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Abstract. This study reported the isolated compound from vetiver grass-root (*Vetiveria zizanioides* Stapf) have bioactivity as a repellant against termites. The compounds were extracted by n-hexane solvent using the soxhletation method. The compounds were tested through bioactivity assay using wood pieces added isolated compound as a sample on the final assay, acetone as a blank, and wood pieces without treatment as a control. The result showed that the isolated compound was acquired by separation and purification in the form of colorless oil 0.0225 g. Of the thin-layer chromatography (TLC) assay using eluents of n-Hexane eluent 100%, n-Hexane : DCM 95%, and n-Hexane : Acetone 70%, were obtained a red-purple spot and Rf values which are 0.27, 0.59, and 0.91, respectively. 2-methyl butane-1-amine ($C_5H_{13}N$) was supposed by the structure elucidation of the isolated compound. The molecular weight was 87.1 g/mol as a precursor terpenoid based on the spectrum examination of FTIR and GC-MS. The bioactivity showed that an isolated compound has acted as a repellent against termite (*Cryptotermes* sp.) with % attractive (0.025 %) and attractiveness index (AI) (-0.039). A negative value of AI indicated that the compound is potential as a repellent against termites.

Keywords: Isolation, Terpenoid, Vetiver grass-root (*Vetiveria zizanioides* Stapf), Repellent, Termites (*Cyrptotermes sp.*).

1. Introduction

Termites are one of the most problematic pests because they are not only damage the structural timber of houses and other materials in structures but also cause crop losses, building damage, and economic losses. However, termites are prominent to the ecosystem in recycling woody and other plant material because they are able to decompose cellulose, the main component of wood [1]. Thirty-nine available family-group names are identified within the insect order Isoptera (termites) around the world [2]. In Indonesia, there are 200 different species of termites, but only several cause problems to properties, which are *Coptotermes gestroi*, *Coptotermes curvignathus*, *Coptotermes havilandi*, *Coptotermes kalshoveni*, *Coptotermes sepangensis*, *Macrotermes gilvus*, *Macrotermes pakistanicus*, *Globitermes*

sulphureus, Schedorhinotermes sp. and *Microcerotermes sp*. About 90% of property damages that were rendered by Coptotermes species making it the serious pests in Indonesia [3].

There are many products inclusive of techniques of chemical, non-chemical, and biological available to manage and prevent dry-wood termites. Synthetic termiticides remain the primary method used to avoid termite attack on wooden structures. Several termiticides contain toxic ingredients such as Copper Naphthenate, Copper Azole, Chromate Copper Arsenate (CCA), and Disodium Octoborate Tetrahydrate (DOT), as well as other active ingredients that have registered under the various brand names around the world [1,4]. However, the persistence of chemical termiticides is at present of environmental concern, and it has resulted in the need to search for plant-derived compounds as an alternative for termite control [5]. A formulation must meet several main criteria to deter termites as repellant effectively. However, a combination of the active compound is the first challenge in process discovery that will provide long-term protection against a wide range of organisms that damage the wood include harmful effects to applicators and the environment, the active ingredients, and solvents used [6].

