



## Antioxidant Activity of Laportea decumana (Roxb) Wedd Ethanol and n-Hexane Extracts

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#### Abstract

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BACKGROUND: Laportea decumana (Robx) wedd was a traditional medicine from Indonesian, especially in Eastern Indonesia, Ambon-Maluku. Based on a literature search, it was found that Laportea decumana (Robx) wedd has very little discussion, and most of its research comes from Indonesia, which is most likely endemic in Indonesia. This makes it interesting to develop it into herbal medicine whose use is based on evidence-based research so that it is safe when used as complementary nursing therapy.

AIM: This study tried to identify the phytochemical composition and investigate the antioxidant activity of Laportea decumana (Robx) wedd extract from Ambon-Maluku

METHODS: Extraction is done by the maceration method with 96% ethanol solvent. Phytochemical tests are performed with thin-layer chromatography (TLC) to look at flavonoids, tannins, triterpenoids, then alkaloids, and saponins using tube tests. This study tested antioxidant activity using the DPPH free radical scavenging method.

RESULTS: Laportea decumana (Robx) wedd phytochemical test positively contains alkaloids, flavonoids, tannins, triterpenoids, and saponins. Laportea decumana (Robx) wedd phytochemical test positively contains alkaloids, flavonoids, tannins, triterpenoids, and saponins. Laportea decumana (Robx) Wedd has a powerfull antioxidant ability which is obtained Laportea decumana (Robx) wedd ethanol extract (LDrW-EE) 22.81 µg/ml, Laportea decumana (Robx) wedd n-hexane extract (LDrW-NHE) 44.89 µg/ml and vitamin C 6.03 µg/ml.

CONCLUSION: The antioxidant activity of LDrW-EE is better than LDrW-NHE but not better than Vitamin C. Laportea decumana (Roxb) wedd extract can be developed as a complementary nursing therapy because it is included in the category of strong antioxidants.

## Introduction

Nature has always been a counterweight in the process of life, including in terms of health. Nature became a provider of medicinal plants that became the basis of many drug discoveries in the medical world. Indonesia is one of the world's countries with a wealth of flora that reaches 20.000 species and 40% of which is endemic Indonesia [1].

The utilization of flora in nursing is part of complementary therapies called biologically based therapies. This therapy includes vitamins, dietary supplements, essential oils, microorganisms, and herbal preparations [2].

In Indonesia, especially Ambon, people know "Itchy Leaves" as traditional medicinal herbal preparations used for generations to overcome aches. Still, no further research has been done on their content and mechanism. However, some research began to

be done to find the proper utilization of itchy leaves. In Latin, itchy leaves are called Laportea decumana (Robx) wedd this includes analgesic, antibacterial, cytotoxic, and antioxidant abilities [3].

Antioxidant activity in the body to ward off free radicals is expected to protect cells from damage. This ability is expected to prevent cancer, heart coroner, premature aging, and a combination of chemotherapy and radiation from triggering cell apoptosis [4], [5].

Antioxidant activity of Laportea decumana (Roxb) wedd in water extract is pro-oxidant and can be used as a source of natural antioxidants [6], [7], [8], [9]. According to the Hestiningtyas (2019), the antioxidant activity of Laportea decumana (Robx) wedd itch is stronger in polar fractions than in non-polar fractions [10].

The research above explains that the antioxidant ability of Laportea decumana (Robx) wedd is very strong, where the antioxidant ability of a plant is often used for cancer therapy because it can reduce

free radicals that trigger protein and DNA damage that lead to cancer [6]. However, further research still needs to be done because the location of growth can affect the content of the compounds in it [7], [8]. Therefore, *Laportea decumana* (Robx) wedd from Ambon-Maluku to be used as herbal medicine in complementary nursing therapy, it is necessary to review its phytochemical composition, including its antioxidant activity.

## **Material and Methods**

#### Sample preparation

Laportea decumana (Robx) wedd has been prepared then washed with water flow to be clean of dirt. Laportea decumana (Robx) wedd is then cut into smaller pieces to faster the drying process. This drying process is quite windy and avoids direct sunlight exposure because high temperatures will cause damage to active substances in the simplicia [11].

