Jurnal Jamu Indonesia (2023) 8(2): 77–83
ISSN 2407-7178
eISSN 2407-7763

Research Article
DOI: https://doi.org/10.29244/jji.v8i2.330

Phytochemical Screening, Cytotoxicity Test, and Antibacterial Activity Test of Wungu Plant Extract (*Graptophyllum pictum* L. Griff)

Skrining Fitokimia, Uji Sitotoksisitas, dan Uji Aktivitas Antibakteri Ekstrak Wungu (*Graptophyllum pictum* L. Griff)

Rini Anggi Arista[1]*, Mohammed A. Jalloh[2], Fahma Alifia Insani[3], Putri Azzahara Balqis[3], Cindy Larasati[3], Barieq Farial Putra Arief[3], Rizka Fatriani[4]

[1]Department of Science Education, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang, Malang, Indonesia
[2]University of Liberia, Liberia
[3]Department of Biochemistry, Faculty of Mathematics and Natural Sciences, IPB University, Bogor, Indonesia
[4]Tropical Biopharmaca Research Center, IPB University, Bogor, Indonesia

Corresponding author: rinianggiarista@gmail.com; (+62)82215483860

Received March 08, 2023; **Accepted** June 23, 2023; **Available online** July 15, 2023

ABSTRACT

Indonesia boasts a rich array of medicinal herbs, including the wungu (*Graptophyllum pictum* L. Griff), which is known for its potential as an anticancer and antibacterial agent. Here, we used qualitative phytochemical assays to identify bioactive compounds in various parts of the wungu plant (leaves, stem, and roots) and their efficacy in combating cancer and bacterial infections. The brine shrimp lethality test (BSLT) was used to measure anticancer properties and antibacterial activity against *Escherichia coli*. We found that the leaves contained flavonoids, triterpenoids, phenols, saponins, and alkaloids, whereas the stem and root contained steroids and alkaloids. Notably, the wungu plant exhibited no toxicity, as shown by the cytotoxicity test, indicating a lack of anticancer potential as all parts showed LC_{50} values exceeding 1000 ppm. However, the antibacterial assays strikingly showed efficacy against bacteria across all parts of the plants, with varying strengths from weak to strong. This study sheds light on the promising antibacterial properties of the wungu plant and its potential as a therapeutic agent.

Keywords: antibacterial, phytochemical analysis, cytotoxicity assessment, wungu plant

ABSTRAK

Tanaman obat di Indonesia sangat melimpah dan terdiri dari berbagai jenis, salah satunya adalah tanaman wungu (*Graptophyllum pictum* L. Griff). Tanaman wungu dapat dimanfaatkan sebagai antikanker dan antibakteri. Oleh karena itu, tujuan dari penelitian ini adalah untuk mengidentifikasi kandungan senyawa aktif dari bagian tanaman wungu dan potensinya sebagai antikanker serta antibakteri. Penelitian ini akan menguji kandungan metabolit pada tiga bagian tanaman wungu (daun, batang, dan akar) menggunakan uji fitokimia kualitatif, uji potensi antikanker menggunakan uji sitotoksisitas metode Brine Shrimp Lethality Test (BSLT), dan uji antibakteri menggunakan bakteri Escherichia coli. Hasil penelitian menunjukkan bahwa tanaman wungu mengandung flavonoid, triterpenoid, fenol, saponin, dan alkaloid pada bagian daun, sedangkan bagian batang dan akar mengandung steroid dan alkaloid. Hasil uji sitotoksisitas menunjukkan bahwa tanaman wungu tidak toksik dengan nilai LC_{50} di atas 1000 ppm pada seluruh bagian tanaman sehingga tidak berpotensi sebagai antikanker. Hasil uji antibakteri menunjukkan bahwa tanaman wungu berpotensi sebagai antibakteri pada setiap bagian tanaman wungu dengan kategori lemah hingga kuat.

Kata Kunci: antibakteri, fitokimia, sitotoksisitas, tanaman wungu

INTRODUCTION

Indonesia's natural landscape is teeming with diverse biological treasures, notably through its rich flora, including a plethora of medicinal plant species thriving in its fertile soil. Among these botanical wonders is the wungu plant (*Graptophyllum pictum* L. Griff), a member of the *Acanthaceae* family with origins tracing back to New Guinea before spreading its roots to regions like India, Mexico, Ghana, Bolivia, and across Asia. The wungu plant has purple and handeuleum leaves, making it easy to spot across Indonesia (Widyowati, 2011).

