 µg/ml), which indicated very strong antioxidant activity. *P. urinaria* leaf extract had stronger inhibition against *Escherichia coli* than *Staphylococcus aureus* (>20 mm) and *P. niruri* leaf extract could inhibit *E. coli* and *S. aureus* bacteria at all concentrations (11-20 mm). This study found that phenolic compounds strongly influenced the antioxidant and antibacterial abilities of *Phyllanthus*, while chlorophyll and carotenoids had only a slight influence. These findings open up opportunities to utilize *P. niruri* and *P. urinaria* as antioxidant and antibacterial agents.

Keywords: antibacterial; antioxidant; bioactive compounds; *Phyllanthus niruri*; *Phyllanthus urinaria*

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INTRODUCTION

Phyllanthus species, plants known for their pharmacological properties, make up the cornerstone of traditional medicine systems worldwide. There are several *Phylanthus* species, including *P. niruri* and *P. urinaria*, and the potential pharmacological activity of these plants is mainly due to their rich phytochemical composition, especially flavonoids and phenolics. The abundance of phenolic compounds and flavonoids in *P. niruri* is quite high (Babu *et al*., 2021), so it has potential as an anticancer, antibacterial, antiallergic, antimicrobial, anti-inflammatory (Hosseinzade *et al*., 2019); Shukla *et al*., 2019; Ferrante *et al*., 2020), antioxidant and tyrosinase inhibitor, as high antioxidant activity often indicates high tyrosinase inhibitory activity (Babu *et al*., 2021).

The presence of flavonoids and phenolic compounds isolated from *P. urinaria* plants indicates pharmacological activities such as anti-inflammatory, antioxidant, antibacterial, anti-cancer, anti-diabetic, antiviral, hepatoprotective, antimicrobial, thrombolytic, cardioprotective, antiallodynic, antioedematogenic and anti-Helicobacter pylori. (Mozaffarian & Wu, 2018); (Wu *et al*., 2013); (Hu *et al*., 2014); (Liu *et al*., 2018). According to research conducted by Choudhury *et al*. (2017), chlorophyll in *P. niruri* leaves showed high antioxidant activity. This allows it to protect the plant from damage caused by oxidative stress. Dewanjee *et al*. (2021) reported that carotenoids from *P. niruri* have the

potential for cancer treatment. In a study conducted by Santos *et al*. (2018), it was found that *P. urinaria* contains chlorophyll, which has antibacterial properties that fight various pathogenic bacteria.

In addition, the ability of antioxidant activity in *P. urinaria* is associated with carotenoid pigments that contribute to health benefits (Liu *et al*., 2024). For the aqueous extract, the IC_{50} was 33.5 ± 0.04 µg/mL for *P. urinaria* and 73.0 ± 0.03 µg/mL for *P. niruri*. For the methanol extract, the IC_{50} was 15.8 ± 0.01 µg/mL for *P. urinaria* and 29.3 ± 0.01 µg/mL for *P. niruri* (Zain & Omar, 2018). Using the disc diffusion method, the *P. niruri* plant showed antibacterial properties. The aqueous extract of *P. niruri* showed properties against gram-positive bacteria *S. aureus* with an inhibition zone of 20 mm and *S. agalactiae* with an inhibition zone of 12 mm. While there was no inhibition zone against gram-negative bacteria *E. coli* and *K. pneumoniae*.

The inhibition zone in the of aqueous extract of *P. urinaria* was 20 mm and 21 mm against *Staphylococcus aureus* and *Streptococcus agalactiae*, the positive control zone was 19 mm and 27 mm (Musuasua *et al*., 2022). On the leaves, there was a 15 mm zone of *Pseudomonas* inhibition. In the positive control, there was a clear zone of 25 mm, and in the negative control, there was no zone of inhibition. For *S. aureus*, the stem, root, and leaf obtained inhibition zones of 10 mm, 7 mm, and 7 mm, while the negative control had no inhibition zone; Proteus gave an inhibition zone of 13 mm on the leaf, and the positive control of 13 mm (James *et al*., 2018). *P. niruri* also has anti-inflammatory potential. Research from Susanti *et al*. (2022), found that from the results of meta-analysis *P. niruri* can have potential as an anti-inflammatory by reducing inflammatory cytokines TNF-α and IL-6, which contribute to the prevention of inflammation.