Natural termite repellent chemicals have been investigated by vetiver extract against termite resistance. For nearly 100 years, until 1999, vetiver's taxonomic classification (that is, the complete accepted scientific name) had been *Vetiveria zizanioides* (L.) Nash. The "L." stands for Linneaus, the great botanist who standardized the use of botanical binomials (two names) in the late 1700s: he placed vetiver in the genus Phalaris, and it changed many times since. The other binomial currently in general use (especially in Europe) has been *V. zizanioides* (L.) Stapf, which refers to precisely the same species of the plant [7]. The Vetiver grass (*Vetiveria zizanioides* L. Nash, now reclassified as *Chrysopogon zizanioides* L Roberty) was first developed by the World Bank for soil and water conservation in India in the mid-1980s [8]. Vetiver planted in more than 120 countries for soil and water conservation, land stabilization, bioremediation, root oil production, and other uses [9]. Vetiver extract is known to possess a complex mixture of over 300 compounds, over 150 of which are sesquiterpenoid compounds and their derivatives. The compounds of Vetiver extract reported that repelling against insects are the ketones-α.-Vetivone, β-Vetivone, Khusimone, and the aldehydes-Zizanal, as well as Epizizanal [10]. Other components of Vetiver oil are Zizanol (or Khusimol), Bicyclovetivenol, and α-Cedrene, including sesquiterpene alcohols, hydrocarbons, and ketones [11]. Secondary metabolites investigated in vetiver extracts. Alkaloids, flavonoids, phenols, saponins, steroids, tannins, sesquiterpenes, terpenoids, and triterpenes were abundant [12, 13]. Vetiver-derived compounds investigated for pest and pathogen management. The repellant activity has potential against multiple organisms, including termite, fungi, and bacterial [14, 15, 16, 17, 18]. It has an earthy fragrant aroma and a high fixative property, so it was potential as raw material for industries such as in the manufacture of perfumes, cosmetics, deodorants, soaps, medicines, as well as an insect repellent [19, 20].

The current study aimed to obtain isolation of terpenoid compounds from vetiver grass-root (*Vetiveria zizanioides* Stapf). The determination of the properties and the compound structures observed through bioactivity assay and elucidation. The extracted compound was carried out by the soxhletation method using n-Hexane as the solvent. The bioactivity assay tested by crude extract and isolated compound as a sample, acetone as a blank, and the pieces wood without treatment as a control. Separation and purification were fulfilled through vacuum liquid chromatography (VLC) with gel silica Merck 60 (10-40 μm) as an impregnation phase and gel silica Merck 60 GF254 (63-200 μm) as a quiescent phase in the column. The purity of the isolated compounds was tested by thin-layer chromatography (TLC) with n-Hexane eluent 100%, eluent of n-Hexane: DCM (95%), and eluent of n-Hexane: Acetone (70%). The elucidation of isolated compounds analyzed by FTIR spectrophotometry and GC-MS methods.

2. Materials and Methods

2.1. *Samples Preparation*

The feedstock of vetiver grass-root (*Vetiveria zizanioides* Stapf) was purchased in the Bringharjo Yogyakarta farmer market, Indonesia.

The feedstock of vetiver grass-root was cleaned with water, chopped into 1 cm pieces, and dried in room temperature (23-25 °C) for 5 to 7 days. The dried material was ground by a milling machine and passed through a sieve with a pore size of 2 mm. The powders were stored for further use [9].

2.2. Extraction and Isolation

To prepare crude extracts, dried vetiver grass-root powder was extracted using the soxhlet method with n-Hexane as a solvent for 20x3 hours at 70 - 80 °C. The mixture was then filtered through cotton filter paper in the funnel, and the filtrate was evaporated in a vacuum rotary evaporator at a rotating rate of 40 - 60 rpm. The small amount of remaining solvent was air-dried, and the extracts were stored for further use [9].

2.3. *Preliminary Assay of Terpenoid*

The preliminary assay of terpenoid was determined with the Liebermann-Burchard reagent (anhydride acetic:concentrated sulfuric acid, 1:1) by thin layer chromatography (TLC) method. By the TLC method, 1 ml of crude extract was taken and affixed to the TLC plate, then the sample eluted with eluent (n-hexane:ethyl acetate) in addition to spray with Liebermann-Burchard reagent to visualize. While the direct test was carried out by taking 1 mL of crude extract, then it added about 3 drops of Liebermann-Burchard reagent. The positive terpenoid content was expressed with red to purple colors.

