Antibacterial and Toxicity Activities Itchy Leaves (*Laportea decumana, Roxb. Wedd*) Extract

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Abstract. Itchy leaves or stinging nettle have been used as medicinal plants in various countries even in Indonesia. Papuans use *Laportea decumana* (Roxb) Wedd as traditional medicines for local people as an anti-fatigue, anti-stiff and anti-fatigue drug. The purpose of this study was to determine the antibacterial and toxicity of the *L. decumana* fraction. Samples were taken from Waibron and Serui, Papua Indonesia. The samples were determined at the Center for Biodiversity Development, Papua State University, Manokwari, West Papua. *L. decumana* leaf was made in the powder of simplicia, extracted with ethanol then fractionated using three solvents namely n-hexane, ethyl acetate, and ethanol. Extracts and fractions were tested for antibacterial against bacteria and cytotoxics test. The results showed that the *L. decumana* fraction had activity antibacterial for *E. coli* and *S. aureus* bacteria. The toxicity test showed that fraction n-hexane and ethyl acetate was toxic while ethanol was not toxic.

1. Introduction

Itchy leaves or stinging nettle have been using as traditional medicine by people in several countries in the world as analgesics, antipyretics, anti-oxidants, anti-diabetic, anti-inflammatory, antibacterial, antiandrogenic, antioestrogenic, pre-gestational potential and others [1-6]. IPNI reported that itchy leaves have 163 species [7] and spread in Asia, Africa, Europe [8-12]. The investigated species were *L. aestuans*, *L. ovafolia*, *L. interrupta*, *L. canadensis*, *L. sinuata*, *L. crenulata* and *L. decumana* [13].

In Indonesia, the study of itchy leaves that have been carried out, namely *L. sinuata* has been tested for toxicity to *Aedes aegypti* with LC50 was 724.43 ppm [14]. *Laportea aestuans* leaf was conducted on the ethanolic extract *Laportea aestuans* (ELA) antibacterial activity test and had moderate antibacterial activity against *E. coli*, *S. aureus* and *S.Typhi* [15]. While LC50 of the ELA was 285 μg / mL and weak antioxidant activity with IC50 was 554.30 μg / mL. According to tail flick methods, the dose of maximum of ELA was 1.2 g / kg resulting in a percent analgesic effect 54.70% and 0.6 g / kg presented 39.62% anti-inflammatory activity [13]. The study of *L. decumana* showed positive phytochemical screening results containing secondary metabolites of flavonoid, tannin, and steroid groups while negative alkaloids [16]. Pharmacognostic data and ash content have been carried out [17]. Extraction with hexane and methanol solutions had good antibacterial activity [18]. Ethanol extract *L.decumana* had antibacterial and toxic activity. Phylogenetic DNA determination was also carried out. The nucleotide sequence of gene Rbcl *Laportea* origin Nabire and Manokwari were successfully determined the sequence with a total length reaching 552 base pairs. The phylogenetic tree analysis showed that samples at 1 in the same haplo type and clade sequences of the genera *Dendrocride spp*, *Discocnide*, and *Laportea*, while the sample at 2 in the same haplotype clade with *Laportea interupta*, *Laportea ruderalisand* Urera sp [19]. Some research had investigated formulation and effectiveness of ointment itch leaf [20].
Figure 1. Itchy leaves [Laportea decumana (Roxb) Wedd]

Itchy leaves, especially the *L. decumana* species, are widely used by the Indonesia Papuans as anti-pain and anti-stiff (Figure 1). This plant is a plant where the shape of the leaves is serrated and has fine hairs along the leaves and stems. These plants are widely used ethnopharmacologically by the seven indigenous Papuan territories (Mamta, Saereri, Domberai, Bomberai, Anim Ha, La Pago, and Meepago) as traditional anti-tired and stiff medicine. Especially the Biak, Wamena, Sentani, and others use this plant to overcome health complaints pain relief as such as pain, stiffness, stomach ache, and effective fatigue [20]. This plant is very effective because it has weapons in the form of hair or stiff hairs (trichomes) which are believed to be hereditary when affixed to parts of the body that are sore, stiff, stiff, pain will get well soon. So far the research on pharmacological activity is still limited to ethanol extract alone. The test for the fraction is still not available, so this study tested the antibacterial activity and toxicity.