Although *P. niruri* and *P. urinaria* species are widely used in pharmacotherapy, comprehensive studies on the phytochemical profiles and related biological activities of many other species are scarce. This study focuses on two *Phyllanthus* species with the aim of dissecting the metabolite landscape and evaluating their antioxidant and antibacterial properties. This study aims to compare the phytochemical antioxidant and antibacterial activities of two *Phyllanthus* species. The findings of this study could open up opportunities to utilize *P. niruri* and *P. urinaria* as antioxidant and antibacterial agents.

METHODS

The research was a quantitative laboratory study in the laboratory of Biochemistry and Molecular Biology, and Microbiology, Faculty of Biology, Satya Wacana Christian University Salatiga, Indonesia. The samples were two varieties of plants from the genus *Phyllanthus*, namely P. niruri and P. urinaria. The samples were taken from a plantation located on Jl. Salatiga - Bringin, Kauman Kidul, Sidorejo Sub-district in August. The sample plants were characterized by the Integrated Laboratory of FMIPA Faculty of Mathematics and Natural Sciences, Semarang State University, Indonesia with letter number 183/UN.37/SHP/Lab. Plant Taxonomy/VIII/2023. The leaves used as samples were selected fresh green leaves with perfect shape and not attacked by disease. Leaf extract was obtained by maceration method using ethanol solvent. Test parameters include qualitative and quantitative tests, total phenolics and flavonoids, chlorophyll and carotene, and antioxidant and antibacterial ability tests.

Preparation of samples

The leaves were cleaned under running water and dried indoors for 72 hours. The dried samples were blended using a blender (Philip HR1538). The maceration process used 96% ethanol solvent (1:5) and kept at room temperature. Every 24 hours the filtrate was filtered, then ethanol was added again. The total maceration time was 3 x 24 hours. The combined filtrate was concentrated using a vacuum rotational evaporator (Rotavapor RE 100 Pro) until a thick green extract was obtained (Kristiani *et al*., 2024).

Qualitative analysis of bioactive compounds

Phenolic Analysis

A total of 0.1 g of extract, dissolved in 10 ml of ethanol, pipette 2 ml of the resulting solution was put into a test tube, and add 3 drops of 1% $FeCl_3$ solution. A positive test for the presence of phenolics is indicated by a change in color to green or blackish.

Flavonoid Analysis

Dissolve 0.1 gram of the extract in 10 ml of ethanol, pipette 2 ml, and place in a test tube. Add 0.1 gram of magnesium powder, 1 ml of concentrated HCl, and 3 ml of amyl alcohol. Shake vigorously, let it separate, and observe the color change to yellow is a sign of the presence of flavonoids.

Quantitative analysis of bioactive compounds

Total Phenolic Content

The Folin-ciocalteu method was used to perform total phenolic analysis (John *et al*., 2014). Gallic acid concentrations were 20, 40, 60, 80 and 100 ppm. In 200 µl of extract, 1000 µl of folin-cioceltaeu reagent and 800 µl of 7% Na_2CO_3 solution were added. Then shaken and incubated for 30 minutes. A UV-Vis spectrophotometer (Shimadzu Mini 1240) was used to measure the absorbance of the mixture at a wavelength of 760 nm and the total phenolic content, expressed as mg Gallic Acid Equivalents (GAE)/g extract, was calculated based on the gallic acid linear regression equation. GAE = c (v/m), where v is the volume of extract (l), c is the total phenolic concentration on the gallic acid standard curve (mg/l), and m is the weight of extract (g).

Total Flavonoid Content

Total flavonoid content was measured using the $AlCl_3$ method (John *et al*., 2014). Quercetin was used as a standard for flavonoid compounds with a concentration variation of 20-100 ppm. A concentration series of 20, 40, 60, 80, and 100 ppm was made, in 200 µl of sample, 600 µl of 95% ethanol, 40 µl of 10% $AlCl_3$, 40 µl of 1 M CH_3COOK, and 560 µl of distilled water were added twice. Then, it was incubated for 30 minutes at room temperature. A Shimadzu Mini 1240 UV-vis spectrophotometer was used to measure the absorbance of the mixture at a wavelength of 517 nm. Total flavonoid content was expressed as mg quercetin equivalent (QE)/g extract, calculated based on the linear regression equation of gallic acid using the equation QE = c (V/m).

