

Bioactivity of *Vernonia arborea* Leaves from Bentian Tribe as Natural Antioxidant and Antibacterial Based on Local Knowledge

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Abstract

The utilization of natural organic materials as raw materials for standardized medicine, including the use of organic materials in medicine manufacture are currently being encouraged by the Government. Based on the information from Bentian Tribe people, *Vernonia arborea* leaf or Kutu Bu'ut leaf has the potential to be developed as natural medicine derived from plants. This study explored the potential of *V. arborea* leaf for its phytochemicals screening by Harborne, Kokate and Senthilmurugan method, antioxidant analysis was evaluated by DPPH radical scavenging assay. Antibacterial analysis was examined using agar well diffusion method against *Escherichia coli* and *Propionibacterium acnes*. The results showed that the *V. arborea* leaf contained alkaloid on n-hexan extract and coumarin on ethyl acetate extract. Ethanol extract from *V. arborea* leaf contained alkaloid, flavonoid, saponin, tannin, and carbohydrate. Antioxidant activity showed that the highest inhibition by 83% at 50 ppm concentration of ethanol solvent. Antibacterial activity of *E. coli* and *P. acnes* showed that the highest inhibition zone by 12 mm and 11 mm at 400 µg/well of ethanol extract. Based on the results, the *V. arborea* leaf contains natural bioactivity and has potential to be further developed as a natural traditional medicine.

Keywords: *Vernonia arborea*; antibacterial; DPPH; traditional medicine.

INTRODUCTION

Indonesia's tropical forest is known to have more than 1000 types of plants used as medicine and only around 300-400 species of plants have been used as traditional medicines. Indonesia is the second highest biodiversity in the world after the Amazon forests (Elfahmi et al., 2014). Most of the studies focused on the prevention of diseases or specific medicinal herbs in Indonesia (SILALAH I et al., 2014). The Indonesian government through the vision and mission of Nawacita 9 priority agenda, provides opportunities for the wider community and researchers to develop the potential of local areas such as in East Kalimantan.

Natural antioxidants from extracted plants are highly recommended in food application for safety. Natural antioxidant recently applied as bioactive nutraceuticals, food additives and bio-pharmaceuticals on daily activities. The antioxidant is used widely to prevent and reduce oxidation in a natural food system. Based on the natural source of local people, the antioxidant of a plant is natural and safe to consume when used in small-scale. Antioxidant also an essential component which plays a vital role in maintaining good health. Many medicinal plants are known as natural antioxidant based on some

research, which mainly depends on an active compound on various biologically (Riaz et al., 2012a). *The compounds of antioxidant can delay or slow down the process of oxidation of a compound. This means the antioxidant can interfere with free radical chain reaction with the propagation reactions, or inhibit the formation of free radicals in the early stages* (Indrianingsih et al., 2015). Kalimantan known as an island for its highest local wisdom. Previous study about local wisdom in Kalimantan, stated more than 250 medicinal plant species from 75 family and more than 100 genera recently used by Dayak Ransa tribe in West Kalimantan, Indonesia. Dayak Kenyah community also recently used about 200 species of forest medicinal plants (Kusuma et al., 2014). Several studies about the utilization of traditional plants in Kalimantan to treat various diseases, for example *Blumea balsamifera*, *Stachytarpheta jamaicensis* and *Vernonia arborea* as an anticandidal against candida of *Candida albicans* (Kusuma et al., 2016). Several studies in recent years about medicinal plants uses, informed that plants were best sources of natural antioxidant such as phenolics, alkaloids and flavonoids compound (Zhao et al., 2018). Antioxidant agent from the plants was a huge resource to scavenging free radicals naturally.

The ones of utilization of local plants use as an external medicine for skin and body health. Local people use traditional plants as they believe for health and it also had no side effect, safe to be consumed and more economical compared to synthetic drugs. East Kalimantan has a wide range of Biodiversity in its territory also had many benefits. This plants potential are efficacious as natural medicines and if used wisely will provide valueless benefits, especially for the health of the nation of Indonesia. The aim of the present study was to explore the potential antibacterial and antioxidant activities, also analyze the secondary metabolites of the plant extracts. Based on the Bentian Tribe people in West Kutai, this plant used as an alternative healthcare treatment such as itching skin.