#### Extraction

Maceration was used as the extraction technique in this study and ethanol 96% as the solvent [12]. 100 gr *Laportea decumana* (Robx) wedd soaked in 1000 ml of ethanol 96%. This soaking process lasts for 5 days and stirs soaking 3 times a day. After obtaining the liquid extract, the next step is to perform the evaporation process on the rotary evaporator machine at a temperature of  $60^{\circ}$ C until the solvent evaporates ideally, which is characterized by the cessation of solvent droplets accommodated in the rotary evaporator. After obtaining a thick extract, do evaporation on the water bath for 1 × 24 h to get a more viscous extract characterized by the extract does not spill when the container is reversed.

#### Fractionation

Fractionation is done with 1 g of ethanol extract obtained in the previous process, then dissolved with n-hexane solvent as much as 50 ml and then shaken until the extract dissolves perfectly, then let stand. The n-hexane fraction can usually thicken perfectly without evaporating over the water bath.

### Phytochemical assay

The method used is the thin layer chromatography (TLC) method [13]. The plate is cut to 3–7 cm for each test to be carried out. The plate used is Aluminum Silica Gel 60 F254 plate. After the slab is ready, the next step is a thick extract that will be tested on the plate that has been prepared. Apply the

methanol-chloroform eluent solution (2:1) and put it in the chamber. The elution process will be carried out in a chamber that already contains eluent, where the TLC plate that has been previously stained with extract will be inserted into the chamber. After the elution process is complete, the next stage is the reagent on the TLC plate to see the color changes that occur. Color stains that appear on the TLC plate can be clarified by spraying with a 4% H<sub>2</sub>SO<sub>4</sub> solution.

#### Flavonoid test

The TLC plate was sprayed with a 10% AlCl3 reagent solution. The positive result is if the stain changes to a light yellow-green color.

#### Tannin test

The TLC plate is sprayed with a 5% FeCl3 reagent solution. The positive result is if the stain changes to a light-dark blue-black color.

#### Terpenoid test

The TLC plate is sprayed with a 10% H<sub>2</sub>SO<sub>4</sub> reagent solution. The positive result is if the stain changes to a light brownish-pink color.

#### Testing using tube tests

#### Alkaloid test

Dissolve  $\pm$  0.2 g extract with 1 mL chloroform in a mortar. After dissolving, 5 mL of chloroform-ammonia was filtered into a test tube. During the filtrate process, add 1–2 drops of sulfuric acid 10%, stir gently for 2–3 min, and form two layers. The top layer was tested using Mayer's reagent in the first and second tubes with Dragendorff reagent. A white residue indicated the presence of alkaloid compounds in the Mayer reaction, and the Dragendorff reagent showed an orange-red precipitate.

#### Saponin test

Put 1 mL of water fraction into the test tube. Then, shake for 1-2 min. Determination of the saponin content if there is a permanent foam.

#### Antioxidant test

The method used in the antioxidant test used the DPPH method [14]. LDrW-EE and LDrW-NHE extracts are made in several concentrations, namely, 80, 40, 20, 10, and 5  $\mu$ g/ml. Vitamin C compared with 10, 8, 6, 4, and 2  $\mu$ g/ml concentrations. The next step is the addition of 5 mL of DPPH solution, and the volume is made up of absolute ethanol. After that, prepare a 5 mL blank of the DPPH solution and makeup to 25 mL in volume. Measurement of absorbance value at a wavelength of 516.35 nm using UV-V is on the PerkinElmer machine LAMDA<sup>™</sup> 365 made in China.

#### Data analysis

The results of measuring the absorbance value are then analyzed for the percent inhibition using the formula [15]:

$$Percent inhibition = \frac{(Abs.Control - Abs.Sample)}{Abs.Control} \times 100$$

IC50 is the sample concentration required to provide 50% inhibition, calculated by making a curve between concentration and percent inhibition [16], [17].

## **Results and Discussion**

Traditional medicine needs to be developed to be used as an additional or complementary therapy. The active substance in medicinal plants depends on the type of plant, how it is harvested, the time, the drying, and the production process.

#### Extraction of Laportea decumana (Robx) Wedd

*Laportea decumana* (Robx) wedd that has been done the extraction process obtained as much as 2 g. The extract is stored in an airtight container and is given silica gel.

Laportea decumana (Robx) wedd used ethanol 96% solvent in the extraction process to dissolve polar and non-polar substances. Ethanol can dissolve the active substances found in extracts in the form of tannins, saponins, glycosides, flavonoids, triterpenoids, and phenols [18].