Chlorophyll and Carotenoid Content

Chlorophyll a, b, and carotenoid tests (Sumanta *et al*., 2014) using a spectrophotometer. The leaves of each *Phyllanthus* were cleaned using tissue. The leaves were cut into small pieces weighing 0.5 g, put into a film bottle, added 0.5 ml dimethyl sulfoxide (DMSO), and incubated in a dark room for 72 hours. The extract was then filtered and the absorbance was measured at 480 nm, 649 nm, and 665 nm wavelengths. To determine the concentration (µg/ml) of chlorophyll a, chlorophyll b, and carotenoids with DMSO solvent at different wavelengths using the equation:

Chlorophyll a (µg/ml) = $(12.47A_{665} – 3.62A_{649})$

Chlorophyll b (µg/ml) = $(25.06A_{649} – 6.5A_{665})$

Carotenoid = $(1000A_{480}$ – 1.29klorofil a – 53.78 klorofil b)/220

Determination of antioxidant activities

Antioxidant activity was measured using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method (Kristiani & Kasmiyati, 2021). Sample concentrations ranged between 5 and 25 ppm. 666 µl of sample extract was added with 666 µl of DPPH twice, shaken then incubated for 30 minutes in a dark room. The absorbance was measured by UV-Vis spectrophotometer (Shimadzu UV mini1240) with a wavelength of 517 nm. Antioxidant ability = (control absorbance - sample absorbance/control absorbance) x100%. For each test concentration, the absorbance value was represented in a linear regression equation, then the IC_{50} value was calculated.

Determination of antibacterial activity

The Kirby-Bauer method was used to identify antibacterial activity (Nurhayati *et al*., 2020). In this study, *Staphylococcus aureus* and *Escherichia coli* bacteria were used with extract concentrations of 75, 150, 500, 750, and 1000 ppm. Fill the wells with 15 µl at each concentration with the extract, pipette 15 µl distilled water used as negative control and tetracyline as a positive control. Incubated at 37 °C for 24 hours and then observed to determine the diameter of the clear zone using a ruler on the diameter of the paper disk width at each concentration.

Data analysis

Qualitative data were analyzed descriptively, while qualitative data were analyzed statistically using the SAS program. The tests carried out include analysis of variance (ANOVA), Pearson correlation analysis using Excel program, and Tukey test.

RESULTS AND DISCUSSION

P. niruri and *P. urinaria* are two weed plants that live wild but are beneficial to human health. Soesanto (2021) said that *Phyllanthus* plants live well in sandy loam soil and black soil, and the plant also lives well at pH 6.5 - 7.5. *Phyllanthus* plants grow well in soils that contain a lot of organic matter such as manure, macro and micronutrients, and balanced soil moisture content (Raihanah, 2014). The sampling location in this study is also an open land area, the soil is black, and slightly wet, so the a possibility of plants growing well in the area.

A B

Figure 1. Habitats of *P. Niruri*, the stem is green (A) and P. *urinaria* the stem is red (B).

The choice of maceration method in extracting samples is because this method is simple and does not use heating, thus reducing the potential damage to active substances in the sample (Sa'adah & Nurhasnawati, 2017). However, the maceration method has the disadvantage of requiring a long time and a large amount of solvent (Susanty & Bachmid, 2016), as in this study, it was carried out for a total of 72 hours. The maceration yield can significantly vary depending on the solvent used. In this study, the extract yield of *P. urinaria* (10.33%) was significantly higher than that of *P. niruri* (9.01%). In a study comparing various techniques of extraction, the maceration method produced the highest extract, that was for *P. niruri* yielding 14.3% using 50% ethanol as the solvent (Kamarudin *et al*., 2016), more than the yield in this study.

