

Phytochemical Analysis and Antioxidant Activity of Pandila Tree Leaves (*Saurauia Tristlyla* Dc) From Tibo Village

Naldiyanto Madao^{a,1,*}, Abdul Hapid^{a,2}, Asniati^{a,3}, Ariyanti^{a,4}, Erniwati^{a,5}, Muthmainah^{a,6}

^a Department of Forestry, Faculty of Forestry, Tadulako University, Jl. Soekarno - Hatta Km. 9, Palu 94148, Indonesia

¹naldiyanto464@gmail.com*

*corresponding author

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ABSTRACT

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Introduction: The Pandila tree known as pandila (*Saurauia tristlyla* DC) is one of the plants that has been traditionally used as a medicinal material. Pandila has been traditionally used by the community as a medicine for external and internal diseases, for example, pandila leaves are believed by the Tibo Village community to absorb congealed blood due to accidents. This study aims to determine the secondary metabolite content and antioxidant activity of pandila leaves. **Method:** The research method used in this study is a descriptive method by looking at changes in color and liquid form in alkaloids, Flavonoid, Tannin, Saponin, Steroid, Terpenoid, and Carotenoid compounds. Phytochemical analysis was carried out on 96% ethanol extract of pandila leaves. The DPPH method was used to determine the antioxidant activity. The results of phytochemical analysis obtained on the leaves of Pandila tree leaves (*Saurauia Tristlyla* DC) using 96% ethanol solvent are old leaf extracts and young leaves of pandila positively contain 3 compounds namely Tannins, Steroids, and Carotenoids. **Results and Discussion:** The results of the antioxidant activity test of Pandila leaf extract using the DPPH method on old leaves have an IC₅₀ of 101, 2844 ppm including in the moderate category, and on young leaves have an IC₅₀ of 76.688 ppm which is included in the strong category. **Conclusion:** Pandila tree leaves are still safe to be used as traditional medicine, by looking at the tannin, steroid, and carotenoid compounds contained in pandila leaves. In addition, the results of antioxidant activity analysis are also a supporting factor where the antioxidant content in old leaves and young leaves of Pandila trees is classified as strong and moderate, so this plant is still safe to be used as a traditional medicinal plant.

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1. Introduction

Forests in addition to providing production in the form of wood, also provide by-products that can be seen from a tree in the form of sap, leaves, bark, fruit, stems, which are non-timber forest products (NTFPs), which can be used as one of the ingredients in making traditional medicines in a particular area, especially in the scope of life of the Tibo Village community, has used medicinal plants, especially Pandila plants as traditional medicines as hereditary medicine [1].

Tibo Village is an area that is included in the Sindue sub-district of Donggala district, Central Sulawesi province. This area has ethnic diversity, namely the Kaili Rai tribe, the Lauje tribe, and

the Taijo tribe. The Kaili Rai tribe is an indigenous tribe and has long settled in Tibo village. The Kaili Rai community uses various types of plants that are used as medicines. Seeing the potential of plants that are useful and used as medicine allows the interaction of the Tibo village community with plants [2]. However, data and information about the types of plants used by the Kaili Rai tribe and the level of interaction have not been widely studied. This is very valuable knowledge where local wisdom on the use of medicinal plants needs to be known and recorded so that traditional knowledge is not lost and passed on from generation to generation [3]. Therefore, the traditional knowledge of the Kaili Rai tribe in using medicinal plants in the vicinity is very important to study.

Pandila tree (*Saurauia tristlyla* DC.) is one type of plant that can grow at a height of 10 meters to 15 meters. Tibo Village is an area that is included in the Sindue sub-district, Donggala Regency, Central Sulawesi Province, where this area has one of the plants used as traditional medicine, especially in leaves. Where the leaves of the Pandila tree are believed to be able to treat internal injuries such as absorbing congealed blood due to accidents. Pandila tree is a plant from the Actinidiaceae family. To determine the chemical compounds and antioxidant activity in Pandila leaves, a phytochemical analysis study was conducted by testing alkaloid compounds, flavonoids, saponins, tannins, steroids, terpenoids, and carotenoids, in pandila leaves and to determine antioxidant activity, the free radical or DPPH silencing method was used. Based on the background description above, the problem formulation of this research is how the content of secondary metabolites, antioxidants, and water content in old leaves and young leaves of Pandila trees (*Saurauia Tristlyla* DC.).