2. Methods and procedure

2.1 Plant material

The leaves of *L. decumana* was obtained from Waibron West Sentani and Yapen island, Jayapura District, Provinsi Papua. The leaves were determined at the Herbarium Universitas Negeri Papua- West Papua Indonesia. The leaves were sun dried and then dried in oven at 50°C. The dried leaves (simplicia) were mashed and made into powder.

2.2 Preparation of plant extract (as crude extract) and fraction

Simplicia were weighed as 500 grams, mixed in 2000 mL 96% ethanol then stirred occasionally. Maceration process were repeated 3 times for 3 days. After concentrated ethanolic extract was obtained, the extract was fractionated using n-hexane. Then fraction was evaporated with rotavop and weighed as recovery. This method also was conducted with same solution ethyl acetate, and next with ethanol.

2.3 Antibacterial activities by agar diffusion

Antibacterial activity testing of ethanol extract of itchy leaves was carried out on *E. coli*, and *S. aureus* bacteria with disc diffusion method. The concentration of ethanol extract test used was 100, 250, 500, 750, and 1000 ppm. Bacterial suspension that has been made, rubbed on agar media. Sterile disc paper was dripped with a 10 µL test solution (extract and fraction) and then allowed for a while. Furthermore, it was placed on the surface, used ciprofloxacin as a positive control, and aquabidest as negative control. The media was then incubated at 37°C for 1x24 hours. Antibacterial activity was shown with the formation of clear zone around the disc paper [21, 22]. The diameter of the inhibitory zone was measured in millimeters (mm) using a caliper with five treatments with four repetitions.
five treatments in question are 4 variations of concentration and positive control. And the level activity antibacterial was shown in Table 1.

| Diameter inhibitory zone of bacteria (mm) | Activity   |
|-------------------------------------------|------------|
| <5 mm                                     | weak       |
| 5-10mm                                    | medium     |
| 10-20mm                                   | strong     |
| >20mm                                     | very strong|

2.4 Toxicity activities by Brine shrimp test

BSLT (Brine Shrimp Lethality Test) was conducted by weighing 1 gram of *Artemia salina* eggs hatched in 3000 mL of sea water for 24 to 48 hours. Then feed as much as one drop of yeast with a concentration of 3 mg in 5 mL of sea water. After two days, *A. salina* eggs will hatch into naupili or *A. salina* larvae and be used for toxicity tests for LC$_{50}$ [23]. The temperature for larvae was between 25 to 28 °C, while for salinity ranges from 10 to 15 ppm, while for pH was alkaline was 8 to 9. The extract and every fraction was weighed and dissolved first, so that the concentration was 10, 50, 100, 250, 500, 750, and 1000 ppm.

From every concentration that has been made, 1 ml was put into vial, evaporated, added with sea water to a volume of 5 mL, and then dropped 10 larvae. The larvae were used 48 hours after hatching. The number of dead larvae was calculated after 24 hours and repeated 3 times [24]. For the percentage of larval mortality can be calculated by the following formula:

\[
\text{Percents of deaths} = \frac{\text{number of dead larvae}}{\text{total of larvae}} \times 100\%
\]

From the results of the study were primary data obtained from *A. Salina* larvae which died after treatment at each concentration of itchy leaf extract. Data were analyzed by probit analysis to determine the LC$_{50}$ with 95% confidence interval. The LC$_{50}$ is the concentration needed to kill 50% of shrimp larvae *A. salina* Leach. If the LC$_{50}$ is <30 ppm then the extract is very toxic and has the potential to contain anticancer active compounds, Harbone mentioned the level of toxicity of an extract as follows [25] Table 2.

| Toxinity     | Concentration (ppm)       |
|--------------|---------------------------|
| Very Toxic   | LC$_{50}$ ≤ 30 ppm        |
| Toxic        | 31 ppm ≤ LC$_{50}$ ≤ 1000 ppm |
| Not toxic    | LC$_{50}$ > 1000 ppm      |