The wugu plant treats various diseases, such as hemorrhoids and constipation, and aids in menstruation. It also has antifungal and anti-inflammatory properties (Widyowati, 2011). The leaves of the wungu plant have many pharmacological activities, including antioxidant, anti-inflammatory, anti-diabetic, analgesic, photoprotective, immunomodulatory, nephroprotective, anti-hemorrhoid, and antibacterial properties (Sartika & Indradi, 2021). Rich in non-toxic phytochemical compounds such as flavonoids, steroids, saponins, and tannins, the wungu plant derives its anti-inflammatory and analgesic properties from these bioactive constituents (Sya'haya & Iyos, 2016). While existing research has shed light on the medicinal potential of the plant's leaves, as shown by Aminah et al. (2016), further research is warranted to unveil the presence of secondary metabolites and explore the therapeutic benefits of its stems and roots. In light of the ubiquitous presence of *Escherichia coli*—a bacterium known for causing infections—research into antibacterial agents is imperative to combat its proliferation effectively. These antibacterial compounds are pivotal in inhibiting bacterial growth and eradicating pathogenic strains like *E. coli* from environments with elevated salt concentrations (Magani et al., 2020).

METHODS

1. Plant Material and Sample Extraction

The research was conducted from August to November 2022 at the Biochemistry Laboratory of the Faculty of Mathematics and Natural Sciences at IPB University. The leaves, stems, and roots of the wungu plant simplicia are extracted using the maceration method. Samples were extracted separately using 70% ethanol solvent. Briefly, 20 grams of simplicia from each part of the plant were dissolved in 150 mL of solvent. The mixture was homogenized in the dark for 1 x 24 hours at 21°C using a water bath shaker (Memmert., Germany). The filtrate was filtered using filter paper, and then the resulting filtrate was evaporated using a rotary vacuum evaporator (Hahnvapor HS-2005v., Korea) at a temperature of 45°C with a speed of 65 rpm until it formed a paste. The resulting paste will be used for phytochemical screening tests, cytotoxicity analysis, and antibacterial activity.

2. Phytochemical Screening Analysis

2.1 Flavonoids test

To conduct a test, take 1 mL of wungu plant sample extract from leaves, stems, and roots, which has been dissolved in 70% ethanol. Then, add Mg powder on the tip of a spatula along with ten drops of 2N HCl. Heat it over a water bath and shake it until evenly mixed. A positive result is indicated by a color change to yellow-red.

2.2 Triterpenoids and steroids test

To determine whether a wungu plant sample contains triterpenoids or steroids, extract 1 mL of the sample (from leaves, stems, and roots) using 70% ethanol solvent. Add ten drops of acetic anhydride, two drops of concentrated sulfuric acid, and 20 drops of acetone to the extract. Shake the mixture and leave it for a few minutes. If the mixture turns red or purple, triterpenoids are present, while green or blue indicates the presence of steroids.

2.3 Quinones test

To test for specific properties in a wungu plant sample, 1 mL of extract from the leaves, stems, and roots, dissolved in 70% ethanol, was mixed with 10-15 drops of NaOH. A positive indication of these properties can be observed as a red color change.

2.4 Phenols test

To test the wungu plant sample extract, 1 mL of the solution (made by dissolving leaves, stems, and roots in 70% ethanol) was mixed with three drops of 5% $FeCl_3$. A positive result can be confirmed by observing a color change to blackish purple.

2.5 Saponins test

A total of 1 mL of wungu plant sample extract from leaves, stems, and roots dissolved in 70%

ethanol was added to 10 ml of hot water and then shaken for 10 seconds. Positive results are indicated by forming stable foam for 10 minutes, 1 cm to 10 cm high.

2.6 Alkaloids test

A total of 1 mL of wungu plant sample extract from leaves, stems, and roots dissolved in 70% ethanol was added with 5 ml of chloroform and two drops of H_2SO_4, then put into a test tube. The chloroform extract in the test tube was shaken with 6 ml of 2M H_2SO_4, and the sample was stirred with a vortex. The acid layer formed on the top of the mixture is separated into another test tube. The acid layer was divided into three on the drop plate, and Mayer, Wagner, and Dragendorff reagents were added to each plate. Positive results can be seen from the color changes to white, brown, and red-orange sequences.

2.7 Tanins test

To test for the presence of tannin in wungu plant extracts, 1 mL of the extract from leaves, stems, and roots was dissolved in 70% ethanol and mixed with three drops of $FeCl_3$. A positive result is indicated by a color change to blue or black. Five drops of gelatin were added to each sample to confirm the presence or absence of tannin until a white precipitate formed.