MATERIALS AND METHODS

Material and chemicals

The leaves of *Vernonia arborea* were collected from Bentian Village, West Kutai, East Kalimantan, Indonesia. The samples were washed thoroughly with water to remove the extraporeneous and dried about 3 days in the laboratory with air conditioned (A.C.) set for 20-25°C. The samples were kept at A.C. room to keep the moisture content stable and milled with a blender. The powdered samples were prepared for further analysis. DPPH (2,2 diphenyl-1-picrylhydrazyl) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Sulfuric acid, hydrochloric acid, acetic anhydride, and potassium iodide were purchased from Merck (Darmstadt, Germany). Ascorbic acid, 1-naphthol and bismuth (III) nitrate were obtained from Sigma (St. Louis, MO, USA).

Procedures

Maceration

About 20 gr powdered samples of *Vernonia arborea* were extracted with n-hexane, ethyl acetate and ethanol

solvent at room temperature with continuous shaking on a shaker for 48 hours. Following filtration of the suspension through Whatman paper No.2 (Maidstone, UK), the crude extracts of *Vernonia arborea* were evaporated in a rotary evaporator at 38-40 °C and put in a vacuum over near dryness to yield the plant extract.

Phytochemical analysis

The n-hexane, ethyl acetate and ethanol extracts of *Vernonia arborea* leaves were subjected to preliminary screening of phytochemical such as alkaloids, flavonoids, terpenoids, tannin, saponin, steroids, carbohydrate and coumarin using some following standard procedures (Sari et al., 2023; Viji et al., 2013).

Antioxidant assay

Test of antioxidant using 5 concentration samples were grouped into 100 ppm, 50 ppm, 25 ppm, 12.5 ppm and 6.25 ppm times of dilution, respectively. Further, 3 mg of *Ascorbic acid* was weighed, then dissolved in 1000 µl of ethanol solvent and regarded as a positive control. While the ethanol solvent was used as a negative control. About 33 µl sample was mixed in glass tube with 467 µl of ethanol was added, and 500 µl of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (Shimizu et al., 2001). The mixing of sample was stopped while volume has reached 1000 µl (1 ml). Samples were incubated for 20 minutes with minimum light and A.C. set for 27-30 °C. The antioxidant activity was determined by decolorization of DPPH with a wavelength of 517 nm using UV Spectrophotometer. Measurement was performed in triplicate examination. The percentage of DPPH free radical was calculated using the following equation:

$$\% \text{ DPPH radical scavenging activity} = \frac{\Delta_{\text{control}} - \Delta_{\text{sample}}}{\Delta_{\text{control}}} \times 100 \quad (1)$$

Antibacterial assay

Testing the antibacterial activity of the n-hexane, ethyl acetate and ethanol extracts of *Vernonia arborea* leaves was carried out using the agar-well diffusion method with slight modifications (Singh et al., 2002). *Escherichia coli* and *Propionibacterium acnes* bacteria were used in this research. About 20 mL of sterile agar media solution was put into a petri dish that had been sterilized for 30 minutes at 121°C in an autoclave. After that, in an antiseptic state (in laminar flow), let the media

harden until it is cold and solid, then inoculate with 100 µL of the bacterial suspension and wipe it evenly over the test medium and let it dry for ± 30 minutes. Then the media was perforated using a 5 mm sterile punch for each sample. In each test hole, 20 µL of sample which had been dissolved with acetone was added as a negative control and chloramphenicol as a positive control in the test. Tests were carried out using test concentrations of 25 µg, 50 µg, 100 µg, 200 µg dan 400 µg.

RESULTS AND DISCUSSION

Yield of extracts

The leaves of *Vernonia arborea* were macerated by n-hexane, ethyl acetate and ethanol at room temperature (Table 1). The weight of extracts maceration range was 0.30-5.93g and yielded of extracts was 1.80-35.17% extracts on the basis of sample dry weight.

Table 1. The yields extract of *Vernonia arborea* leaves.

| Solvent | Weight (g) | Yield (%) |
|---------------|------------|-----------|
| n-Hexane | 0.30 | 1.80 |
| Ethyl acetate | 5.93 | 35.17 |
| Ethanol | 4.87 | 28.91 |

*Yield was calculated on the basis of sample dry weight

Table 2. Phytochemical Analysis of *Vernonia arborea* Extract.

| Sample | Test | n-Hexane | Ethyl Acetate | Etanol |
|-------------------------|--------------|----------|---------------|--------|
| <i>Vernonia arborea</i> | Alkaloids | + | - | + |
| | Flavonoids | - | - | + |
| | Saponins | - | - | + |
| | Tannin | - | - | + |
| | Steroids | - | - | - |
| | Terpenoids | - | - | + |
| | Carbohydrate | - | - | + |
| | Coumarins | - | + | + |

The test results showed that the *Vernonia arborea* plant in the n-hexane extract only contained alkaloid compounds and the ethyl acetate extract only contained coumarin compounds. In the ethanol extract, the *Vernonia arborea* plant contains alkaloids, flavonoids, terpenoids, saponins, tannins, carbohydrates and coumarins. In several studies it is known that the presence of secondary metabolite compounds in a plant will affect the bioactivity contained in the plant (Lim et al., 2014).

