Antioxidant and anti-termite activities of the ethanol extract of cibotium barometz (L.) J. Sm.

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Abstract. The antioxidant and anti-termite activities have been considered on the ethanol extract of Cibotium barometz (L.) J. Sm rhizome. An antioxidant assessment was carried out using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method and measured the absorbance at λ = 517 nm. An anti-termite assay was carried out by the force-feeding method using concentrations of 0, 10, 20, and 30 ppm. The results of phytochemical screening indicated that the extracts contained secondary metabolites of flavonoids, polyphenols, tannins, saponins, steroids, and triterpenoids. The antioxidant trial for which data were processed by the linear regression showed IC\textsubscript{50} values of 8.20 and 5.04 ppm for the extracts and the vitamin C (as a standard) respectively. The IC\textsubscript{50} value of the ethanol extract of the C. barometz rhizome exhibited the strongest antioxidant activity. The anti-termite assay whose data was processed by the Trimmed Spearman-Karber (TSK) program showed an LC\textsubscript{50} value of 28.88 ppm. The termite mortality data for 96 hours were analyzed statistically using one way ANOVA with α = 0.05 demonstrated that the given treatments exposed very significant differences in termite mortality \[F(3, 16) = 293, p <0.0001\]. Based on the results of the study, it might be firmed that the ethanol extract of C. barometz rhizome could be exploited as antioxidant and anti-termite stuff.

1. Introduction
Termites are insect caused severe damage to buildings. The economic losses caused by termites are estimated to reach 8.6 trillion rupiah in 2015 and increase from year to year in Indonesia [1]. So far, people have used a synthetic termiticide to control it. The termiticides used are mostly not environmentally friendly and toxic. If the termiticides continue to be used, the active ingredients which are toxic will accumulate in nature and seriously endanger the survival of humans and ecosystems. To overcome this issue, the community should use natural termiticides derived from natural ingredients, one of which is Cibotium barometz (L.) J.Sm. plant.

The C. barometz is a species of fern. This plant is found in Viet Nam, Thailand, Taiwan, Philippines, Papua New Guinea, Myanmar, Malaysia, Japan, Indonesia, India, Hong Kong, and China [2]. The plant is known by several regional names, such as Pakis Simpi (Sumbar), Bulu Jambe (Madura), Jampi (Bali), Gou Ji (China), Scythian Lamb (English) [3] and Pakou Kamat (Aceh). According to information from the people of Tanggal sub-district, this plant can cure fresh or festering wounds and as a termiticide. Secondary metabolites that function as anti-termite, namely flavonoids, tannins, saponins [4], triterpenoids, polyphenols, and steroids [5]. Flavonoids have the ability as antioxidants [6] showed an antifeedant activity (inhibitory feeding) [7]. Termites avoid wood containing antioxidant substances because they can interfere the digestive process of lignocellulose [8].
2. Experimental

2.1. Materials
The instruments employed in this research were glassware, knives, cutting tables, analytical scale, jars, filters, rotary evaporator, volume pipettes, stopwatch, cotton buds, glasses, baskets, gauze, pincers and UV-Vis Shimadzu spectrophotometers 1800.

2.2. Biomaterial
Sample of *C. barometz* was obtained from the Neubok Badeuk region, Tangse Sub-district – Pidie District, and the *Cryptotermes brevis* termites were got from Mon Ikeun, Lhok Nga village, Aceh Besar District, Aceh Province, Indonesia. This research was conducted at the Chemistry Education Laboratory of Faculty of Teacher Training and Education, Syiah Kuala University.

2.3. Chemicals
The chemicals used in this study were 70% ethanol, pro-analysis ethanol, Mayer’s reagent, Dragendorff’s reagent, Wagner’s reagent, Liebermann-Burchard’s reagent, Trim-Hill’s reagent, ammonia, chloroform, 2 M sulfuric acid, aqua dest, Mg powder, concentrated hydrochloric acid, 1% hydrochloric acid, amyl alcohol, ether, iron (III) chloride 1%, DPPH, vitamin C, tissue, sand, plastic, rubber bands, and aluminum foil.

2.4. Extraction
The *C. barometz* rhizome was cut, weighed and dried at room temperature. Sample in weight of 650 grams was extracted by a maceration method using 70% ethanol in the total volume of 4 L for 2 x 24 hours. The filtrate was evaporated with a rotary evaporator and obtained the brown extract as much as 20.158 grams.

2.5. Phytochemical screening
The secondary metabolite testing was carried out by the phytochemical screening methods [9, 10]. Alkaloids were identified using Mayer’s, Dragendorff’s, and Wagner’s reagents. Terpenoids were identified using Trim-Hill’s reagent, and triterpenoids and steroids were identified using Liebermann-Burchard’s reagent. Identification of saponins by foam test, and polyphenols and tannins were carried out with the addition of 1% FeCl₃. Identification of flavonoids was carried out via Shinoda’s test by the addition of Mg powder and HCl.