3. Cytotoxicity Analysis

3.1 Sample preparation

0.04 grams of paste sample was weighed and mixed into 20 mL of seawater. The mixture was then stirred with a vortex machine and sonicated for 10 minutes. Samples are ready for BSLT testing.

3.2 Larvae preparation

1.5 liters of sea air and put it into a large tube. We then added one teaspoon of powder containing Artemia larvae eggs into the tube. The tube containing the larval eggs was left for 48 hours while aerated using an aerator and illuminated using a lamp.

3.3 Cytotoxicity Analysis

We carried out BSLT testing based on the method of Meyer et al. (1982). We prepared clean microplates and filled each microplate well with 10 Artemia larvae and 1 mL of seawater. We then filled the microplate well with sample and sea air up to 2 mL with a final concentration of 0, 10, 50, 100, 500, and 1000 ppm. Each concentration was carried out three times. The sample was left for 24 hours, after which we counted the number of live larvae.

4. Antibacterial Activity

4.1 Preparation of McFarland Standard Solution (Andrews, 2008)

The McFarland standard solution is made by mixing 1% $BaCl_2$ and 1% H_2SO_4. To prepare this solution, take 0.05 mL of 1% $BaCl_2$ and mix it with 9.95 mL of 1% H_2SO_4. Shake the mixture until it becomes homogeneous. Measure the turbidity of the solution at a wavelength of 450 nm using distilled water as a blank. The absorbance value of the standard solution should fall between 0.08 and 0.13. McFarland standard solution 0.5 is equivalent to a bacterial cell suspension with a concentration of 1.5 x 108 CFU/mL and has an absorbance value of 0.138.

4.2 Rejuvenation of Test Bacteria

E. coli and *S. aureus* were introduced into NA media to revitalize the bacteria and then incubated at 37°C for 24 hours. Once colonies had developed on the media, they were transferred into 10 mL of NB media using aseptic techniques. The bacterial suspension's absorbance was adjusted with McFarland 0.5 standard solution to obtain a bacterial suspension with a cell number of 1.5 x 108 CFU/mL.

4.3 Antibacterial Activity Testing

Antibacterial compounds were added to the wells of micro dishes and then diluted to various concentrations using sterile NB media. The compounds used were antibiotics with a 1 mg/mL stock concentration. The concentration series included 100, 200, and 500 ppm, a 10% DMSO blank, and 50 ppm antibiotics. Then, 5 µL of the test bacterial suspension, standardized for cell number with 0.85% NaCl, was added to the wells of the micro dishes. The dishes were then placed in a 37°C incubator for 24 hours. The total volume of the antibacterial compounds, NB media, and bacterial cell suspension was 200 µL. After 24 hours, the micro cups were observed based on the turbidimetric principle. The minimum inhibitory concentration was determined as the clearest (not cloudy) concentration. To determine the minimum kill concentration, 50 µL of the precise suspension was subcultured into NA media and observed after 24 hours. The minimum kill concentration was the

concentration that killed most bacteria. The negative controls included media and test bacteria. Finally, the precise zone concentration designated as KHTM was observed, and its diameter was measured.

RESULTS & DISCUSSION

1. Phytochemical Screening Analysis

The phytochemical test aims to identify the chemical compounds contained in a plant qualitatively. The research results were shown by changes in color in the wungu plant sample solution from the leaves, stems, and roots after treatment. Qualitative phytochemical results of wungu plant samples in the leaves, stems, and roots can be seen in **Table 1**.

Table 1. Phytochemical Screening results of wungu plant leaves, stems, and roots extracts

Phytochemical Test	Parts of Wungu Plants		
	Leaves	Stems	Roots
Flavonoids	+	-	-
Triterpenoids	+	-	-
Steroids	-	+	+
Quinones	-	-	-
Phenol	+	-	-
Saponins	+	-	-
Alkaloids	+*	+*	+*
Tannin	-	-	-

Note: * (positive dragendorff and wagner); - (negative); + (positive)

The results of the qualitative phytochemical tests shown in **Table 1** show that extracts from the leaves, stems, and roots of the wungu plant had different results. These different results depend on the secondary metabolite compounds found in each part of the plant, which are characterized by changes in color, indicating the presence of secondary metabolites found in the plant (Oktari et al., 2014).