The purpose of this study was to determine the content of chemical compounds through phytochemical analysis, antioxidants, and water content of old leaves and young leaves of Pandila trees (*Saurauia Tristlyla* DC.) With this research, it is hoped that it will be able to provide insight and information related to Pandila leaves (*Saurauia Tristlyla* DC.) which are used as traditional medicines, especially phytochemical analysis and antioxidant activity, with this research, it will provide the latest insights regarding whether or not this plant is suitable to be made as a broad medicine for all people, and also as additional information for researchers who will conduct the same or similar research.

2. Method

In this study, the method used was maceration for leaf extraction [4], the qualitative method in phytochemical test, and the DPPH method for antioxidant activity testing research, from Pandila tree leaves [5].

1.1. Procedure

Pandila leaves that have been taken are washed using running water, after the next rare clean, namely, the leaves are dried through direct sunlight until the leaves are completely dry and brittle. After drying, the next step is that the leaves are pulverized using a blender and then the powder is sieved to get a smooth sample.

Determination of moisture content of old and young leaves of Pandila tree using the Association of Official Analytical Chemist standard (AOAC 2006). Samples of old leaves and young leaves of the Pandila tree obtained were weighed. The sample in the cup was then oven to 105°C for 3 hours, after which it was put into a desiccator for 15-20 minutes and then weighed. Next, it was reheated for 1 hour until a constant weight was obtained. Moisture content can be calculated with the following formula:

$$Ka = \frac{\text{initial weight}x}{\text{final weight}} \times 100 \quad (1)$$

Pandila leaves that have been powdered are then extracted by maceration. By soaking the sample in 96% ethanol solvent with a size of 1: 5 for 24 hours to get the results of pandila leaf extract. After filtering which aims to get the filtrate [6]. After getting the filtrate, remaceration with ethanol is again carried out, and the obtained is evaporated, which aims to obtain ethanol extract [7].

2.1 Observation Parameters

1. Phytochemical Test of Pandila Leaf

After obtaining thick extracts from maceration results, further testing of alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, and carotenoids was carried out.

a) Alkaloid Test

1.0 ml of pandila leaf extract was put into a test tube and 2-3 drops of reagent were added. In this observation, it can be said to be positive for alkaloids if there is a color change to an orange color [8].

b) Flavonoid Test

This test is done by entering the sample solution in a test tube with Magnesium powder added and also a few droplets of concentrated HCL (Shinoda reagent). In this test if the color changes to red, pink, and orange, it can be said that the positive results contain flavonoids [9].

c) Tannin Test

This test is done by inserting Pandila leaf extract with a dose of 1 ml into a test tube, then adding a few drops of FeCl_3 as much as 5%. If in this test there is a color change to brownish green or blackish blue, it can be said that pandila leaves are positive for tannins [10].

d) Saponin Test

Pandila leaf extract is again measured as much as 1 ml which is put into a test tube, and distilled water is added, then the next stage is shaken for 15 minutes, and in this test, if there is foam as high as 1 cm in a period of 5 minutes, it can be stated that pandila leaves are positive for saponins [10].

e) Terpenoid Test

A total of 1 ml of pandila leaf extract was mixed with 0.5 ml of chloroform, and then 1.5 ml was concentrated to form a layer and added with H_2SO_4 . In this stage, if there is a change in color to reddish brown on the surface, it can be stated that pandila leaves are positive for terpenoids [11].

f) Steroid Test

A total of 1 ml of pandila leaf extract was dissolved into 5 ml of chloroform, and 6 ml of sulfuric acid was added. In this situation, if there is a color change in the upper layer, it becomes red, and the lower layer shows yellow and green colors, it can be said that pandila leaves contain steroids [12].

g) Carotenoid Test

A total of 1 ml of pandila leaf extract was mixed with 5 ml of chloroform in a test tube, then shaken and filtered, and mixed again with 85% sulfuric acid. In this stage, if there is a blue color change on the surface, it shows that pandila leaves contain carotenoids [13].