2.4. Bioactivity Assay

Bioactivity assays were conducted with modified methods from [9]. For our studies, three pieces of wood samples (approximately 2 - 3 mm) were placed in 3 plates with diameter of 25 cm, respectively. The treatments tested were: (1) three-wood samples added with crude extract as a sample test (2) three-wood samples added with acetone solvent as a blank and (3) three-wood without treatment as a control. Into each plate 20 termites (*Cyrptotermes sp.*) previously fasted for 1 h were added. Each treatment on the plates was left and observed for 10 hours. Each treatment on the plates was left and observed for 10 hours inclusive of the attractiveness index as well as % attractive was calculated using the formula equation 1 and equation 2, respectively.

Equation (1) to determine % attractive :

$$\% \text{ Attractive} = \frac{\sum (a)}{\sum (n)} \text{ x } 100\%$$

Equation (2) to obtain the attractiveness index (AI) :

$$\text{AI} = \frac{\sum (a) - \sum (b)}{\sum (n) - \sum (b)}$$

Where ∑(a) is the number of interested insects, ∑(n) is the number of released insects, and ∑(b) is the number of insects on the blank.

The interpretations of the attractiveness index for the bioactivity assay are developed from the equation's [9] : ≥0.2 indicated an attractant; between 0.2 and 0.1, a weak attractant; from 0.1 to −0.1, without effect; between −0.1 and −0.2, a weak repellent; and ≤−0.2, a repellent.

2.5. Fractionation and Purification

Separation and purification of crude extract were fulfilled through vacuum liquid chromatography (VLC) with gel silica Merck 60 (10-40 μm) as an impregnation phase and gel silica Merck 60 GF_{254} (63-200 μm) as a quiescent phase in the column using an appropriate separation solvent system. After obtained the fractions from VLC, the purity of the isolated compounds was tested using TLC with n-Hexane eluent 100%, eluent of n-Hexane : dichloromethane DCM (95%), and eluent of n-Hexane : Acetone (70%). Then, the sample was affixed from the bottom on the gel silica 60 F_{254} 0.25 mm pre-coated TLC plate. After that, the sample fractions were sprayed with Liebermann-Burchard reagent to visualize of characteristic compounds. The collected fraction was also evaluated through R_f values and color spot by TLC. Finally, the sample was stored in a desiccator for further use as a biology activity assay to evaluate as same as procedures previously [10].

2.6. Structural Properties

2.6.1 Fourier Transform Infrared (FTIR) Spectrophotometer

The isolated sample of vetiver grass-root was mixed with KBr and made into pellets for analysis preparation. The functional groups were determined according to IR spectrum absorption.

2.6.2 Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analysis of the isolation sample of the vetiver grass-root was carried out using an Agilent 6890 N GC instrument coupled with a 5975 B mass selective detector. The instrument was equipped with an HP-5 MS capillary column. The oven temperature was maintained at 50°C for 5 min and ramped to 230 - 235 °C at a temperature gradient 4 °C/min. The components were identified by comparing mass spectra with the NIST mass spectra library in the GC/MS data system [9].

3. Results and Discussion

3.1. Sample Preparation, Extraction, and Preliminary Assay of Terpenoid

In this research, as much as 3 kg of vetiver grass-root feedstock was acquired, approximately 1 kg of dry root powder. The extraction of grass-root veriter dry powder using n-Hexane solvent through the soxhletation method produced a crude extract of 14.26 g in the non-polar phase and 4.28 g in the polar phase. The crude extract was evaporated to further use for preliminary assay of terpenoid determination. The extract of vetiver was the positive levels of secondary metabolites like steroids was indicated by direct test using Lieberman-Burchard reagent in which it was demonstrated by the green-black colors on the non-polar phase as well as the polar phase. Meanwhile to identify terpenoid be able to use Lieberman-Burchard reagent (Anhydrate Acetic Acid : Concentrated Sulfuric Acid, 1:1), which is the positive terpenoid content, was showed from the red to purple colors using the reagent assay, respectively. The contents of secondary metabolites are comparable according to [13]. The color attributes of the sample were expressed according to each content.