The result showed that the ethyl acetate solvent gave the higher yielded extracts while the lowest yielded was obtained from n-hexane solvent.

Phytochemical analysis

Phytochemical analysis was carried out to determine the content of secondary metabolites contained in plant extracts. Based on information from the local community about the utilization of the *Vernonia arborea* plant or known by the people of Bentian Village by the local name Daun Kutu Bu'ut, it is necessary to analyze the content of secondary metabolites before conducting other bioactivity tests.

Antioxidant activity

Antioxidant analysis was carried out to determine the free radical absorption of *Vernonia arborea*. Tests were carried out using the Shimizu et al (Shimizu et al., 2001) method with modifications. Tests were carried out using a DPPH solution and a UV Spectrophotometer with a wavelength of 517 nm. Test concentrations are 6.25 ppm, 12.5 ppm, 25 ppm, 50 ppm and 100 ppm. Ascorbic acid used as a positive control. The test results can be seen in Figure 1 as follows.

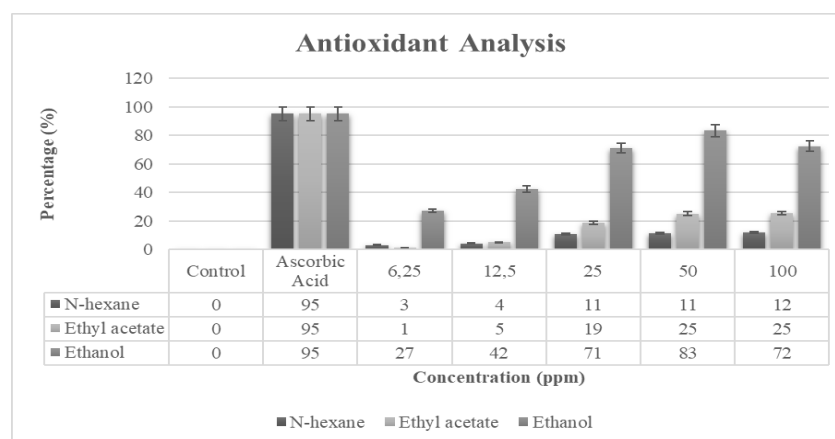


Figure 1. Antioxidant Activity of *Vernonia arborea* Extract against DPPH.

The test results showed that the *Vernonia arborea* plant has potential as a natural antioxidant, this can be seen from the results of testing the DPPH free radical which was used as a negative control in the test and vitamin C as a positive control. The n-hexane extract has a percentage of 3-12 % in the antioxidant test. The ethyl acetate extract has a percentage of 1-25 % while the ethanol extract has a percentage of 27-83 % in the test. The ethanol extract had a percentage of 83 % at a concentration of 50 ppm, whereas at a concentration of 100 ppm the inhibition percentage was only 72%. This is influenced by the concentration of the sample in the test, so that the absorption obtained is not read properly and causes a decrease in the percentage.

In the phytochemical analysis carried out, it was found that the *Vernonia arborea* plant contains various secondary metabolite compounds including polyphenols (tannins and flavonoids). Plants containing polyphenols

are an important source of antioxidants because they have an ideal chemical structure to scavenge free radicals. Several studies reveal the potential of antioxidants in reducing the risk of various chronic and acute diseases such as heart disease, cancer and stroke by reducing free radical compounds involved in the pathogenesis of various diseases (Riaz et al., 2012b).

Antibacterial activity

Antibacterial analysis was carried out to determine the potential of *Vernonia arborea* against bacterial attack. Tests were carried out using the method of Singh et al (Singh et al., 2002) with modifications. *Escherichia coli* and *Propionibacterium acnes* bacteria were used in the test. Sample test concentrations were 25, 50, 100, 200 and 400 µg/well using Chloramphenicol as a positive control.