These two plants have almost the same shape: their petioles have small round green fruits and their stems are branched with a yellowish-white taproot. They also grow well in slightly wet, black soil. A very notable difference between these two plants is that *P. niruri* has thin green leaves, green stems, and is taller than *P. urinaria*. In contrast, *P. urinaria* has thick reddish-brown leaves, is leafy, flares out laterally, and has red stems.

Bioactive compounds analysis

At the initial stage, phytochemical screening of extracts was carried out to determine the qualitative content of phytochemicals in this study, especially phenolics and flavonoids (Table 1). Phytochemical screening is used to identify biochemical compounds in plant extracts, this technique is cheap, fast, and simple. This technique aims to identify secondary metabolite compounds present in plants (Setyowati *et al*., 2014).

Table 1. Flavonoids and phenolics compounds assay of ethanol extract of *P.niruri* and *P. urinaria*

Ethanol extract from plant leaves	Phytochemical assay	
	Flavonoids	**Phenolics**
P. niruri	+	+
P. urinaria	+	+

+: the sample positive contained of phytochemical assay

Table 1 shows that both types of extracts contain phenolic compounds and flavonoids. The reaction between $FeCl_3$ and phenolic compounds forms a solution into intense green, red, purple, blue, or black (Kurniawan & Wardany, 2021). In the flavonoid test, to reduce the benzopyrone contained in the flavonoid carbonyl group, Mg powder, and HCl are added to form a red-orange flavylium salt (Illing *et al*., 2017).

Based on the results of the qualitative phytochemical screening, quantitative determination (Table 2) of phenolic and flavonoid levels in the extract was continued to determine the levels of both types of compounds in the extract. In addition to these two compounds, the levels of chlorophyll and carotenoids in the extract were also determined. The levels of phenolic compounds in *P. urinaria* (80.8 ± 7.5 mg GAE/gram extract) were significantly higher than *P. niruri* (54.8 ± 6.5 mg GAE/gram extract). On the other hand, the levels of other test compounds, namely flavonoids, chlorophyll-a, chlorophyll-b, and total chlorophyll in *P. niruri* were significantly higher than *P. urinaria*, namely 12.2 ± 1. 9, 30.0 ± 2.6, 13.1 ± 3.1, and 43.2 ± 4.8 (in units respectively) in *P. niruri* and 8.8 ± 1.4, 24.1 ± 3.4, 10.2 ± 2.9, and 34.3 ± 5.6 (in units respectively) in *P. urinaria*. In contrast to other compounds, the carotenoid content was not different at both of extracts.

Table 2. Levels of total phenolic compounds and flavonoids, chlorophyll, and carotene pigments in ethanol extracts of *P. niruri* and *P. urinaria* leaves.

Compounds	Unit	Ethanolic extract of	
		P. niruri	***P. urinaria***
Phenolic	mg GAE/gram ekstrak	54.8 ± 6.5[b]	80.8 ± 7.5[a]
Flavonoid	mg QE/gram ekstrak	12.2 ± 1.9[a]	8.8 ± 1.4[b]
Chlorophyll a	(µg/ml)	30.0 ± 2.6[a]	24.1 ± 3.4[b]
Chlorophyll b	(µg/ml)	13.1 ± 3.1[a]	10.2 ± 2.9[b]
Total Chlorophyll	(µg/ml)	43.2 ± 4.8[a]	34.3 ± 5.6[b]
Carotenoids	(µg/ml)	11.9 ± 2.1[a]	11.9 ± 0 .9[a]

Notes: Different letters a and b in the same row indicate significant differences (P<0.05 and P<0.01). GAE: gallic acid equivalent; QE: quercetin equivalent

In both ethanol extract samples, phenolic content was higher than flavonoid content. These results are in line with the research (Zain & Omar, 2018), namely the methanol extract of *P. urinaria* contains more phenolics (308.71 ± 0.04 mg GAE/g extract) than flavonoids (35.86 ± 0.04 mg QE/g extract) while in *P. niruri*, phenolics are 159.13 ± 0.02 mg GAE/g extract and flavonoids 22.08 ± 0.04 mg QE/g extract. Different results were obtained in the research of Kristiani & Kasmiyati (2021), in their study found that the levels of *P. niruri* from different growing locations with this study had lower phenolic content (39.6 ± 3.23 mg GAE/gram extract) compared to flavonoid content (105.0 ± 6.25 mg QE/gram extract). This shows that the high and low content of phenolics and flavonoids can be related to the living environment of both *Phyllanthus*.