3.2. Bioactivity Assay of Crude Extract

The stock solution of the crude extract both non-polar phase and polar phase was prepared by dissolving in acetone. Bioactivity assay used to evaluate the repellent effect of the crude extract from vetiver grass-root was repelled by 20 termites (*Cyrptotermes sp.*). The monitoring result of the bioactivity assay was presented in **Table 1** and **2**, crude extract of the non-polar phase and polar phase, respectively.

Based on observation, the bioactivity assay of the crude extract non-polar phase with parameters of % attractive and AI at different times has resulted in very consistent by an overall average of 0%, and AI = -0.10 for 1-10 hours, respectively (see **Table 1**). Meanwhile, bioactivity assay on the crude extract

polar phase with the same parameters demonstrated an overall average of 0.05%, and AI = 0.02 for 1-10 hours, respectively (see **Table 2**). Be able to infer that the bioactivity of the crude extract non-polar phase is higher more than on the crude extract polar phase in which the crude extract non-polar phase is effectively influenced as a weak repellent against termites because of AI value between −0.1 and −0.2, was categorized as a weak repellent according to [9].

Table 1. Bioactivity assays of the crude extract non-polar phase as a repellent against termites

Times (Hours)	S	C	B	L	% Attractive	Attractiveness Index (AI)
1	0	1	2	0	0	-0.11
2	0	2	1	0	0	-0.05
3	0	3	4	0	0	-0.25
4	0	1	1	1	0	-0.05
5	0	0	2	0	0	-0.11
6	0	2	2	4	0	-0.11
7	0	1	4	2	0	-0.25
8	0	1	1	4	0	-0.05
9	0	1	0	4	0	0
10	0	1	1	5	0	-0.05
∑ (Total)	0	13	18	20	0	-1.04
Average	0	1	2	2	0	-0.10

Annotation: S is the number of insects on the sample; C is the number of insects as a control; B is the number of insects on the blank; L is the number of die insects.

Table 2. Bioactivity assays of the crude extract polar phase as a repellent against termites

Times (Hours)	S	C	B	L	% Attractive	Attractiveness Index (AI)
1	0	0	0	0	0	0
2	1	4	0	1	0.05	0.05
3	1	5	1	0	0.05	0
4	2	1	1	1	0.10	0.05
5	2	3	0	2	0.10	0.10
6	1	4	1	4	0.05	0
7	1	6	1	1	0.05	0
8	1	6	0	4	0.05	0.05
9	1	5	1	0	0.05	0
10	0	1	1	5	0	-0.05
∑ (Total)	10	35	6	18	0.50	0.20
Average	1	4	1	2	0.05	0.02

Annotation: S is the number of insects on the sample; C is the number of insects as a control; B is the number of insects on the blank; L is the number of die insects.

3.3. Fractionation, and Purification

Since the non-polar phase of the crude extract possesses bioactivity as a repellent termite, the sample was separated by vacuum liquid chromatography (VLC). The fractionation by VLC was selected using an appropriate separation solvent system which is eluents of n-Hexane 100 %, n-Hexane : Ethyl Acetate (20 - 95 %), Ethyl Acetate 100 % and Methanol 100 %. The elusion process was carried out in order with an eluent of n-Hexane 100%, n-Hexane : Ethyl Acetate (98 %, 95 %, 92 %, 90 %, 85 %, 80 %, 75 %, 70 %, 65 %, 60 %, 55 %, 50 %, 40 %, 30 %, and 20 %), Ethyl Acetate 100 % and Methanol 100 %. The selection was based on the solvent system to identify the chemical compositions, which are obtained approximately 31 fractions, as shown in **Figure 1**.

