

# The Potential of Katuk Hutan (*Phyllanthus reticulatus*) as a Medicinal Plant

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## ABSTRACT

Katuk Hutan (*Phyllanthus reticulatus*) is a pioneer plant that grows in Papua. This plant belongs to the Phyllanthaceae family, which is commonly used as a medicinal plant. This study aimed to determine the potential of katuk hutan (*P. reticulatus*) as a medicinal plant. The method used in phytochemical testing. A colour-changing test did a phytochemical analysis. Antioxidant activity was tested by DPPH radical rinsing activity mechanism method, and antimicrobial testing was used the diffusion method using *Propionibacterium acnes*, *Streptococcus sobrinus*, *Escherichia coli* and *Candida albicans* fungi. To test the toxicity of *P. reticulatus* leaves using the Brine Shrimp Lethality Test (BLST) method. *P. reticulatus* with a yield of 12.48-27.60%, containing alkaloids, flavonoids, steroids, saponins, tannins, triterpenoids, carotenoids, coumarin; and is a powerful antioxidant with an IC value of <50. *P. reticulatus* can inhibit *P. acnes*, *E. coli* and *C. albicans* fungi and cannot inhibit *S. sobrinus* bacteria. The leaves of *P. reticulatus* have a very high toxicity potential as a cancer drug.

**Keywords:** *Phyllanthus reticulatus*, Antioxidant, Bacteria and Fungi, Toxicity, Medicinal Plant

## 1. INTRODUCTION

Secondary metabolism is a compound resulting from the biogenesis of primary metabolism. In the secondary metabolite process in a plant, plants can produce toxic compounds and can be used to treat various types of diseases in humans. Secondary metabolite compounds are alkaloids, flavonoids, saponins, tannins, steroids and triterpenoids [1]. Phytochemicals or the content of bioactive compounds found in plants can provide health to the human body.

Researchers often carry out various studies on medicinal plants to determine the potential of different medicinal plants. This research is widely carried out because currently, there are still many medicinal plants whose potential is unknown, and there is no scientific study of these medicinal plants.

Katuk hutan (*Phyllanthus reticulatus*) is thought to have potential as medicinal plants. This is known from the local knowledge of the Papuan people who use the forest katuk plant as traditional medicine, which effectively treats various types of diseases, including breast cancer, uterine cancer, myoma, and cysts, asthma,

and anti-allergies. Therefore, it is necessary to do scientific research on the katuk hutan (*P. reticulatus*).

## 2. MATERIALS AND METHODS

### 2.1. Extraction

The sample was collected from the area of Pantura District and West Manokwari District, Manokwari Regency. Maceration of the extraction method was applied by using methanol. Samples that were put into the maceration process were varies based on what collected from the field. The samples were dissolved in methanol with comparison 1:4. This process took up to 3 days. After that, the samples were stained by using the Büchner funnel. The maceration product was then concentrated or steamed using a rotary vacuum evaporator at a temperature of 30 – 40<sup>0</sup>C. The next step has stored the product in a vacuum oven until crude extract is obtained.

### 2.2. Chemist Component Testing

Phytochemical analysis was done by the colour-changing test that was referring to Sofowora (saponin),

Harborne (terpenoid and steroid), Senthilmurugan (coumarin and carotenoid) and Kokate (alkaloid, flavonoid and tannin) [1-4].

**2.3. Antioxidant**

The method was used to test antioxidant [5]. This test uses a UV-Vis spectrophotometer at room temperature (25<sup>0</sup> C) with a wavelength of 514nm and uses DPPH solution (1,1-diphenyl-2picrylhydrazyl). Antioxidant activity was determined through decolorizing of DPPH using a wavelength of 514 nm that customs UV-Vis spectrophotometer. On this test, Vitamins C used as a positive control.

**2.4. Antimicrobial**

*Propionibacterium acnes*, *Escherichia coli*, *Streptococcus sobrinus*, and *Candida albicans fungus* are microbial types used as microorganism for testing. The diffusion Jelly well method was used for testing the antimicrobial activity. This method referred to [6]. The well that was made by using cork borer on each medium contained 20 µL of the sample with acetone as a negative control, chloramphenicol as a positive control, and extract with a concentration of 125 µg/well, 250 µg/well, and 500 µg/well. If there was a clear zone around the well, there was an antimicrobial inhibition activity by plant extract.