Researchers also measured other compounds, namely chlorophyll and carotenoids. Both compounds are reported to in addition to photosynthesis also function as antioxidation, which serves as an antioxidant (Dewi *et al*., 2023). Photosynthetic pigments such as chlorophyll and carotenoids in *P. niruri* and *P. urinaria* vary depending on environmental factors of the analyzed plant parts (Woerdenbag *et al*., 2014).

Antioxidant activity

The ability of a chemical to prevent oxidation and neutralize free radicals is known as antioxidant activity. This ability shields biological systems from oxidative stress. DPPH methods, which are used in this study are one of the methods for antioxidant activity. The IC_{50} value is the concentration of a compound required to inhibit 50% of the oxidizing activity of a material (Katrin & Bendra, 2015). In *P. niruri* and *P. urinaria*, the pigments that play an important role in antioxidant ability are chlorophyll and carotenoids which allow them to capture free radicals and reduce oxidative stress. Thus, there is a positive correlation between pigment concentration and antioxidant activity (Srianta *et al*., 2017). Table 3 presents the IC_{50} values of both types of extracts measured using the DPPH method.

Table 3. IC_{50} values and antioxidant activity of *P. niruri* and *P. urinaria* leaves

Samples	IC_{50} value* (µg/ml)	The antioxidant power
P. niruri	17.72 ± 0.80	Very strong
P. urinaria	6.16 ± 0.42	Very strong
Ascorbic acid[**)]	21.04 ± 0.10	Very strong

[*)]The IC_{50} value is very high if it is less than 50 ppm, the value is high if it is 50 – 100 ppm, medium if the value is between 100 – 150 ppm, and low if it is between 151 – 200 ppm (Ginting *et al*., 2017), [**)](Kristiani & Kasmiyati, 2021)

Plants bioactivity is greatly impacted by the phytochemicals they contain which are generally known as secondary metabolites, and have a variety of medicinal uses. These substances are becoming more widely acknowledged for their potential to cure a variety of illnesses, especially as synthetic medication substitutes. Numerous biochemical pathways, such as the antioxidant and anti-inflammatory ones, are used by phytochemicals to produce their effects. These pathways are essential for the management of chronic illnesses (Umezinwa *et al*., 2021). *Phyllanthus* plants contain various types of secondary metabolites, including phenolics and flavonoids (Kaur *et al*., 2017), which were measured in this study. As shown in Table 3, based on the antioxidant strength category (Ginting *et al*., 2017), both extract was have antioxidant strength in the very strong category, comparable to the antioxidant strength of standard ascorbic acid (Kristiani & Kasmiyati., 2021).

This is according to research Giribabu *et al*.

(2014), the high antioxidant activity in *P. niruri* is related to the active flavonoid and polyphenol compounds contained in the plant. Da'i *et al*. (2016) found that ethanol extract of *P. niruri* from the local market in India showed antioxidant potential in both in vitro and in vivo tests. In the study, the IC_{50} value in the in vitro test of the extract was 14.21 ± 0.73 µg / ml. The results are also not much different from the methanol extract of *P. niruri* which in the 2,2-azinobis-3-Ethylbenzothiazoline-6-Sulfonic Acid test showed higher antioxidant activity compared to the aqueous extract, with IC50 values ranging from 11.2-26.0 µg/mL (Zain & Omar., 2018).

In a study of *P. niruri* by Kristiani & Kasmiyati. (2021), the antioxidant activity of the extract was inactive with an IC_{50} value of 508.5 ± 16.9µg/ml. *P. urinaria* has pharmacological activities such as antioxidant, antibacterial, anticancer, cardioprotective, immune system enhancement, and anti-inflammatory due to the presence of flavonoid and phenolic content (Tungmunnithum *et al*., 2018), (Locatelli *et al*., 2018), (Rupasinghe *et al*., 2015). Phenolic and flavonoid compounds are the main compounds that act as antioxidants (Tungmunnithum *et al*., 2018). Ascorbic acid, more familiarly known as vitamin C, is used as a natural antioxidant compound. Vitamin C is a group of non-enzymatic antioxidants also called exogenous antioxidants whose mechanism can inhibit the occurrence of free radical chain reactions (Dontha, 2016). By ethanol maceration method, *P. niruri* and *P. urinaria* showed antioxidant IC_{50} less than 50 (µg/ml) categorised as high.