2. Antioxidant Activity Test of Pandila Leaves

The concentrated extract of Pandila leaves is determined by UV spectroscopy activity with the DPPH method [14], the sample extract is calculated as much as 10 mg, then put into a 10 ml volumetric flask, then determined with ethanol solvent, until a solution concentration of 1000 ppm is obtained, then dissolved in small series, until a solution of 20, 40, 60, 80, 100 ppm is obtained, and the solution that has been made is pipetted as much as 1 ml and added with 3 ml of $50\mu\text{M}$ DPPH solution, the mixture is homogenized and allowed to stand for 30 minutes in a dark place, then the absorbance is measured at 517 nm. Tests were also carried out on the DPPH value of the solution [15]. The absorbance obtained was used to determine the % inhibition using the following equation:

$$\%inhibisi = \frac{Abs\ DPPH + Abs\ sampel}{Abs\ DPPH} \times 100\% \quad (2)$$

2.2 Data Analysis

The data obtained is presented descriptively by looking at changes in color and shape of the liquid tested after going through the phytochemical test stage. And from the results of the phytochemical test of Pandila leaf extract can be presented in the form of images or documentation and tables [16]. Data obtained from the Antioxidant Activity test of Pandila leaves were analyzed using regression equations.

3. Results and Discussion

3.1 Water Content

The results of testing the water content of old leaves and young leaves of the Pandila tree (*Saurauia tristlyla* DC) in this study obtained the water content in old leaves is 12.8%, while in young leaves it is 12.7%. In this study, the sample was weighed 5 grams, then oven for 3 hours at 105°C with 3 repetitions to obtain a constant weight. The value of water content can be known from the difference between the weight of the sample before and after the oven [17]. The moisture content of Pandila leaves can be presented in the following table:

Table 1. The moisture content of old and young leaves of Pandila tree

Sample	Water Content
Old pandila leaf	12,8%
Young pandila leaf	12,7%

The high water content will accelerate microbial growth and can also facilitate the occurrence of Nhidrolisa to its chemical content so that it can result in a decrease in the quality of traditional medicine. So the purpose of removing water content by a certain amount is useful to extend the durability of the material during storage [18]. High water content in plant materials can cause instability when made into preparations, which will encourage the growth of microbes, and fungi, and can cause damage to compounds through the hydrolysis process [19].

3.2 Extraction Results of Old Pandila Leaves and Young Pandila Leaves

The extraction of old Pandila leaves and young Pandila leaves was carried out by soaking the sample using the maceration method, where maceration is a method or technique used to extract or take the desired compounds from a solution or solid by soaking the extracted material [20]. The maceration method has simple procedures and equipment and does not require heating of the sample so that natural components are not damaged. A total of 5 grams of old leaf powder and young leaves of the Pandila tree were put into a container in the form of a measuring flask, then soaked with ethanol solvent in a ratio of 1: 5, then soaked for 24 hours.

After soaking for 24 hours, the next step is to separate the residue and filtrate of old Pandila leaves and young Pandila leaves using filter paper. After that, the next step is that the resulting filtrate is then evaporated with a tool in the form of a rotary evaporator with a vacuum at a temperature of 29°C. This method is used to separate the leaf extract with ethanol solvent.

3.3 Phytochemical Test Results of Old Pandila Leaves and Young Pandila Leaves

The results of phytochemical analysis of ethanol extracts of old leaves and young leaves of Pandila trees (*Saurauia Tristlyla* DC) are shown by a change in the reaction on the sample can be seen in the following.