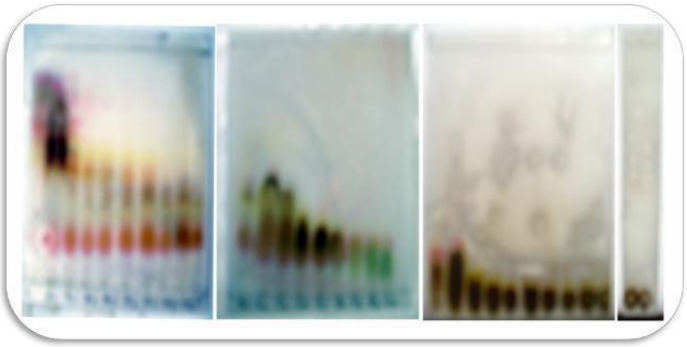

Figure 1. Profiles of TLC chromatogram of crude extract non-polar phase fractionation using an eluent of n-Hexane : Ethyl Acetate 95 %

The 31 fractions were evaporated using a vacuum rotary evaporator. After this fraction was dried, all fractions which are highlighted the similarities tracks on the same plate that the similarities track fractions were merged into 5 fractions i.e. fractions of A (1-10); B (11-17); C(18-20); D (21-23); and E (24-31). Based on the track profile of TLC, only fractions of A and B posses that are red-orange to purple spot what indicate the existence of the terpenoid compound. Both fractions A and B were purified using VLC. Because the fraction A express properties very non-polar phase in which it is difficult to purify using an appropriate solvent system. Therefore, fraction B was selected to further purification corresponding to the solvent system.

To purify fraction B about 3.09 gram, it was conducted using several eluents which is eluent of n-Hexane : Ethyl Acetate (85 %), and n-Hexane : Chloroform (40 -50 %). The result was shown in **Figure 2**. Purification using an eluent of n-Hexane : Chloroform 40 % provides separation effectively. Wherefore purification of fraction B further was taken on using eluents of n-Hexane 100 %, n-Hexane : Chloroform (98 %, 95 %, 90 %, 85 %, 80 %, 75 %, 70 %, 65 %, 60 %, 55 %, 50 %, and 40 %), in sequence. As much as 26 fractions was aquired from fraction B as shown in **Figure 3**. Of 26 fractions that have the same TLC pattern, it is combined and concentrated using a vacuum rotary evaporator. The combination of the fractions is gained 5 fractions which is fractions of B_1 (1-2), B_2 (3-6), B_3 (7-19), B_4 (20-23), and B_5 (24-26). The TLC results (see **Figure 3**) showed that only fraction B_1 still contained terpenoids that be marked with 2 red spots.

A B c

Figure 2. Profiles of TLC chromatogram of fraction B using eluents:

(a) n-hexane : ethyl acetate 85 %;

(b) n-hexane : chloroform 50 %;

(c) n-hexane : chloroform 40 %

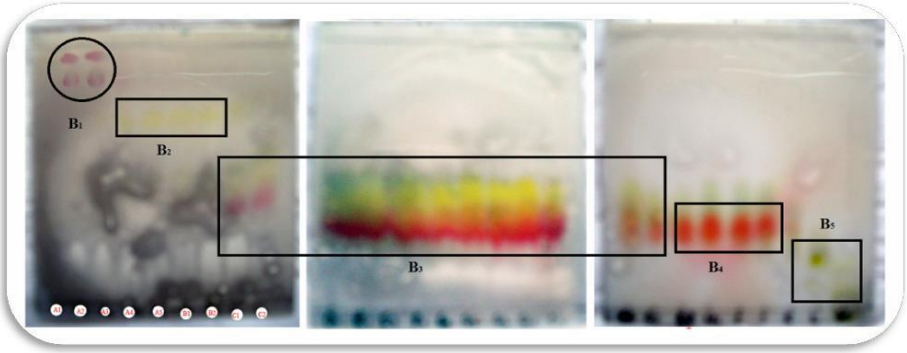

Figure 3. Profiles of TLC chromatogram of fraction B using an eluent of n-hexane: chloroform 50 %