#### Fractionation

As much as, 1 g of *Laportea decumana* (Robx) wedd extract ethanol 96% is partitioned by the liquid method by adding n-hexane solution as much as 50 mL and obtained n-hexane compound as a compound much as 0.45 g.

# Identification of bioactive compound Laportea decumana (Robx) wedd

Phytochemical tests are performed using the TLC method and tube tests. As presented in Table 1.

Table 1: Phytochemical test *Laportea decumana* extract (Robx) Wedd with thin-layer chromatography method

Serial	Type of	Reagents	Test results	Information
number	testing	riougonio	rootrootato	
1	Alkaloid	Dragendorff	+ (positive)	Discoloration to reddish-orange
2	Flavonoid	AICI, 10%	+ (positive)	Discoloration to greenish-yellow
3	Tannin	FeCl <sub>3</sub> 5%	+ (positive)	Color change to blue
4	Triterpenoid	H,SŎ, 10%	+ (positive)	Discoloration to brownish pink
5	Saponin	2 4	+ (positive)	Formed foam that is quite permanent

This study showed that *Laportea decumana* (Robx) wedd from Ambon-Maluku contains alkaloids, flavonoids, tannins, triterpenoids, and saponins. This is supported by the previous research, which explains that *Laportea decumana* (Robx) wedd composition saponins, tannins, polyphenols, glycosides, flavonoids, steroids/triterpenoids, and alkaloids [10], [19].

Laportea decumana is a traditional medicine widely used by the people of Indonesia, especially the Maluku community, which is used as an anti-fatigue, analgesic, antibacterial, cytotoxic, and antioxidant [8], [10], [20], [21].

#### Antioxidant test

An extract can be done in several ways in antioxidant testing, such as the DPPH test, ABTS test, RP test, and FRAP test. Of the several test methods above, DPPH has high sensitivity [14].

Measurement of antioxidant activity is done by spectrometry at a wavelength of 516.35 nm. The DPPH method is assessed based on the decrease in absorbance caused by the change in color of the DPPH solution. This change occurs due to the reaction of DPPH solution with hydrogen atoms from free radical damping compounds that make up stable hydrazine DPPH. This color changes from purple to yellow with the intensity according to the antioxidant ability of the compound.

Observations were made on three samples: LDrW-EE, LDrW-NHE, and Vitamin C as a comparison. Concentrations in LDrW-EE and LDrW-NHE are 5, 10, 20, 40, and 80  $\mu$ g/ml, while in Vitamin C is 2, 4, 6, 8, and 10  $\mu$ g/ml. In Table 2, it can be obtained that any 100% increase in LDrW-EE concentration will lead to an average decrease in absorbance values of 0.170 with an increase in average inhibition ability of 14.02%.

Table 2: Absorbance measurement results *Laportea decumana* (Robx) wedd ethanol extract

Concentration (µg/ml)	Absorbance	Percentage inhibition
5	0.897	26.14
10	0.702	42.25
20	0.528	56.51
40	0.364	70.07
80	0.216	82.22

In Table 3, it can be obtained that any 100% increase in LDrW-NHE concentration will lead to an average decrease in absorbance values of 0.185 with an increase in average inhibition ability of 15.20%.

Table 3: Absorbance measurement results *Laportea decumana* (Robx) wedd extract N-hexane

Concentration (µg/ml)	Absorbance	Percentage inhibition
5	1.024	15.71
10	0.937	22.85
20	0.84	30.86
40	0.609	49.84
80	0.285	76.49

In Table 4, it can be obtained that any increase of 2  $\mu$ g/ml of Vitamin C concentration will lead to an average decrease in absorbance value of 0.143 with an increase in average inhibition ability of 11.76%.

The results of the DPPH test in this study showed that LDrW-EE (22.81  $\mu$ g/ml) has better antioxidant capabilities than LDrW-NHE (44.59  $\mu$ g/ml). The results of this study are different from the results of the previous studies, which explained that the antioxidant ability of the intact compound (90.31  $\mu$ g/ml) and the polar fraction (34.61  $\mu$ g/ml) was higher than that of the non-polar compound (208.76  $\mu$ g/ml) [10].