Antibacterial activity

The potential for antibacterial activity is determined by observing the growth inhibition zone after the bacteria have been grown for a certain time. In a study conducted by Suvorov *et al*. (2021), found that *P. urinaria* contains chlorophyll an important role in antimicrobial activity through various mechanisms, such as generation of reactive oxygen species (ROS) and termination of bacterial development. Carotenoids in both *Phyllanthus* species also contribute to antibacterial activity. This is related to lycopene and β-carotene which have bacteriostatic properties against several pathogens (Kot *et al*., 2016). In this study, bacteria were used *Staphylococcus aureus* from the gram-positive group and *Escherichia coli* from the gram-negative group and the bacterial growth period for 24 hours (Table 4 and 5). The inhibition zones formed by *E. coli* and *S. aureus* varied between concentrations, which means that the ability of the extract to inhibit the growth of the test bacteria varied.

In inhibiting the growth of *E. coli* bacteria (Table 4), the ability of *P. urinaria* extract (inhibition zone in the range of 23 – 29 mm) is significantly stronger than the ability of *P. niruri* that were inhibition zone in the range of 17-19 mm, inhibition zone in the range of 23 -29, except at a concentration of 150 mg/L (inhibition zone was 25.5 mm). However, in general, the strength of *P urinaria* is below the standard strength of tetracycline (inhibition zone in the range of 34.8 ± 1.3 mm). Based o Surjowardojo *et al*. (2015), the antibacterial power of *P. urinaria* is very strong (inhibition zone was ≥ 20 mm) while *P. niruri* classified as strong (inhibition zone in the range of 11 – 20 mm).

The antibacterial ability against *S. aureus* bacteria (Table 5) of both types of *Phyllantus* extract at all test concentrations (11–20 mm) was lower than the standard antibiotic tetracycline. Both types of extracts have antibacterial ability classified as strong, with an inhibition zone in the range of 14 - 20 mm, except for *P. urinaria* extracts at concentrations of 500 and 1000 which are classified as very strong (Surjowardojo *et al*., 2015). Study Farzaneh *et al*. (2018) show that *P. urinaria* has a high antibacterial ability against *E. coli*.

Table 4. Antibacterial activity of *Phyllantus* extract against *Escherichia coli*

Extract concentration (mg/L)	Inhibition zone of extract (mm)	
	P. niruri	***P. urinaria***
Tetracycline*	34.8 ± 1.3^{a}	
75	17.3 ± 2.7^{e}	28.2 ± 1.4^{bcd}
150	25.5 ± 5.0^{cd}	23.3 ± 1.7^{cde}
500	19.0 ± 4.3^{e}	23.0 ± 0.4^{de}
750	18.0 ± 3.7^{e}	26.5 ± 1.6^{cd}
1000	18.0 ± 2.5^{e}	29.3 ± 3.4^{bc}

*****: tetracycline as a standard antibiotic, at a concentration of 150 (mg/L). The different superscripts font behind the inhibition zone shows the significant differences between the values ($P<0.05$ dan $P<0.01$)

Table 5. Antibacterial activity of *Phyllantus* extract against *Staphylococcus aureus*

Extract concentration (mg/L)	Inhibition zone of extract (mm)	
	P. niruri	***P. urinaria***
Tetracycline*	34.8 ± 1.3^{a}	
75	14.3 ± 0.74^{de}	19.3 ± 1.43^{bc}
150	18.8 ± 2.30^{bcd}	13.2 ± 1.63^{e}
500	16.5 ± 3.74^{cde}	23.2 ± 3.63^{b}
750	19.3 ± 1.47^{bc}	19.5 ± 0.61^{bc}
1000	20.2 ± 3.19^{bc}	20.3 ± 1.74^{bc}