Table 2. Phytochemical analysis test results of old and young Pandila leaf extracts

No	Compound	Ethanol solvent test results	
		Old leaves	Young leaves
1	Alkaloids	-	-
2	Flavonoids	-	-
3	Tannin	+	+
4	Saponins	-	-
5	Steroids	+	+
6	Terpenoids	-	-
7	Carotenoids	+	+

Description:

+: Positive (Contains compound)

-: Negative (Not contained compound)

3.4 Alkaloid Compound Testing Results

In testing alkaloid compounds carried out on old pandila leaf extracts and young pandila with ethanol solvents showed negative results. This is due to the absence of orange precipitate changes in old and young leaves, as in the following figure:



Figure 1.

- Old Pandila leaf extract and assay results of alkaloid compounds
- Young Pandila leaf extract and assay results for alkaloid compounds.

Alkaloids are compounds found in plant tissue or in animals, which are most likely to be alkaline and contain nitrogen atoms (N), in heterocyclic or aromatic structures [21], alkaloids have a role in the treatment of several diseases such as diarrhea, diabetes, and as antimicrobial agents [22].

The results of this study are also the same as the results of research on peridot leaf extract (*Saurauia vulcani* Korth) conducted by [23]. The results showed that the ethanol extract of peridot leaves did not contain alkaloid compounds.

3.5 Flavonoid Compound Testing Results

In the flavonoid test carried out on the extract of old Pandila leaves and young leaves, it was declared negative, this is because there was no change in the color orange, pink or red, as shown below:



Figure 2.

- a. Old Pandila leaf extract and flavonoid compound testing results
- b. Young Pandila leaf extract and flavonoid compound assay results

The results of this study are different from the results of research conducted by [24]. Determination of chemical compound levels in concentrated extracts of Kesambi leaves (*Schleira Oleosa*), using the sample immersion method, and with research conducted for 3 repetitions to obtain valid results, and the results show that qualitatively flavonoid compounds are proven to exist in Kesambi leaves. In this

flavonoid test if the color changes to red, pink, and orange, it can be said that the positive results contain flavonoids [25].

3.6 Tannin Compound Testing Results

The study of tannin compounds carried out on extracts of old leaves and young leaves of Pandila trees showed positive results containing tannin, this is due to changes in brownish green or blue-black color, as in the following figure:



Figure 3.

- a. Old Pandila leaf extract and tannin compound test results
- b. Young Pandila leaf extract and tannin compound testing results

The results of this study are in line and the same as research conducted by [26], where the results research conducted to determine the tannin content in ethanol extracts of mahogany bark (*Swietenia Mahagoni Jacq*), and from these results it is known that mahogany bark positively contains tannin compounds, where there is a change in color after research on the tannin test.

Similarly, research conducted by [27] on mahogany leaves and seeds, where the results of research through phytochemical analysis, especially on tannin compounds, obtained positive results. Tannin compounds are compounds that are useful as antibacterials and antioxidants and even have potential as antidiabetics.

3.7 Saponin Compound Testing Results

The saponin test on the sample extract of old Pandila leaves and young Pandila leaves shows negative results because there is no change in the form of foam or foam on old or young leaves, as in the following figure:



Figure 4.

- a. Old Pandila leaf extract and test results for saponin compounds
- b. Young Pandila leaf extract and test results for saponin compounds

The results of research conducted by [28], on black wood leaves were different from the results of this study, where research conducted by Guli et al, black wood leaves contain saponin compounds, which are seen from the shape of the foam that appears at the top during the shaking process Saponin compounds in plants are one of the compounds that are very important for a plant, where saponin compounds act as a defense system, many saponin compounds are contained in roots, skin, leaves, and seeds [29].