2.6. Antioxidant trial via DPPH method
An aqueous solution of the extract of *C. barometz* was prepared to a concentration of 100 ppm in 10 mL. This solution was pipetted as much as 0.5, 1, 1.5, 2, and 2.5 mL into a 25 mL volumetric flask each to obtain the concentration of the sample test solution 2, 4, 6, 8, and 10 ppm. Each of the sample test solutions was pipetted as much as 3 mL into a 5 mL volumetric flask and then was added 1 mL of 0.1 mM DPPH solution to prepare a mixed solution. A blank solution was made by adding 3 mL of p.a ethanol into 1 mL of 0.1 mM DPPH solution. The mixed solution was incubated for 30 minutes. DPPH absorbance was measured by visible spectrophotometer at λ = 517 nm [11]. The same process was repeated for vitamin C as a comparison.

2.7. Anti-termite assessment
The anti-termite assessment was carried out using the force-feeding method [12] with some modifications. A colony was taken from wood attacked by *Cryptotermes brevis* termites. The termites and dry wood as food source were inserted into an adaptation jar covered with wire gauze and left for 72 hours. Furthermore, the termites were inserted into a trial jar as many as 50 individuals consisted of 45 individuals of worker and 5 individuals of soldier. The termites were left for an hour and then ready to apply the extract. Based on the preliminary test, the extract was administrated to several
concentrations of 0, 10, 20 and 30 ppm with 5 repetitions. The dry wood obtained from the place of termite colony was cut into small pieces. Each piece of the wood was soaked in the extract with various concentrations and then was dried. The pieces of wood that have been soaked with extract were inserted into the trial jar. Observation on an anti-termite assessment was carried out every 24, 48, 72, up to 96 hours.

2.8. Data analysis
The absorbance of the antioxidant trial was transformed to inhibition (%) with the following formula [11]:

\[
\text{Inhibition (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of extract}) \times 100}{\text{Absorbance of control}}
\]

The anti-termite test study was carried out using a completely randomized design (CRD). The analysis used was one way ANOVA with \( \alpha = 0.05 \) and further analyzed using the Tukey method. Furthermore, the median lethal concentration of the extract (LC50) was analyzed using the Trimmed Spearman-Karber (TSK) program [13].

3. Result and discussion
The C. barometz rhizome was extracted by a maceration method using the ethanol in order to get all active ingredients. The phytochemical screening informed several secondary metabolites in the extract (Table 1). Flavonoids have properties as antioxidants, antidiabetic, antibacterial, anti-inflammatory, antihypertensive and anticancer [14]. The terpenoids, phenolics, tannins and flavonoids are responsible for the antihypertensive effect [15]. Phenol compounds have antioxidant and hypoglycemic activities [16].

| No. | Phytochemical screening | Qualitative result | Colour of test result | Positive colour |
|-----|------------------------|--------------------|-----------------------|-----------------|
| 1   | Alkaloids              | -                  | colourless solution   | red precipitate |
|     | - Mayer                | -                  | brown precipitate     | red precipitate |
|     | - Dragendorff          | -                  | orange-brown solution | brown precipitate|
|     | - Wagner               | -                  | solution              | red solution    |
| 2   | Terpenoids             | +                  | yellow solution       | golden yellow   |
| 3   | Triterpenoids          | +                  | solution              | brown ring formed|
| 4   | Steroids               | +                  | golden yellow solution| solution        |
| 5   | Saponins               | +                  | solution              | brown ring formed|
| 6   | Tannins                | ++                 | brown ring formed     | stable foam     |
| 7   | Polyphenols            | ++                 | stable foam           | white precipitate|
|     |                        |                    | white precipitate     | bluish black solution|
|     |                        |                    | bluish black solution |                 |
| 8   | Flavonoids             | +++                | red solution          | red solution    |

Note: +++ (high), ++ (moderate), + (low), - (negative)

The antioxidant trial was carried out using the DPPH method. The principle of this test is the reaction between compounds that can donate hydrogen atoms to DPPH. Thus, radical DPPH can be neutralized. An indicator has occurred the neutralization is marked by the change in the colour of the DPPH solution from purple to yellow [17]. The C. barometz extract demonstrated an antioxidant activity at varying concentration tested as well as of the ascorbic acid as presented in Table 2. The half-maximal inhibitory concentration (IC50) was calculated based on parameters of curve relationship between concentrations...
and inhibition as exposed in Figure 1. IC\textsubscript{50} values of the ethanol extract of the \textit{C. barometz} and ascorbic acid were 8.20 and 5.04 ppm, respectively. A substance has a weak antioxidant activity if the IC\textsubscript{50} value <151-200 ppm, a moderate antioxidant activity if the IC\textsubscript{50} value <100-150 ppm, a strong antioxidant activity if the IC\textsubscript{50} value <50-100 ppm, and a most potent antioxidant activity if the IC\textsubscript{50} value <50 ppm [18]. Referring to the categories, the property of the ethanolic extract of the \textit{C. barometz} rhizome was the strongest antioxidant activity.