*****: tetracycline as a standard antibiotic, at a concentration of 150 (mg/L). The different superscripts font behind the inhibition zone shows the significant differences between the values ($P<0.05$ dan $P<0.01$)

According to Razali & Fauzi (2023), *P. niruri* extract showed significant antibacterial activity against *E. coli* and *S. aureus* bacteria with inhibition zones measured at 10 mm and 10 mm, respectively. Because the active compounds contained in *Phyllanthus* leaves such as flavonoids and phenolics make these two *Phyllanthus* have antibacterial properties. *P. urinaria* has the ability to stop the activity of *S. aureus* bacteria. Bioactive compounds, especially phenolics contained in *P. urinaria* are responsible for this ability. According to Octaviani *et al*. (2019), the inhibition zone produced by the antibacterial activity of the test material because it contains secondary metabolites such as flavonoids, phenolics, and terpenoids. Although *P. niruri* only strongly inhibits *E. coli* and *S. aureus* bacteria, based on the research of Ifandi *et al*. (2016), interview findings from the Tompu community in Kaili District showed that *P. niruri*, with the local name Panuntu, has long been used for the treatment of diarrhea, cough, fever, and gallbladder stones. Not only that, but *P. niruri* is also used to treat diabetes mellitus in Beutong District, Nagan Raya Regency based on interviews with local people (Putrimarlin *et al*., 2022).

From this research results, *P. niruri* and *P. urinaria* can inhibit *E. coli* and *S. aureus* bacteria strongly and very strongly using only the maceration method. The maceration method has been effectively used to inhibit both bacteria, although in inhibiting *S. aureus* bacteria, both *Phyllanthus* have no stronger inhibitory power, perhaps an extract boiling method is needed to inhibit. Study of Ihuma *et al*. (2022), boiling is the best way to stop *Staphylococcus aureus* bacteria.

The correlation of compound content and bioactivity

Among the observed parameters, a correlation test was performed using the Pearson correlation test (Table 6-9).

The correlation between phenolic content and antioxidant activity was a strong positive correlation, with antibacterial activity against *E. coli* being moderate positive, but low positive against *S. aureus*. This is in accordance with the results of this study that an increase in phenolic content is followed by an increase in the ability to inhibit the oxidation process. This is in line with research conducted Khadijah *et al*. (2017), which states phenolic compounds correlate with antioxidant compounds. According to Isnindar & Luliana. (2020), phenolic compounds, and antioxidant activity are positively correlated in *Phyllanthus.*

The correlation of phenolic compound content to antibacterial activity against *S. aureus* is weaker as indicated by the ability of both types of extracts is lower than tetracycline, while against *E. coli* in the moderate category as indicated by the ability of *P. urinaria* extract is equivalent to tetracycline. Suheri *et al*. (2015) also stated that *Phyllanthus* extract has weaker antibacterial properties against *S. aureus* compared to antibiotics such as tetracycline and ampicillin whose inhibition zones are 25.58 mm and 36.64 mm, respectively. According to Seko *et al*. (2021), not all phenolic compounds show consistently strong antibacterial activity in inhibiting *S. aureus*. These properties may vary depending on the specific compound and test conditions. *P. urinaria* had higher phenolic content (80.8 mg/g), while *P. niruri* had higher flavonoid content (12.22 mg/g). The IC_{50} values of *P. urinaria* (6.16 ± 0.42 μg/ml) and *P. niruri* (17.72 ± 0.80 μg/ml), which indicated the same very strong antioxidant activity.

Table 6. Correlation between phenolics, antioxidants, and antibacterial

	TPC	*FRS*	*Inhibition of EC*	*Inhibition of SA*
TPC	1			
FRS	0.650	1		
DHEC	0.582	0.323	1	
DHSA	0.176	0.316	0.266	1

Note: r of N = 60, TPC = total phenolics, FRS= antioxidant, DHEC = Inhibition *E. coli*, DHSA = inhibition of *S.aureus*. Correlation is significant at the 0.05 and 0.01 levels (2-tailed)

Table 7. Correlation between flavonoids, antioxidants, and antibacterial

	TFC	*FRS*	*Inhibition of EC*	*Inhibition of SA*
TFC	1			
FRS	-0.451	1		
DHEC	-0.480	0.323	1	
DHSA	-0.112	0.316	0.266	1

Note: r of N = 60, TFC = total flavonoids, FRS= antioxidant, DHEC = Inhibition *E. coli*, DHSA = inhibition of *S.aureus*. Correlation is significant at the 0.05 and 0.01 levels (2-tailed).