The result of TLC chromatogram profiles was indicated that fraction B_1 contains an abundance of terpenoid compounds with a track spot on the TLC chromatogram. Therefore, it is further separated using VLC and impregnated with gel silica Merck 60 GF_{254} as a reserved-phase. As much as 0.33 gram of fraction B_1 sample was purified with an appropriate solvent system which is eluents of n-Hexane 100 %, n-Hexane : Dichloromethane (DCM) (98 %, 96 %, 94 %, 92 %, 90 %, 88 %, 86 %, 84 %, 82 %, 80 %, 78 %, 76 %, 74 %, 72 %, and 70 %), in order. After that, the VLC result is acquired 29 fractions in which several samples are carried out to the TLC assay that is shown in **Figure 4**.

The same of TLC tracks were merged from 29 fractions in which it is obtained 12 fractions of $B_{1.1}$ (1-2), $B_{1.2}$ (3), $B_{1.3}$ (4-7), $B_{1.4}$ (8-11), $B_{1.5}$ (12), $B_{1.6}$ (13), $B_{1.7}$ (14), $B_{1.8}$ (15-16), $B_{1.9}$ (17-18), $B_{1.10}$ (19), $B_{1.11}$ (20-21), and $B_{1.12}$ (22-29). Of all that, a red spot on the TLC profile is to be a parameter to identify the terpenoid compound. Through investigation on the TCL tracks, fractions of $B_{1.3}$, $B_{1.5}$, and $B_{1.11}$ are selected according to the red spot with R_f value in sequence 0.86, 0.78, and 0.34 using an eluent of n-Hexane : DCM 50 %, respectively. Meanwhile, at a fraction of $B_{1.4}$ be discovered, that the fraction is a combination between a fraction of $B_{1.3}$ and a fraction of $B_{1.5}$, which can be separated and merged furthermore (see **Figure 5**).

a B c

Figure 4. Profiles of TLC chromatogram of fraction B_1 using eluents:

(a) n-hexane : chloroform 60 %;

(b) n-hexane : chloroform 40 %;

(c) n-hexane : DCM 70 %

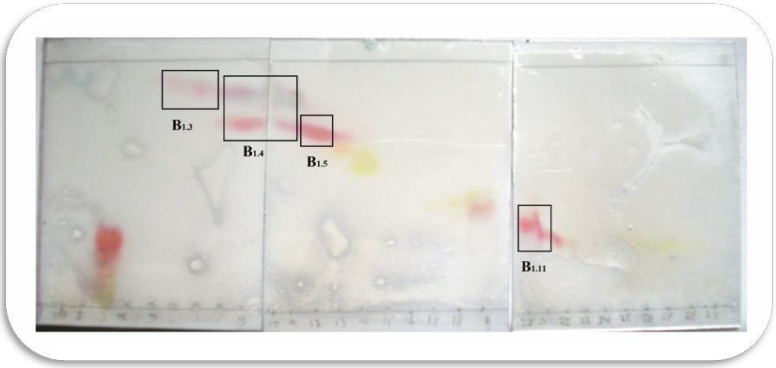

Figure 5. Profiles of TLC chromatogram of fraction B_1 using an eluent of n-Hexane: Dichloromethane (DCM) 70 % ($B_{1.3}$, R_f = 0.86; $B_{1.4}$, R_f = 0.86 and 0.78; $B_{1.5}$, R_f = 0.78; $B_{1.11}$, R_f = 0.34)

As much as 0.023 g of fraction $B_{1.4}$ sample was separated to identify an appropriate separation solvent system. The TLC pattern result has represented that eluent of n-Hexane 100 % is effective to separate. Therefore, the elusion process using VLC has obtained 5 fractions, which each 10 mL of the several samples is carried out to the TLC assay shown in **Figure 6**. Of the 5 fractions through evaluation of the TLC pattern and the R_f value of fraction $B_{1.4}$, that it can be merged as a fraction of $B_{1.4.1}$ (1-3) which is combined with fraction $B_{1.3}$ as well as a fraction of $B_{1.4.2}$ (4-5) what is merged with fraction $B_{1.5}$ (see **Figure 7**).