Wungu plant leaf extract contains flavonoids, triterpenoids, phenols, saponins, and alkaloids in Dragendorff and Wagner compounds. Wungu plant stem extract contains steroids and alkaloids in Dragendorff and Wagner reagents. Meanwhile, the root extract of the wungu plant contains the same compounds as the stem, namely steroids and alkaloids in the Dragendorff and Wagner reagents. This aligns with research by Sya'haya & Iyos (2016), who found that the leaves of the wungu plant contain alkaloid, flavonoid, and saponin compounds.

Positive results in the flavonoid test were only in the leaf extract of the wungu plant, while the stem and root extracts showed negative results (**Table 1**). This is in line with research conducted by Aulia et al. (2018) that shows that the leaves of the wungu plant (*Graptophyllum pictum* (L.) Griff) positively contain flavonoids. The positive result is indicated by the change in color of the solution to red after treatment, namely a few drops of HCl and Mg powder.

The phytochemical test results were positive for triterpenoids in the leaf extract, while the stem and root extracts showed positive results for steroid compounds (**Table 1**). The triterpenoid and steroid tests are tests where the two are interrelated. Holil & Griana (2020) explained that the color change in the sample would depend on the compounds contained. If the sample changes color from red to purple, it indicates that it is positive for triterpenoids, whereas if the color changes from blue to green, it indicates that the sample is positive for steroids. According to research by Sekti et al. (2022), the leaves of the wungu plant contain triterpenoids characterized by the color changing to blue after being treated with acetic acid. Positive results for steroids in the stem and root extracts of the wungu plant indicate other compounds in these parts. Tukiran et al. (2014) state that the wungu plant contains steroid compounds in n-hexane and chloroform extracts because the plant is non-polar.

The wungu quinone test showed negative results for all leaf, stem, and root extracts (**Table 1**). This is the opinion of Murtie (2013) the wungu plant contains flavonoids, alkaloids, pectin, tannin, steroids, saponins, glycosides, formic acid, and chlorophyll. Quinones are colored phenolic derivatives and have rough chromophores. Positive results for quinones are indicated by a color change to red, violet, or red-purple (Harborne, 1987).

Based on the results of the phytochemical test, positive results for alkaloids were found in all samples, namely in leaf, stem, and root extracts (**Table 1**). This is characterized by a brown precipitate forming with Wagner's reagent and an orange-red precipitate with Dragendorff's. This is by research conducted by Poh-Yen et al. (2018), who found that the leaves of the purple plant (*Graptophyllum pictum* (L.) Griff) were positive for containing alkaloids. In this alkaloid test, H_2SO_4, which functions to form alkaloid salts, is added. This is because alkaloid compounds are essential, so they are extracted with acidic solvents (Puspa et al., 2017). The alkaloid compounds in the wungu plant have anti-inflammatory and analgesic properties (Sya'haya & Iyos, 2016).

The phenolic or phenol hydroquinone test adds a 5% $FeCl_3$ reagent (Saragih & Arsita, 2019). A positive phenol test result is indicated by a change in color to green, red, purple, dark blue, blackish blue, or blackish green (Wardhani et al., 2018). Based on the results of the phenol test on the wungu plant extract, positive results were only found in leaf extract samples (**Table 1**). This is characterized by a change in color to blackish purple. These results are the same as the opinions of Juniarti et al. (2021) that the leaves of the wungu plant contain phenols, tannins, alkaloids, flavonoids, and saponins.

Saponin is a surface active compound that is detected through the formation of foam. In an extract, the presence of saponin is positive if foam forms 1–10 cm high for 10 minutes (Wahid & Safwan, 2020). Based on the saponin phytochemical test results, positive results were obtained only for the leaf extract, while the stem and root extracts showed negative results (**Table 1**). This is in line with research conducted by Sekti et al. (2022) that shows that the leaves of the purple plant (*Graptophyllum pictum* (L.) Griff) contain positive saponins. The formation of stable foam indicates this positive result after shaking for 10 minutes. The foam is formed because saponin compounds can dissolve in water (hydrophilic) and dissolve in nonpolar solvents (hydrophobic) (Sulistyarini et al., 2019).

A negative result of the tannin test is indicated by no change in color to blackish blue in the sample tested (Ikalinus et al., 2015). Based on the results of phytochemical tests, negative tannin results were found in all wungu plant samples, namely in leaf, stem, and root extracts (**Table 1**). These results differ from the phytochemical tests conducted by Fauziah et al. (2022). Fauziah et al. (2022) state that wungu leaf extract contains tannin compounds. The difference in test results could be caused by the small number of metabolite compounds in the extract, so they were not detected (Oktari et al., 2014).