**2.5. Brine Shrimp Lethality Test**

Toxicity analysis was done by BLST [7]. Some amount of shrimp eggs was put into a beaker glass 1000 ml contained seawater. In making the primary solution, it needed 30 mg rough extract put into 3 ml ethanol solution and mixed until homogeny so that it had 10.000 ppm as a start concentration. The testing was done with one concentration by taking 500 µL of the primary solution and added it with seawater up to 5 ml so that the final concentration was 1000 ppm, and did it again until three times. On this test, seawater used as a negative control, and *Gallic acid* used as a positive control.

The next step was from each of the solutions above need to be laid off to evaporate. This process could be about a day or so. Each of the dried samples was dissolved into 2 ml of seawater. If some extract was difficult to dissolve, then added with a maximum of 10% of DMSO out of the total solution. Put one day of *A. salina* (nauplii) into vials, and each vial consists of ten nauplii. Then, the small bottles containing extract and nauplii were stored in a place with enough lights for 24 hours. From there, then artemia was counted to see how many were alive and dead. The compound was called toxic if it had LC50 (concentrate that could kill 50% of shrimp larva).

**2.6. Data Analysis**

All the data collected was demonstrated in tables. The results of the tests were compared toward positive control and negative control in each of testing due to the potential of bioactivity found in types of rough extract plants.

**3. RESULT AND DISCUSSION**

**3.1. Rendemen**

The community uses Katuk hutan (*P. reticulatus*) from Pantura District and West Manokwari District as a medicine for asthma, allergies, myoma, cysts, breast cancer and heat. The parts used as medicine are the leaves and skin.

**Table 1.** Sample of Katuk Hutan (*Phyllanthus reticulatus*) from several locations

No	Species	Part of tree	Type of disease
1	<i>Phyllanthus reticulatus var. glaber</i>	leave	asthma, allergies, myoma, cysts, breast cancer
2	<i>Phyllanthus reticulatus var. reticulatus</i>	leave	asthma, allergies, myoma, cysts, breast cancer
3	<i>Phyllanthus reticulatus var. reticulatus</i>	bark	fever
4	<i>Phyllanthus reticulatus var. glaber</i>	leave	asthma, allergies, myoma, cysts, breast cancer
5	<i>Phyllanthus reticulatus var. reticulatus</i>	leave	asthma, allergies, myoma, cysts, breast cancer

The yield of forest katuk (*P. reticulatus*) is 12.48% - 27.60% (Table 2), the type of solvent much influences this yield. Following the result's opinion depending on the type of solvent used, the more polar the solvent, the higher the product produced [8]. The amount of yield is also influenced by the maceration process's length, the prolonged contact with the solvent, more compounds dissolved [11].

**Table 2.** The yield of Katuk Hutan (*Phyllanthus reticulatus*) extract

No	Species	Part of use	Rendemen (%)
1	<i>Phyllanthus reticulatus</i> var. <i>glaber</i>	leave	27,60
2	<i>Phyllanthus reticulatus</i> var. <i>reticulatus</i>	leave	12,48
3	<i>Phyllanthus reticulatus</i> var. <i>reticulatus</i>	bark	17,65
4	<i>Phyllanthus reticulatus</i> var. <i>glaber</i>	leave	27,16
5	<i>Phyllanthus reticulatus</i> var. <i>reticulatus</i>	leave	17,08

### 3.2. Phytochemical Analysis

Katuk Hutan (*P. reticulatus*) contain flavonoids, saponins, tannins, triterpenoids, steroids, keratinoid and coumarin (Table 3).