3. Results

3.1 Antibacterial Test of Hexane, Ethyl Acetate, and Ethanol Fractions.

The sample was dried and mashed into simplicia to reduce the water content in the plant [25]. The grinding process into a powder simplicia will make all of the active compounds that contained in each part of the plant completely dissolved with solvent. The extraction process depended on the texture and water content of the extracted plant material [25].
**Table 3.** Result of Extract And Fraction Itchy Leaves (*Laportea decumana* (Roxb) Wedd).

| Part of plant | Extract | F. n-hexane | F. ethyl acetate | F. ethanol |
|---------------|---------|-------------|------------------|-----------|
| Leaves *L. decumana* | 5.937 gr | 1.187 % | 1.389 gr | 23.396 % | 1.079 gr | 18.174 % | 3.131 gr | 52.737 % |

The recovery of ethanol extract can be seen in Table 3 that showed total extract was weighed 5.937 g (1.187%) from 500 g of initial simplicia. From fractionation showed that the secondary metabolites contained in itchy leaf plants were commonly found in polar solvent (52.737%). Based on Table 1 the results of fractionation obtained from itchy leaf extract with n-hexane solvent was 1.389 gr (23.396%), ethyl acetate solvent was 1.079 gr (18.174%), and ethanol solvent was 3.131 gr (52.737%). This showed that itchy leaf extract had potential secondary metabolit in polar compounds (ethanol fraction), followed semi-polar and non-polar compounds.

**Figure 2.** The results of antibacterial activity test of itchy leaf extract ethanol against *E.coli* bacteria

**Figure 3.** The results of antibacterial activity test of itchy leaf n-hexane fraction against *E.coli* bacteria

**Figure 4.** The results of antibacterial activity test of itchy leaf ethyl acetate fraction against *E.coli* bacteria
Figure 5. The results of antibacterial activity test of itchy leaf ethanol fraction against *E. coli* bacteria

Figure 6. Results of measurement of inhibitory zones of Ciprofloxacin for *E. coli* and *S. aureus*

In Table 4, inhibitory zones against *E. coli* bacteria had medium category. They were ethanol extract and fraction in 100 ppm was 8-9 mm (Figure 2-5). However, the measurement results of inhibitory zones against *S. aureus* bacteria n-hexane fraction at a concentration of 500 ppm (11 mm) and ethanol (10 mm) was categorized as strong activity. Ethyl acetate fraction belong to the medium category (Figure 7-10).

Figure 7. The results of antibacterial activity test of itchy leaf extract ethanol against *S. aureus* bacteria

Figure 8. The results of antibacterial activity test of itchy leaf n-hexane fraction against *S. aureus* bacteria
Figure 9. The results of antibacterial activity test of itchy leaf ethyl acetate fraction against *S. aureus* bacteria

Figure 10. The results of antibacterial activity test of itchy leaf ethanol fraction against *S. aureus* bacteria

The positive control that used in this antibacterial activity test was ciprofloxacin. Ciprofloxacin is a broad-spectrum antibiotic that can inhibit gram-positive and gram-negative bacteria. Ciprofloxacin is the class of fluoroquinolones which acts as bactericides in inhibiting DNA gyrase in bacteria [27]. The inhibition zone of positive control was shown in Figure 6.

| Table 4. Results Measurement of Inhibitory Zones for *E. coli* and *S. aureus* |
|---------------------------------------------------------------|
| **Doses (μg/mL)** | **The average diameter of the inhibitory zone (mm) to** | **E. coli** | **S. aureus** |
| Dose | Extract | N-hexane | Ethyl acetate | Ethanol | Extract | N-hexane | Ethyl acetate | Ethanol |
|------|--------|----------|-------------|--------|--------|----------|-------------|--------|
| 100  | 8      | 9        | 9           | 8      | 7      | 9         | 9           | 9      |
| 250  | 8      | 9        | 9           | 7      | 8      | 10        | 9           | 9      |
| 500  | 7      | 8        | 9           | 8      | 7      | 11        | 9           | 10     |
| 750  | 7      | 8        | 9           | 8      | 8      | 10        | 9           | 9      |
| 1000 | 8      | 7        | 9           | 8      | 8      | 10        | 9           | 9      |
| C (+) | 34    | 34       | 34          | 34     | 27     | 27        | 27          | 27     |
| C (-) | 0     | 0        | 0           | 0      | 0      | 0         | 0           | 0      |