Table 8. Correlation between total chlorophyll, antioxidants, and antibacterial

	TotlChlo	*FRS*	*Inhibition of EC*	*Inhibition of SA*
TotlChlo	1			
FRS	-0.444	1		
DHEC	-0.430	0.323	1	
DHSA	-0.038	0.316	0.266	1

Note: r of N = 60, TotlChlo= total chlorophyll, FRS= antioxidant, DHEC = Inhibition *E. coli*, DHSA = inhibition of *S.aureus*. Correlation is significant at the 0.05 and 0.01 levels (2-tailed)

Table 9. Correlation between carotenoids, antioxidants, and antibacterial

	Carotene	*FRS*	*Inhibition of EC*	*Inhibition of SA*
Carotene	1			
FRS	0.008	1		
DHEC	0.068	0.323	1	
DHSA	-0.010	0.316	0.266	1

Note: r of N = 60, Caroten= carotenoids, FRS= antioxidant, DHEC = Inhibition *E. coli*, DHSA = inhibition of *S.aureus*. Correlation is significant at the 0.05 and 0.01 levels (2-tailed)

The correlation of flavonoids and chlorophyll to antioxidant and antibacterial activity showed the same pattern: antioxidant activity was moderately negatively correlated, while antibacterial activity against *E. coli* was moderately positively correlated, but very low positively correlated against *S. aureus* (Tables 7 and 8).

Manik *et al*. (2014) stated that the overall flavonoid content was related to antibacterial activity. Research from Solikhah *et al*. (2019), showed that there was no correlation between chlorophyll content and antioxidant activity. In addition, chlorophyll a and carotenoid pigment levels did not show values that were directly proportional to their antioxidant activity.

Statistical analysis showed that the correlation between carotenoid content and antioxidant and antibacterial activity was very low. It can be observed that the carotenoid content in both extracts is the same but the antioxidant and antibacterial abilities are different. This suggests that these abilities are influenced by other compounds such as phenolics which are positively correlated.

According to Fitriansyah *et al*. (2018), the correlation of carotenoid content with antioxidants is weaker than that of phenolics and flavonoids. Another study showed that although carotenoid content contributes to the overall health of *Phyllanthus* plants, the role of carotenoids in antioxidant and antibacterial activity is lower than that of phenolics and flavonoids (Rao *et al*., 2023). This study found that the antioxidant and antibacterial abilities of *Phyllanthus* were strongly influenced by phenolic compounds. While

flavonoid compounds had a significant effect on these two activities, chlorophyll and carotenoids had only a slight effect on the antioxidant activity but not on the antibacterial activity. This suggests that the ability is influenced by phenolic compounds, and perhaps not pigments, but flavonoid compounds. The advantage of this study encourages the use of *P. urinaria* and *P. niruri* as traditional medicine, especially for antibacterial and antioxidant purposes.

CONCLUSION

In addition, *P. niruri* had higher chlorophyll than *P. urinaria*. The carotene of both *Phyllanthus* species was the same, 11.9 µg/ml. *P. urinaria* leaf extract has a very strong inhibition against *E. coli*, which is more than 20 mm, while *P. niruri* leaf extract has a strong inhibition against both bacteria around 11-20 mm. *P. urinaria* can inhibit *S. aureus* at a high concentration of 500 mg/L. The relationships between phenolics, flavonoids, antibacterials, and antioxidants were negatively correlated. The correlation between flavonoids and total chlorophyll with antioxidant activity was quite high. *E. coli* bacteria had a moderate relationship with total phenolics and flavonoids.

Further research could be focused on the extraction of phenolic compounds to enhance the antioxidant and antibacterial capabilities of the extract. Another opportunity could be researched by treating the cultivation of both plants to increase the production of phenolic compounds.

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