Figure 6. Profiles of TLC chromatogram of fraction $B_{1.4}$ using an eluent of n-Hexane 100 %

Figure 7. Profiles of TLC chromatogram of fraction $B_{1.4}$ using an eluent of n-Hexane : DCM 50 % ($B_{1.4.1}$, R_f = 0.78 and $B_{1.4.2}$, R_f = 0.86)

Via the purifications that have been carried out, several fractions containing terpenoid compounds are fractions of $B_{1.3}$, $B_{1.5}$, and $B_{1.11}$. However, of the three fractions, only fraction $B_{1.3}$ was abundant to carry out the purity assay, bioactivity assay, and spectrometry analysis. Therefore, to purify of fraction $B_{1.3}$, it is conducted TLC assay using eluents of n-Hexane 100 %, n-Hexane : DCM 95 %, and n-Hexane : Acetone 70 % as well as sprayed Liebermann-Burchard reagent and used UV lamp by a wavelength of 254 nm to visualize of the spot. After the visualization process using a UV lamp at a wavelength of 254 nm, it demonstrated that there was no spot on the TLC pattern. Whereas in the visualization with Liebermann-Burchard reagent, the TLC pattern was discovered a red spot of it using these eluents inclusive of different R_f values were acquired in the sequence, which is 0.27, 0.59, and 0.91, respectively. The results are shown in **Figure 8**.

Based on the result, fraction $B_{1.3}$ is able to conclude that it contains the terpenoid compound in the form of colorless liquid oil with a weight of 0.0225 g. Therefore, to ensure the terpenoid compound of the fraction $B_{1.3}$, it carried out structural elucidation using analysis of GC-MS and FTIR spectrophotometer and bioactivity assay as a repellent against termites.

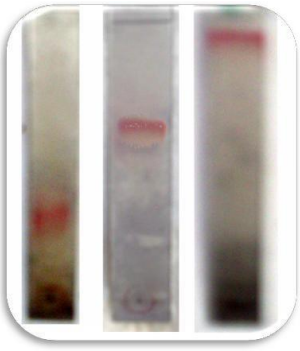

Figure 8. Profiles of TLC chromatogram of fraction $B_{1.3}$ using eluents:

(a) n-Hexane 100 %; Rf = 0.27

(b) n-Hexane : DCM 95 %; Rf = 0.59

(c) n-Hexane : acetone 70 %; Rf = 0.91

3.4. Bioactivity Assay of Isolation Compound

To determine the bioactivity of terpenoid compounds isolated from vetiver grass-root as fraction $B_{1.3}$, therefore, it was carried out assay as a repellent against termite using the same method as the bioactivity test for crude extract. Vetiver grass-root isolated compound was also active against termite (*Cryptotermes sp.*) in our study (see **Table 3**). These conclusions, based on parameters of % attractive and attractiveness index (AI), which is an average value of 0.025% and (-0.039), respectively. A negative value of AI indicates the compound effective as a repellent against termites because of AI value ≤ -0.2, categorized as a repellent according to [9].

Table 3. Bioactivity assays of the isolated compound as a repellent against termites

Times (Hours)	S	C	B	L	% Attractive	Attractiveness Index (AI)
1	1	2	2	0	0.05	-0.05
2	1	2	3	0	0.05	-0.11
3	0	4	2	1	0	-0.11
4	0	1	1	4	0	-0.05
5	0	2	1	5	0	-0.05
6	1	2	0	0	0.05	0.05
7	1	1	1	5	0.05	0
8	0	3	0	0	0	0
9	1	2	1	0	0.05	0
10	0	3	1	5	0	-0.05
∑ (Total)	5	22	12	20	0.25	-0.39
Average	1	2	1	2	0.025	-0.039

Annotation: S is the number of insects on the sample; C is the number of insects as a control; B is the number of insects on the blank; L is the number of die insects.