**Table 3.** Phytochemical result of medical plants species

No	Species	Plant's Part	Phytochemical compound
1.	<i>Phyllanthus reticulatus</i> var. <i>glaber</i>	L	Fla, Sap, Tan
2.	<i>Phyllanthus reticulatus</i> var. <i>reticulatus</i>	L	Tan, Tri
3.	<i>Phyllanthus reticulatus</i> var. <i>reticulatus</i>	B	Flav, Tan, Car
4.	<i>Phyllanthus reticulatus</i> var. <i>glaber</i>	L	Tan, Ste, Car, Cou
5.	<i>Phyllanthus reticulatus</i> var. <i>reticulatus</i>	L	Fla, Tan, Car

**Alk:** alkaloid, **Fla:** flavonoid, **Sap:** saponine, **Tan:** tannin, **Tri:** triterpenoid, **Ste:** steroid, **Car:** carotenoid, **Cou:** coumarin, **L:** Leaves, **B:** Bark, **R:** Roots

Flavonoids in plants generally function as growth regulators, photosynthesis regulators, antimicrobials and

antivirus. Flavonoids, as natural antioxidants, can change or reduce free radicals and as anti-free radicals. Flavonoid compounds are compounds that have a significant role in the field of proven medicine [12]. Furthermore, he said, based on flavonoid compounds have been widely researched, and even some compounds have been produced as anti-cancer, anti-viral, anti-allergic and anti-cholesterol. Saponins are natural glycosides that are bound to steroids or triterpene. Saponins have broad pharmacological activities, including immune- modulators, anti-tumour, anti-inflammatory, anti-viral, anti-fungal, can kill shellfish. Saponins increase the ciliated epithelium activity, an event that triggers a cough to expel phlegm, while the local irritant effect of saponins can cause sneezing. Steroids found in nature are derived from triterpenoids, triterpenoids and steroids, both structurally and biogenetically, and there is no simple definition to differentiate them [27]. Carotenoids provide a significant contribution to various life sectors, especially as a source of vitamin A which is beneficial for the visual organs, food colouring, food additives, red blood cell enhancers, antioxidants, anti-bacterial, enhancing immunity, and replacements for damaged cells [15]. Coumarin compounds and their derivatives have many biological activities, including blood anticoagulants, antibiotics and some that show activity to inhibit carcinogenic effects. In addition, coumarin is also used as a base for making perfumes and as a fluorescent ingredient in the textile and paper industry [20]. The function of tannins is very complex, ranging from protein to metal inhibitor; in addition to the role of tannin antioxidants in medicine, it functions as a diarrhoea treatment, hemostatic (to stop bleeding).

### 3.3. Antioxidant

The antioxidant activity test using the DPPH method showed that the *P. reticulatus* extract had an IC50 of 25-47. This indicates that the extract has powerful antioxidant activity because it has an IC50 of less than 50 (Table 4) [9].

**Table 4.** Antioxidant activity based on IC50 [9]

Value of IC50	Antioxidant Activity
< 50	Very strong
50 – 100	Strong
100 – 150	medium
151 – 200	weak

The test results can be seen in Table 5. Compared with the antioxidant activity of vitamin C, which has a value of IC50 15, the antioxidant activity of *P. reticulatus* extract is still lower. But in this study, it was still in the form of crude extracts, so there was still a possibility that the pure compounds contained had more

robust free radical scavenging activity than the extracts. The DPPH method was chosen because it is simple, easy, fast and sensitive and only requires a small sample. Antioxidant compounds will react with DPPH radicals through the hydrogen atom donation mechanism and cause colour decay [10].

The phytochemical analysis results indicate that the samples tested have the potential to be developed as traditional medicines because they have secondary metabolites that support disease healing. The content of flavonoids is known to have anti-cancer, anti-viral, antioxidant, anti-bacterial and anti-inflammatory agents. Coumarins have also been shown to play an essential role in many useful applications including, antioxidant, analgesic, anti-cancer, anti-HIV, anti-inflammatory, antibiotic, anticoagulant and anti-tumour [17].