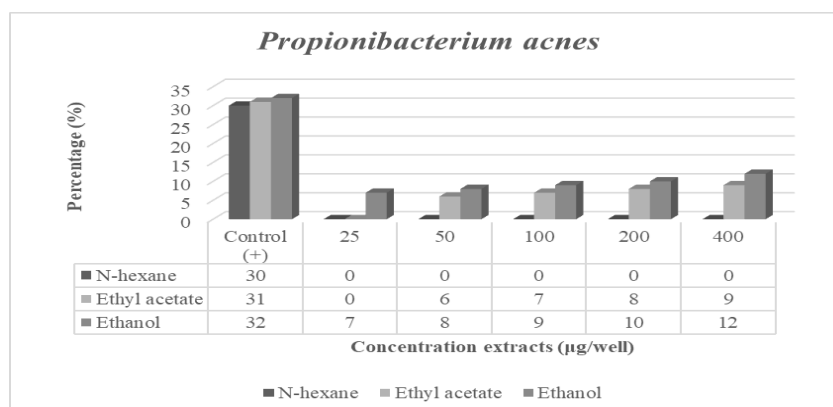


Figure 2. Antibacterial Activity of *Vernonia arborea* Extract against *Propionibacterium acnes*.

Testing of the *Vernonia arborea* plant extract against *Propionibacterium acnes* bacteria as shown in Figure 2 shows that the n-hexane extract has no inhibition, the ethyl acetate extract has an inhibition diameter of 0-9 mm or the equivalent of 0-28% and is included in the medium category. In the ethanol extract there is an

inhibition diameter of 7-12 mm or equivalent to 21-36% and is included in the medium-strong category. This shows that the ethanol extract has the potential to be developed as a natural antibacterial against *Propionibacterium acnes* bacteria.

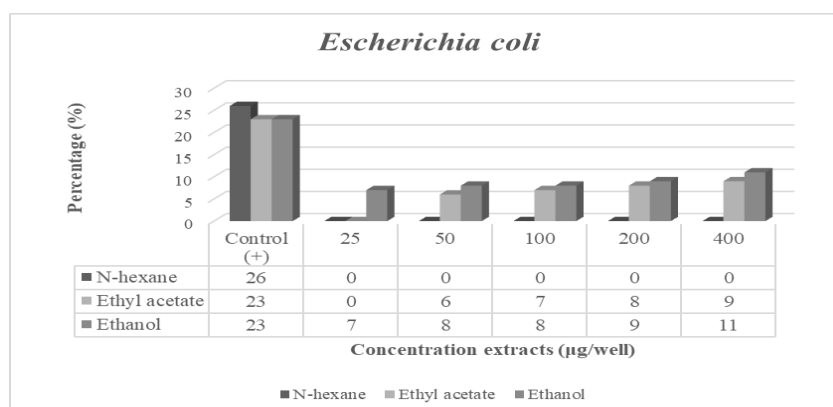


Figure 3. Antibacterial Activity of *Vernonia arborea* Extract against *Escherichia coli*.

Testing of the *Vernonia arborea* plant extract against *Escherichia coli* bacteria as shown in Figure 3 shows that the n-hexane extract has no inhibition, the ethyl acetate extract has an inhibition diameter of 0-9 mm or the equivalent of 0-39% and is included in the medium category. In the ethanol extract there is an inhibition diameter of 7-11 mm or equivalent to 33-47% and is included in the medium-strong category. This shows that the ethanol extract has the potential to be developed as a natural antibacterial against *Escherichia coli* bacteria.

The presence of secondary metabolite compounds in the *Vernonia arborea* plant extract, namely alkaloids, tannins, flavonoids and coumarins which are known to act as antimicrobials is one of the factors that makes the *Vernonia arborea* plant extract able to inhibit bacterial growth. Several studies have stated that the presence of compounds from the alkaloid and flavonoid groups can cut and denature proteins and prevent the digestive process of bacteria (Ahmed & Wang, 2021; Heliawati et al., 2022).

CONCLUSIONS

Based on the results, the *Vernonia arborea* leaves or Daun Kutu Bu'ut medicinal plants used by Tende people from East Kalimantan contains natural bioactive and has potential to be further develop as a natural antioxidant and antibacterial, also give a scientific basic to the traditional uses of the investigated plants.

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Authors' Contributions: In this research, Nur Maulida Sari was designed the research, supervised all process and wrote the manuscript. Humairo Aziza was observing, taking research samples and supervised research data analysis. Farida Aryani supervised all research data analysis and manuscript writing. Murdianto controlled the samples and preparation of materials also controlled research data analysis.

Competing Interests: There is no conflict of interest in this research.

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