2. Cytotoxicity BSLT (Brine Shrimp Lethality Test) Analysis

Sample toxicity is determined by determining the LC_{50} value. To obtain the LC_{50} value, the mortality value is first determined from Artemia larvae exposed to the sample for 24 hours. After that, the mortality value was analyzed using probit to determine the LC_{50} value. In the BSLT test, LC_{50} is the compound concentration that kills up to 50% of the Artemia larvae population. After probit analysis, the LC_{50} value is calculated using linear regression calculations $y = a + bx$. The LC_{50} calculation results can be seen in **Table 2**.

Table 2. BSLT test results for extracts of leaves, stems, and roots of the wungu plant

Parts of Wungu Plants	LC_{50} (ppm)
Leaves	> 1.000
Stems	> 1.000
Roots	> 1.000

Based on the BSLT toxicity test results, all samples had LC_{50} values above 1000 ppm. This shows that a sample concentration of more than 1000 ppm is needed to kill up to half of the Artemia larvae population. According to Lestari et al., (2015), for an extract to be said to be toxic or bioactive, the extract must have an LC_{50} value below 1000 ppm. Based on this, samples originating from all parts of the wungu plant are non-toxic.

3. Antibacterial Activity

The antibacterial activity of the wungu plant is carried out from various parts of the plant, namely the leaves and roots. It stems, which are extracted and then made into a standard solution. The antibacterial activity test on wungu plants was determined by the appearance of a clear zone around the paper disc whose diameter was measured against the growth of Escherichia coli with an incubation period of 1 x 24 hours at a temperature of 37°C. The research results can be seen in **Table 3**.

Table 3. Test results of the antibacterial activity of leaves, stems, and roots extracts of the wungu plant on *Escherichia coli*

Parts of Wungu Plants	Average Zone of Inhibition (mm)
Leaves	21.8 ± 0.00b
Stems	2.70 ± 0.00b
Roots	5.30 ± 3.51b
Ampicillin	2.36 ± 1.50a

The inhibitory power of wungu plant leaf, stem, and root extracts has antibacterial activity against Escherichia coli, which can be seen from the diameter of the clear zone around the paper disc. This indicates that bioactive compounds in each extract can inhibit bacterial growth (Angelika et al., 2014). According to Sari et al. (2019), if the apparent zone diameter is < 5 mm, it is indicated that the inhibitory power is in the weak category, the inhibition zone diameter is 5–10 mm, it is indicated as moderate, the apparent zone diameter is 10–19 mm, it is indicated

as being in the medium category, and the apparent zone diameter is >20 mm, it is indicated categorized as vital. Based on the results of the antibacterial activity test, it was found that the most excellent antibacterial activity was found in the leaf extract with an average apparent zone diameter of 21.8 mm, which was categorized as vital. Meanwhile, the antibacterial activity was categorized as weak in the stem extract, with an average apparent zone diameter of 2.70 mm. In comparison, the average apparent zone diameter of the root extract was 5.30 mm, which was categorized as medium. The greater the diameter of the clear zone, the greater the inhibitory power, which is directly proportional to its antibacterial activity (Emelda et al., 2021). Thus, the wungu plant leaf extract has more excellent antibacterial activity than the other two samples, which can be seen from the highest average diameter value of the clear zone.

CONCLUSION

The wungu plant (*Graptophyllum pictum* (L.) Griff) contains flavonoids, triterpenoids, phenols, saponins, and alkaloids in the leaves, while the stems and roots contain steroids and alkaloids. The antibacterial activity of the wungu plant is different in each part. The leaves of the wungu plant have the most potent antibacterial activity compared to the roots and stems. The wungu plant, including its leaves, stems, and roots, is not toxic because it has a high LC_{50} value, so it does not have the potential to be an anticancer drug.

ACKNOWLEDGMENT

Thanks are expressed to the laboratory assistants and laboratory technicians of the Department of Biochemistry who have provided technical assistance in this research.

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Citation Format: Arista, R. A., Jalloh, M. A., Insani, F. A., Balqis, P. A., Larasati, C., Arief, B. F. P., & Fatriani, R. (2023). Phytochemical Screening, Cytotoxicity Test, and Antibacterial Activity Test of Wungu Plant Extract (*Graptophyllum pictum* L. Griff). *Jurnal Jamu Indonesia, 8*(2), 77–83. https://doi.org/10.29244/jji.v8i2.330