3.8 Testing Results of Terpenoid Compounds

Terpenoid tests on sample extracts of old Pandila leaves and young Pandila leaves show negative results due to the absence of reddish-brown color changes on the surface, which can be seen in the following figure:

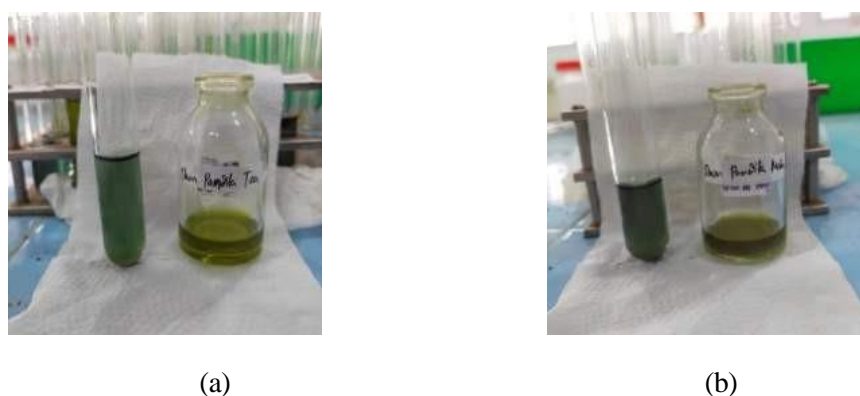


Figure 5.

- a. Old Pandila leaf extract and terpenoid compound assay results
- b. Young Pandila leaf extract and terpenoid compound assay results

This study is different from the research conducted by [28] where in his research the black wood leaf extract after testing the terpenoid compounds results showed positive for terpenoid compounds.

Terpenoids are one of the compounds present in plants where terpenoid compounds are able to provide a distinctive aroma in plants [30].

3.9 Steroid Compound Testing Results

From the results of testing steroid compounds against sample extracts of old leaves and young leaves of the Pandila tree, it is positive for steroids, it can be seen in the formation of a red top layer and the bottom layer of sulfuric acid shows yellow and green colors, as in the following



Figure 6:

(a)

(b)

- a. Old Pandila leaf extract and steroid compound testing results
b. Young Pandila leaf extract and steroid compound assay results

These results are in line with the results of research conducted by [31] which shows the formation of a red top layer and the bottom layer looks yellow and green in the test results of steroid compounds of bay stems with ethanol solvents. Steroids are compounds that play a very important role in a plant where steroids play a role in inhibiting leaf Pandila so that leaves do not fall quickly [32].

3.10 Carotenoid Compound Testing Results

The test for carotenoid compounds on the sample extract of old leaves and young leaves of the Pandila tree is positive, this can be seen when a precipitate is formed on a brownish-blue surface, as for the following picture:



(a)

(b)

Figure 7.

- a. Old Pandila leaf extract and carotenoid compound assay results
b. Young Pandila leaf extract and carotenoid compound assay results

Carotenoids are one of the compounds that are also owned by plants and also in animals, where carotenoid compounds have the function that is to absorbing light energy to be used in photosynthesis [33].

The results of this study are in line with research conducted by [34], from the results of his research, the carotenoid content is contained in Surian leaf extract, where when testing carotenoid compounds there is a form of precipitate with a brownish-blue color on the surface of the extract tested.

3.11 Antioxidant Activity Test Results of Old and Young Leaves of Pandila

The concentrated extract of the sample was determined to be antioxidant activity using the spectrophotometric method with DPPH reagent. Sample extracts of old Pandila leaves and young Pandila leaves weighed as much as 10 mg and then put into a 10 ml volumetric flask, then diluted with ethanol solvent to obtain a solution concentration of 1000 ppm. Then a dilution series was carried out to obtain a solution of 20, 40, 60, 80, and 100 ppm. The solution that has been made is pipetted as much as 1 ml and added with 3 ml of 50 μ M DPPH solution. The mixture was homogenized and left for 30 minutes in a dark place. Then the absorbance was measured at a wavelength of 517 nm. The test was also conducted on the DPPH solution. The following results of the antioxidant activity test of old leaves and young leaves of the Pandila tree can be seen in the table as follows:

Table 3. Antioxidant Activity Test Results of Old Pandila Leaves

Concentration (ppm)	ABS 517nm	% Inhibition	IC ₅₀
20	0,358	32,32514	
40	0,336	36,48393	
60	0,311	41,20983	101,2844
80	0,289	45,36862	
100	0,266	49,71645	

The following is the regression equation curve of % inhibition against ethanol concentration of old Pandila leaves. Regression analysis curve of % inhibition against the concentration of ethanol extract of old Pandila leaves.