| Control (EtOH, A) | Concentration (ppm) | Ascorbic acid | Inhibition (%) | IC\textsubscript{50} (ppm) | \textit{C. barometz} Absorbance | \textit{C. barometz} Inhibition (%) | \textit{C. barometz} IC\textsubscript{50} (ppm) |
|------------------|---------------------|---------------|----------------|----------------|---------------------------------|-----------------------------|---------------------|
| 0.415            | 2                   | 0.292         | 0.384          | 29.64          | 7.47                            | 5.04                        | 8.20 |
|                  | 4                   | 0.238         | 0.334          | 42.65          | 19.52                           | 5.04                        | 8.20 |
|                  | 6                   | 0.179         | 0.277          | 56.87          | 33.25                           | 5.04                        | 8.20 |
|                  | 8                   | 0.128         | 0.218          | 69.16          | 47.47                           | 5.04                        | 8.20 |
|                  | 10                  | 0.066         | 0.148          | 84.10          | 64.34                           | 5.04                        | 8.20 |

**Figure 1.** Relationship graph between concentration (ppm) and inhibition (%)

Before an anti-termite bioassay was administrated, termite colonies were maintained for one month [4], and adapted first so that only active and healthy termites were used in the study [19]. Anti termite test was carried out by the force-feeding method. This feeding technique forces termites to catch food source that has been soaked with sample extract. This study used wood as a food source because termites like materials that contain high cellulose. Cellulose is the main food source for termites.
Table 3. Results of one way ANOVA analysis.

| Source of variation       | SS    | df | MS    | F     | P-value |
|---------------------------|-------|----|-------|-------|---------|
| Between Groups (treatment)| 2639  | 3  | 880   | 293   | <0.0001 |
| Within Groups (residue)   | 48    | 16 | 3     |       |         |
| Total                     | 2687  | 19 |       |       |         |

Table 4. Data for calculating the LC$_{50}$ value using the Trimmed Spearman-Karber program.

| Concentration (ppm) | Number of termites (individual) | Average mortality (individual) | 95% lower limit | 95% upper limit | LC$_{50}$ (ppm, 96 hrs) |
|---------------------|---------------------------------|-----------------------------|-----------------|---------------|-------------------------|
| 0                   | 50                              | 0.6                         |                 |               |                         |
| 10                  | 50                              | 17                          | 25.11           | 33.21         | 28.88                   |
| 20                  | 50                              | 25                          |                 |               |                         |
| 30                  | 50                              | 31                          |                 |               |                         |

Different letters indicated statistically significant differences in mortality of termite among the treatments (p<0.0001, Tukey’s post hoc following one-way ANOVA)

The mortality data obtained were then processed statistically using one-way ANOVA with α = 0.05 and analyzed the effect of each extract concentration on termite mortality using the Tukey method. Mortality data processed by one-way ANOVA (Table 3) showed that the given treatment showed a very significant difference in the mortality of termites [$F(3, 16) = 293, p <0.0001$]. Further analysis using the Tukey test showed that extract concentrations of 10 and 20 ppm were not significantly different in mortality of termites, but the mortality of termites differed significantly in the administration of extracts with a concentration of 30 ppm rather than concentrations of 10 and 20 ppm.

Secondary metabolites of flavonoids, polyphenols, tannins, saponins, steroids, and triterpenoids contained in the ethanol extract of the $C. barometz$ rhizome were predicted to be toxic to termites. These compounds could cause eating termites to be disrupted. The flavonoid, polyphenol, and tannin substances exhibited toxic properties to termites through feeding deterrent mechanisms [7, 20, 21]. Saponins were reported for cytotoxic effects to termites [22]. Steroid compounds showed a property as insect repellent [9]. Triterpenoids function as stomach poisons, which could kill protozoa in the digestive tract of termites. This bacterium was a Symbion produced cellulose enzymes. This cellulose enzyme function was to digest and to convert it into a simple sugar used by termites as an energy source [23].

This study observed that giving extracts at a concentration of 30 ppm caused a mortality of 62% of the tested termite (Table 4). This finding was in line with the conclusion of Tafsir et al. [4] which stated that the higher the concentration of a given extract raised higher mortality. According to Clarkson’s toxicity index, the toxicity evaluation of plant extract was classified in the following order: extract with LC$_{50}$ above 1000 ppm was non-toxic, extract with LC$_{50}$ of 500 - 1000 ppm was low toxic, extract with LC$_{50}$ of 50 - 500 ppm was medium toxic, and extract with LC$_{50}$ of 0 - 100 ppm was highly toxic [24]. The LC$_{50}$ value of the extract analyzed using the TSK program showed results of 28.88 ppm as revealed in Table 4. Referring to the index, the ethanol extract of the $C. barometz$ rhizome has a highly toxic to the tested termites so that it could be used as a termiticide.

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