**Table 5.** Antioxidants of Katuk Hutan (*Phyllanthus reticulatus*)

No.	Species	% DPPH	Part of use	IC50
1	Vitamin C	94.36		15
2	<i>Phyllanthus reticulatus</i> var. <i>glaber</i>	70.45	leave	< 25
3	<i>Phyllanthus reticulatus</i> var. <i>reticulatus</i>	66.23	leave	< 25
4	<i>Phyllanthus reticulatus</i> var. <i>reticulatus</i>	67.51	bark	< 50
5	<i>Phyllanthus reticulatus</i> var. <i>glaber</i>	73.12	leave	< 25
6	<i>Phyllanthus reticulatus</i> var. <i>reticulatus</i>	67.51	leave	< 25

### 3.4. Anti-Bacterial and Fungal

Katuk hutan (*P. reticulatus*) originating from Susweni village, West Manokwari district, which has moderate inhibition of *Propionibacterium acnes* (5-10 mm), although in general, the phytochemical content of forest katuk has the same components (flavonoids, tannins and carotenoids).

**Table 6.** Anti-bacterial activity of Katuk hutan (*Phyllanthus reticulatus*) extract against *Propionibacterium acnes*

No	Local Name	Species	Part of tree	Level of Resistance (mm)
1	Katuk hutan putih	<i>Phyllanthus reticulatus</i> var. <i>glaber</i>	leave	-
2	Katuk Hutan Merah	<i>Phyllanthus reticulatus</i> var. <i>reticulatus</i>	leave	-
3	Sautrame	<i>Phyllanthus reticulatus</i> var. <i>reticulatus</i>	bark	8,22
4	Katuk Hutan Putih	<i>Phyllanthus reticulatus</i> var. <i>glaber</i>	leave	-
5	Sautrame	<i>Phyllanthus reticulatus</i> var. <i>reticulatus</i>	leave	5,78

Phytochemical compounds in plants will function differently if the parts used and their origin is different and other factors [29]. This study found that study even though it has the same phytochemical compounds but has extra antimicrobial power. For example, the sautrame leaves from Saubeba have antimicrobial properties, while the Red Forest Katuk leaves from Susweni are not antimicrobial, although they both have tannin compounds.

**Table 6.** Level of inhibition of medicinal plants against bacteria and fungi [32]

Resistance value (mm)	Resistance level
> 20	Very strong
10-20	Strong
5-10	Moderate
< 5	weak

The content of flavonoids can inhibit gram-positive bacteria such as *S. aureus* [16]. Types of plants with strong inhibitory power contain secondary metabolites in the form of flavonoids, saponins, tannins and triterpenoids. Tannin has an anti-bacterial activity related to its ability to activate microbial cell adhesion and activate enzymes and interfere with protein

transport in the inner layer of cells [14]. The mechanism of action of flavonoids as anti-bacterial is to form complex compounds with extracellular and dissolved proteins that can damage the bacterial cell membrane and is followed by the release of intracellular compounds [14,23]. Saponins' mechanism of action as anti-bacterial reduces surface tension, resulting in increased permeability of cell leakage and resulting in intracellular compounds to be released [22]. According to [21], these compounds diffuse through the outer membrane and susceptible cell walls, then bind to the cytoplasmic membrane and disrupt and reduce the stability. This causes the cytoplasm to leak out of the cell resulting in cell death. Antimicrobial agents that interfere with the cytoplasmic membrane are bactericidal.

Katuk hutan (*P. reticulatus*) did not have inhibitory power against *S. sobrinus* (Table 7). Medium to strong katuk hutan (*Phyllanthus reticulatus*) had inhibitory control against *Escherichia coli* (Table 8). Based on the phytochemical analysis, this type contains saponins, tannins, triterpenoids, flavonoids, steroids and carotenoids (Table 3). Plants are a source of various kinds of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids etc., which have been found to have antimicrobial properties in vitro [28].