The presence of antibacterial activity of ethanol extract and n-hexane, ethyl acetate, also ethanol fraction of *L. decumana* against *E. coli* and *S. aureus* (Table 3 and 4) bacteria can be caused by secondary metabolites contained in *L. decumana* such as group compounds flavonoids, tannins, and steroid [16]. The results of this study are supported by previous studies which ethanol extract of itchy leaves (*L. aestuans*) was able to inhibit *E. coli* bacteria (8.55 mm), *S. typhi* (9.02 mm), and *S. aureus* bacteria.
In the presence of tannins and steroids can increase antibacterial activity [28]. Flavonoid compounds have a tendency to bind proteins that can interfere with metabolic processes by damaging bacterial cell membranes, deactivating enzymes, binding to adhesin and damaging cell membranes [29,30].

Honestly significance difference (HSD) analysis test on the diameter of inhibitory areas of E. coli and S. aureus bacteria in Table 3 showed significant differences in positive control and fifth concentration of ethanol extract and n-hexane, ethyl acetate, ethanol fraction. Positive control showed significant differences in the HSD test, because it produced the greatest antibacterial activity against the test bacteria compared to the five concentrations of ethanol extract and n-hexane, ethyl acetate, ethanol-water fraction. Thus it can be said that the HSD_{0.05} and HSD_{0.01} tests at concentrations of 100 ppm, 250 ppm, and 500 ppm, 750 ppm and 1000 ppm have medium inhibition zone against E. coli and S. aureus bacteria, and there were differences significant with other concentrations.

3.2 Toxicity Test of n-hexane, ethyl acetate and ethanol

Toxicity testing of n-hexane fraction, ethyl acetate and ethanol was carried out three times replication (Table 5). The three fractions obtained were tested for toxicity using the BSLT (Brine Shrimp Lethality Test) method with concentrations of 10, 50, 100, 250, 500, 750, 1000 ppm. The results showed that LC_{50} n-hexane fraction had the most toxic effect on shrimp larvae A. salina with LC_{50} at101.64 μg / ml (toxic), ethyl acetate with LC_{50} at 293.69 μg /ml (toxic), ethanol fraction at 6835 μg/ml (non-toxic).

Based on the results of BSLT testing, it showed that fraction of L. decumana at various levels of concentration gave increasing the death of larvae. The higher concentration of each fraction gave the higher mortality rate of larvae [33]. The highest mortality concentration was shown at 1000 ppm concentrations where almost all test animals died. This proved that the death of larvae was pure because of the effect of extract. Meyer et al., (1982) have reported that an extract showed the toxicity activity in BSLT if the extract can cause the death of 50% of test animals in a concentration test of less than 1000 ppm [24]. Ethyl acetate and n-hexane of L. decumana on larvae which has LC_{50} value was toxic and can be continued in research using cancer cell culture.

| Concentration (ppm) | Percentage of death | LC_{50} (ppm) |
|---------------------|---------------------|---------------|
|                     | N-hexane            | Ethyl acetate | Ethanol       |
| 10                  | 27                  | 13            | 3             |
| 50                  | 37                  | 20            | 7             |
| 100                 | 23                  | 17            | 10            |
| 250                 | 57                  | 27            | 10            |
| 500                 | 73                  | 53            | 17            |
| 750                 | 90                  | 63            | 20            |
| 1000                | 93                  | 93            | 47            |
|                     | 145.45 (toxic)      | 542.68 (toxic)| 6835.96 (non-toxic) |

4. Discussion

The difference in inhibitory zone diameter between E. coli and S. aureus bacteria was due to the difference in the speed of the extract diffusing to the agar medium. Another factor that causes the difference in diameter of the inhibitory zone of the extract was the difference in the concentration of the active compound contained in the extract. The size of the inhibitory zone was influenced by several things, such as the sensitivity level of the test organism, the speed of diffusion of antibacterial
compounds and the concentration of antibacterial compounds [26]. The results of antibacterial activity ethanol extract and fraction itchy to E.coli and S. aureus bacteria can be seen in the Table 4.