Tale 4. Antioxidant Activity Test Results of Young Pandila Leaves

Concentration (ppm)	ABS 517nm	% Inhibition	IC ₅₀
20	0,355	32,89225	76,68896
40	0,321	39,31947	
60	0,292	44,80151	
80	0,259	51,03970	
100	0,228	56,89981	

The following is the regression equation curve of % inhibition against ethanol concentration of young Pandila leaves:

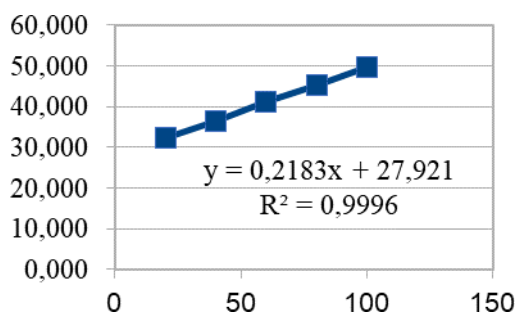


Fig 8. Regression analysis curve of % inhibition against the concentration of ethanol extract of young Pandila leaves.

Based on the results of antioxidant activity testing in Tables 3 and 4, from the five concentrations at a wavelength of 517 nm, it shows that each concentration has a change in absorbance, where the higher the concentration of the test solution, the lower the absorbance value, this can be interpreted that DPPH which acts as a free radical can be soaked free radicals by antioxidants contained in the test sample solution.

The IC value (*inhibition concentration*) obtained from the antioxidant activity test of the old pandila leaf extract using 96% ethanol solvent is classified as moderate, which is 101.2844 ppm, while the young pandila leaf is classified as strong, which is 76.68896 ppm. The ability activity of ethanol extracts of Pandila leaves to inhibit free radicals is said to be strong and moderate because it obtains a low IC₅₀ value. Specifically, a compound is said to be a very strong antioxidant if the IC₅₀ value is <50 ppm, strong 50-100 ppm, moderate 100-150 ppm weak 150-200 ppm, and very weak if IC₅₀ >200 ppm.

In contrast to the research conducted by [35], in his research the method used was in line with this study and the results of his research showed that the antioxidant power of chopstick leaf extract ethanol extract obtained an IC value of 152.227 ppm and included in the very weak category.

4. Conclusion

Based on the results of research conducted in the research laboratory of the chemistry department, faculty of mathematics and natural sciences, it can be concluded that:

- 1) The results of the phytochemical test analysis of 96% ethanol solvent on old Pandila leaves and young Pandila leaves contain 3 compounds including tannins, steroids, and carotenoids.
- 2) The results of the antioxidant activity test of 96% ethanol extract of Pandila leaves using the DPPH method in old leaf types have IC₅₀ of 101.2844 ppm which is classified as moderate, and in young leaf types IC₅₀ of 76.688 ppm is classified as strong.
- 3) From the results of this study, it can also be concluded that the water content contained in the old leaves of the Pandila tree is 12.8%, while in the young leaves of the Pandila tree is 12.7%.
- 4) From the results of phytochemical analysis research and antioxidant activity that has been carried out on old leaves and young leaves of Pandila trees, it can be concluded that Pandila tree leaves are still safe to be used as traditional medicine, by looking at the tannin, steroid, and carotenoid compounds contained in Pandila leaves. In addition, the results of antioxidant activity analysis are also a supporting factor where the antioxidant content in the old leaves and young leaves of Pandila trees is classified as strong and moderate, so this plant is still safe to be used as a traditional medicinal plant.

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