**Table 7.** Anti-bacterial activity of Katuk Hutan *Phyllanthus reticulatus* extract on *Streptococcus sobrinus*

No.	Local Name	Species	Part of tree	Level of Resistance (mm)
1	Katuk hutan putih	<i>Phyllanthus reticulatus var. glaber</i>	leave	-
2	Katuk Hutan Merah	<i>Phyllanthus reticulatus var. reticulatus</i>	leave	-
3	Sautrame	<i>Phyllanthus reticulatus var. reticulatus</i>	bark	-
4	Katuk Hutan Putih	<i>Phyllanthus reticulatus var. glaber</i>	leave	-
5	Sautrame	<i>Phyllanthus reticulatus var. reticulatus</i>	leave	-

Plants rich in various kinds of secondary metabolites such as tannins, alkaloids and flavonoids, which have been found in vitro to have antimicrobial properties, can treat infectious diseases with fewer side effects and reduce toxicity. Saponin compounds in plants and some show the presence of tannins, glycosides and flavonoids. All of these extracts have the potential to anti-bacterial *E. coli*. Furthermore, it is said that the combined form of the four extracts is traditionally used to treat diarrhoea because it is believed that each of them supports its active component for optimal efficacy [30].

**Table 8.** Anti-bacterial activity of Katuk Hutan (*Phyllanthus reticulatus*) extract against *Escherichia coli*

No.	Local Name	Species	Part of tree	Level of Resistance (mm)
1	Katuk hutan putih	<i>Phyllanthus reticulatus var. Glaber</i>	leave	6.30
2	Katuk Hutan Merah	<i>Phyllanthus reticulatus var. reticulatus</i>	leave	-
3	Sautrame	<i>Phyllanthus reticulatus var. reticulatus</i>	bark	9.93
4	Katuk Hutan Putih	<i>Phyllanthus reticulatus var. Glaber</i>	leave	-
5	Sautrame	<i>Phyllanthus reticulatus var. reticulatus</i>	leave	12.81

Meanwhile, the medicinal plant *Trema guineensis*, which contain alkaloid compounds, tannins, saponins, flavonoids and anthraquinones, can inhibit *S. aureus*, *E. coli*, and *S. enteritidis* bacteria with a zone of inhibition [18]. The ethanol extract of *Origanum elongatum* leaves was most effective against *Escherichia coli* with an inhibition zone diameter of 30.33 mm. There was a clear correlation between the level of phenolic compounds in the *O. elongatum* plant and anti-bacterial activity [19].

The higher the extract concentration, the greater the inhibitory power against *Escherichia coli* bacteria. Following the research results [25,31], the higher the concentration of the extract used causes, the higher the number of compounds in the extract. According to the research results of Lestari et al., 2016, Nipah leaves contain polyphenols, flavonoids, triterpenoids/steroids, saponins and alkaloids that have anti-bacterial activity against Gram-negative and positive bacteria. [16] Phytochemical screening of *Baekkea frutescens* L extract showed alkaloids, flavonoids, steroids,

terpenoids, phenols, and carbohydrates. The presence of alkaloids is of interest because of their practical use as antimalarial, analgesic and stimulant—flavonoids are known to prevent tumour growth and are also used to protect against infection.

Katuk Hutan (*P. reticulatus*) had moderate to strong inhibition against *C. albican* (Table 9). Katuk hutan' ability to inhibit the growth of the fungus *C. albicans* is thought to be due to the presence of secondary metabolites. Secondary metabolites' content is thought to inhibit fungi' growth, especially the fungus *C. albicans* [13]. The range of secondary metabolites in plants with moderate to strong inhibition power are flavonoids, tannins, saponins and triterpenoids. Saponins are complex glycoside compounds that contain sugar groups, also have active polar and non-polar groups. Saponins are surfactants that are polar in shape and can be an anti-fungal by reducing the surface tension of the sterol membrane from *C. albicans*' walls to increase permeability. The expanded permeability results in more concentrated intra-cellular fluid being pulled out of the cell so that *Candida albicans* cells die because the cells swell and burst [30].