Table 4: Absorbance measurement results of Vitamin C

Concentration (µg/ml)	Absorbance	Percentage inhibition
2	0.899	25.96
4	0.738	39.28
6	0.616	49.26
8	0.468	61.47
10	0.327	73.06

Two factors affect antioxidant activity, namely, the type of solvent and temperature [22]. Hestiningtyas research (2019) used methanol solvent, while this study used 96% ethanol. Non-polar solvents are commonly used to remove polyphenols from water; ethyl acetate solvents; and diethyl ethers extract lower molecular weight phenols, while ethanol and water are widely used for health reasons, and their abundant amounts are [22]. On extraction using an aquadest solvent, Laportea decumana (Robx) wedd extract had a chelation capacity of 6.50 times Fe<sup>+2</sup> ion when compared to Vitamin C at the same rate. Still, in this study, the IC50 value in Vitamin C (6.03µg/ml) is more potent than Laportea decumana (Robx) wedd extract. However, there are no binding rules about the use of solvent types in the maceration process; it is necessary to test each plant to find out the suitable solvent in each plant because, in addition to the two factors above, the demographic location of the plant also affects the content of compounds contained in it [7], [23].

#### **Determination of IC50**

Based on Figure 1, it can be obtained the linear regression equation LDrW-EE Y = 0.6642X + 34.847, LDrW-NHE Y = 0.7981X + 14.41 and Vitamin C Y =5.8195X + 14.889. The IC50 value of each sample is determined based on the results of linear regression calculations obtained. The calculation results were obtained by LDrW-EE 22.81 µg/ml, LDrW-NHE 44.59 µg/ml, and Vitamin C 6.03 µg/ml, as in Table 5. The IC50 value is powerful if the value is < 50 µg/ml; it is said to be strong if 50–100 µg/ml and is said to be weak if 150–200 µg/ml [24].

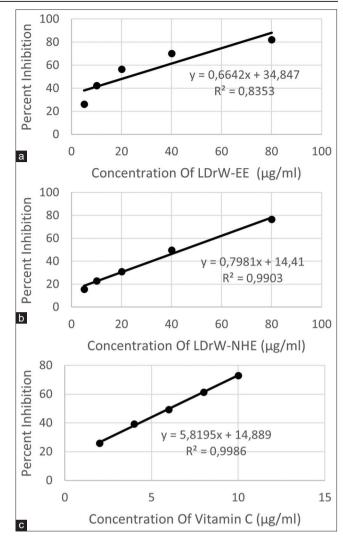


Figure 1: Association of concentration with percent inhibition: (a) Laportea decumana (Robx) wedd Ethanol Extract (LDrW-EE), (b) Laportea decumana (Robx) wedd n-Hexane Extract (LDrW-NHE), and (c) Vitamin C

The average value of absorbance and inhibition capability in LDrW-EE is compared to LDrW-NHE as presented in Tables 2 and 3. In that case, it can be seen if the ability of LDrW-NHE is better than the LDrW-EE, this is in line with the value of  $R^2$  in Figure 1, which is the value of  $R^2$  in the LDrW-EE of 0.8353, while in LDrW-NHE, the value of  $R^2$  of 0.9903 is very close to the value of  $R^2$  Vitamin C which is 0.9986. R2 is a combination of independent variables that jointly affect the value of the dependent variable. In this case, the relationship between the value 1 [25].

 Table 5: Laportea decumana (Robx) wedd antioxidant test

 method 2,2- diphenyl-1-picrilhydrazil

Sample	IC <sub>50</sub> (μg/ml)	Antioxidant power	
LDrW-EE	22.81	Powerful	
LDrW-NHE	44.59	Powerful	
Vitamin C	6.03	Powerful	

LDrW-EE: Laportea decumana (Robx) wedd ethanol extract, LDrW-NHE: Laportea decumana (Robx) wedd extract N-hexane These results explain that the antioxidant abilities of LDrW-EE and LDrW-NHE fall into the category of very strong although not as robust as the Vitamin C used as a comparison.

## Conclusion

The study showed that LDrW-EE and LDrW-NHE had a vigorous antioxidant activity of 22.81  $\mu$ g/ml and 44.59 $\mu$ g/ml, and 6.03  $\mu$ g/ml Vitamin C among others. *Laportea decumana* (Robx) wedd from Ambon-Maluku can be developed into complementary nursing therapy because it has strong antioxidants and contains alkaloids, flavonoids, tannins, triterpenoids, and saponins.

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