The mechanism of tannins as antibacterial are precipitated protein by reaction with cell membranes, enzyme inactivation and inactivation of genetic material functions. Tannins inhibit the reverse transcriptase and topoisomerase DNA enzymes so that bacterial cells can not form. Tannins has antibacterial activity which is related to its ability to activate adhesion of microbial cells, activate enzymes, and interfere with protein transport in the inner layer of cells. Tannins also have a target on polypeptides so that the formation becomes is not form. The worst formation make cells lysis due to osmotic or physical pressure then bacterial cells will die. Also, complexity of iron ions with tannins can explain tannin toxicity. Microorganisms that grow under aerobic conditions require iron for a variety of functions, including reduction of ribonucleotide DNA precursors. Reverse transcriptase enzymes and bacterial cell topoisomerase DNA can not be formed by strong iron binding capacity by tannins [31].

The mechanism of steroids as an antibacterial is related to lipid membrane and sensitivity to steroid components which cause leakage of liposomes. Steroids can interact with membrane phospholipid cells which are permeable to lipophilic compounds, causing membrane integrity to decrease and morphology of cell membranes which cause fragile cells and lysis [33].

The toxicity test with the BSLT method is an acute toxicity test in which the toxic effects of a compound are determined in 24 hours after the administration of the test dose [24]. The BSLT toxicity test was carried out by determining the LC$_{50}$ value of the active component activity of the plant against A. salina larvae. LC$_{50}$ (Lethal Concentration 50) is a concentration of substances that can cause death in 50% of A. salina shrimp larvae as test animals. The results of the analysis carried out by determining the LC$_{50}$ value of the ethanol extract of itchy leaves (L.decumana) indicated that it had a toxic effect on A. salina larvae. An extract is toxic based on the BSLT method if the extract can cause the death of 50% of the test animals at concentrations of less than 1000 ppm [24]. This method is often used to determine the toxicity of natural ingredients / plant extracts and to screen anticancer compounds because of the positive correlation.

Phytochemical screening results showed that itchy leaves contained flavonoid, tannins, steroids compounds which are known to function as antioxidants that is very good for cancer prevention. The effects of consumption of flavonoids made anti-inflammatory, anti-allergic, antimicrobial, hepatoprotective, antiviral, antithrombotic, cardioprotective, capillary strengthening, antidiabetic, anti-cancer and antineoplastic effects [34]. Flavonoids obtain active components to treat liver dysfunction and possibly as antimicrobial and antiviral. The mechanism of flavonoids as anticancer there are several theories. (1) Flavonoids as antioxidants has activation mechanisms apoptosis pathway of cancer cells. The mechanism is the result of DNA fragmentation that begins releasing of the proximal DNA chain by oxygen compounds reactive like hydroxyl radicals. This compound is formed from the redox Cu (II) reaction. This copper compound is mobilized by both flavonoids from extra cells or intra cells, especially from chromatin. (2) Flavonoids are inhibitors of tumor / cancer proliferation, one of which is by inhibiting the activity of protein kinases, thereby inhibiting the signal transduction pathway from the cell membrane to the cell nucleus. (3) The inhibiting receptor tyrosine kinase activity increase tyrosine kinases play a role in malignant growth [35].

Tannins naturally precipitate proteins. Tanin and steroid are procarcinogenic or anticarcinogenic and both mutagenic or antimutagenic. However, except for such extreme cases betel quid chewing, which increases mitochondrial DNA accumulation (mtDNA) removal of oral cells [36].

5. Conclusion

The results showed that the L. decumana fraction had activity antibacterial for E. coli and S. aureus bacteria. That fraction was more effective as antibacterial for S. aureus bacteria. That were on average in strong inhibitory zone with the best antibacterial activity using n-hexane fraction at 500 ppm (11mm). The toxicity test showed that fraction n-hexane and ethyl acetate was toxic while
ethanol was not toxic. LC$_{50}$ of n-hexane fraction was 145.45 ppm (toxic), and ethyl acetate fraction with LC$_{50}$ values of 542.68 ppm (toxic) and ethanol fraction (not toxic) with LC$_{50}$ values of 6835.96 ppm.

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