**Table 9.** Inhibition of *Candida albican* in Katuk Hutan (*Phyllanthus reticulatus*)

No.	Local Name	Species	Part of tree	Level of Resistance (mm)
1	Katuk hutan putih	<i>Phyllanthus reticulatus</i> var. <i>Glaber</i>	leave	-
2	Katuk Hutan Merah	<i>Phyllanthus reticulatus</i> var. <i>reticulatus</i>	leave	-
3	Sautrame	<i>Phyllanthus reticulatus</i> var. <i>reticulatus</i>	bark	11.00
4	Katuk Hutan Putih	<i>Phyllanthus reticulatus</i> var. <i>Glaber</i>	leave	-
5	Sautrame	<i>Phyllanthus reticulatus</i> var. <i>reticulatus</i>	leave	8.19

Plants that have the strongest inhibitory power are the skin of *P. reticulatus* from subeba followed by leaves of *P. reticulatus* from saubeba. Inhibition of the skin of *P. reticulatus* against *C. albican* bacteria 11 mm. The compounds found in the skin are flavonoids, carotenoids and tannins. The inhibition of *Phyllanthus*

*reticulatus* leaves was 8.19 mm and the compounds were flavonoids, carotenoids and tannins.

This result is still better than the results of the study by [24], which found that the zone of growth inhibition of *Candida albicans* was  $9.6 \pm 0.43$  mm in the ethanol extract of *Aloe vera*. The anti-fungal activity of the ethanol extract of *Aloe vera* is due to the presence of tannins and phenolic acid polypropols, quercetin, flavones, flavanols, alkyds, terpenoids, lectins, polypeptides, and complex mixtures. According to [24] the main compounds that can produce inhibitory power in *Candida* strains are tannins (*A. chica* and *R. officinalis*), anthocyanins (*A. chica*), flavonoids (*A. chica* and *R. officinalis*), naphthoquinones (*T. avellanadae*), menthol and menthone (*M. piperita*), diterpenoid casearvestrin (*C. sylvestris*), and terpenoid b-eudesmol (*A. lappa*), which are known to have antimicrobial properties against microorganisms.

### 3.5 Toxicity

The LC50 value of the extract or compound tested for less than 1,000 µg/mL (ppm) is considered to indicate biological activity, so this test can be used as an initial screening for bioactive compounds that are thought to have anti-cancer properties [26].

**Table 10.** Toxicity (LC50) Katuk Hutan (*Phyllanthus reticulatus*) extract

No.	Local Name	Species	Part of tree	IC50
1	Katuk hutan putih	<i>Phyllanthus reticulatus</i> var. <i>Glaber</i>	leave	-
2	Katuk hutan merah	<i>Phyllanthus reticulatus</i> var. <i>reticulatus</i>	leave	≤ 10
3	Sautrame	<i>Phyllanthus reticulatus</i> var. <i>reticulatus</i>	bark	-
4	Katuk hutan putih	<i>Phyllanthus reticulatus</i> var. <i>Glaber</i>	leave	-
5	Sautrame	<i>Phyllanthus reticulatus</i> var. <i>reticulatus</i>	leave	-

The results of the toxicity test for medicinal plant extracts can be seen in Table 10. From this table, there are six types of plants that have a toxicity effect on *Artemia salina* Leach, potentially a cancer drug. This is supported by research by Mayer (1982), which states that an extract shows toxicity activity in the Brine

shrimp lethality test (BSLT) if the extract can cause the death of 50% of the test animals at a concentration of LC50 <1000 ppm. Phenolic compounds (flavonoids, terpenoids and tannins) are poisonous which are antitoxic, meaning they have the potential for cancer drugs [16], stated that phenolics are toxic compounds that have the potential to be anti-cancer, specifically for *P. reticulatus var. reticulatus* has very high toxicity with a value of <10 ppm, this is following what is found in the community that *P. reticulatus var. reticulatus* is used as an anti-cancer for breast and cervical cancer.

#### 4. CONCLUSION

*P. reticulatus* with a yield of 12.48-27.60%, containing alkaloids, flavonoids, steroids, saponins, tannins, triterpenoids, carotenoids, coumarin; and is a powerful antioxidant with an IC value of <50. *P. reticulatus* can inhibit *P. acnes*, *E. coli*, and *C. albicans* fungi and cannot inhibit *S. sobrinus* bacteria. The leaves of *P. reticulatus* from Susweni have a very high toxicity potential as a cancer drug.

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