3.5. Structural Properties

3.5.1. Fourier Transform Infrared (FTIR) Spectrophotometer

The FTIR spectra of isolated compounds of fraction $B_{1.3}$ are shown in **Figure 9**. By the spectrum examination, it gives an absorption band of N-H stretching vibrations (primer amine) at 3415.30 cm^{-1} and 3477.16 cm^{-1} regions that are supported by N-H bending at 1618.56 cm^{-1} regions. In other areas, it shows the absorption of C-H stretching vibration (short-chain alkanes) at 2924.85 cm^{-1} that are supported by uptake at 1386.14 cm^{-1} and 1457.43 cm^{-1}, which is C-H absorption of methyl and methylene. This result corresponds to the fragmentation spectrum of GC-MS spectrometry measurement. For detail, the bonding properties of FTIR are summarized in **Table 4**.

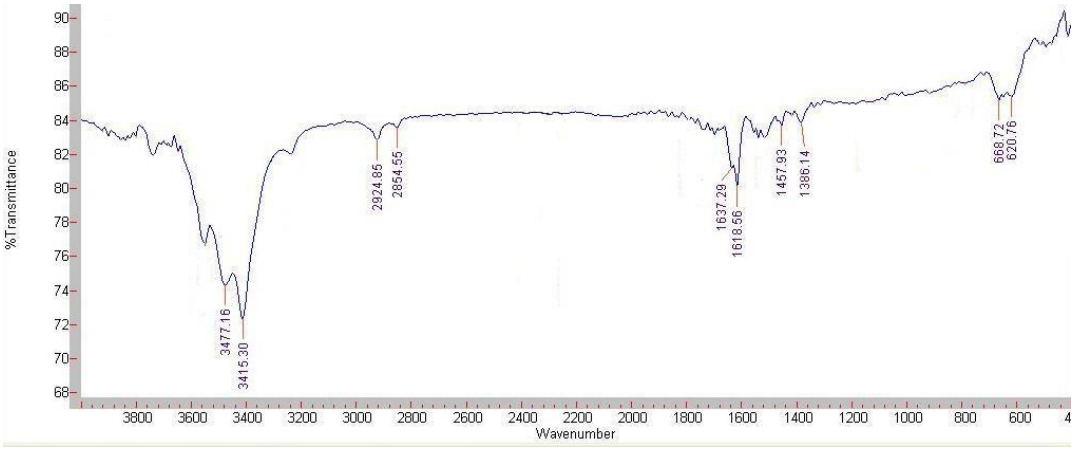

Figure 9. FTIR spectra of isolated compounds of fraction $B_{1.3}$

Table 4. FTIR absorption areas of the isolated compound of fraction $B_{1.3}$

Absorption areas (cm^{-1})	Vibrations of functional groups
1386.14	C-H bending (Methyl)
1457.43	C-H bending (Methylen)
1618.56	N-H bending (Primer Amine)
2924.85	C-H stretching (Alkane)
3415.30 and 3477.16	N-H strecthing (Primer Amine)

3.5.2. Gas Chromatography-Mass Spectrometry (GC-MS)

Figure 10 shows that a typical MS fingerprint of the isolated compound of fraction $B_{1.3}$ extracted from Vetiver grass-root gives data to interpret. The mass spectrum of the sample is mainly characterized by abundant ion peak M^+ = 57.2 (100%) as a base peak and m/e = 87.1. The ion fragment peaks are appeared at m/e = 71.1; m/e = 57.2; m/e = 55.2; m/e = 43.1; m/e = 41.1; m/e = 39.1; m/e = 29.1; and m/e = 27.1, respectively. In this study, the retention times are obtained 2.783 minutes based on gas chromatography measurement. Related to FTIR spectra and functional groups vibration, and also based on Mass spectra fingerprint of the sample in which it was implied that the isolated compound of fraction $B_{1.3}$ sample has $C_5H_{13}N$ chemical formula.

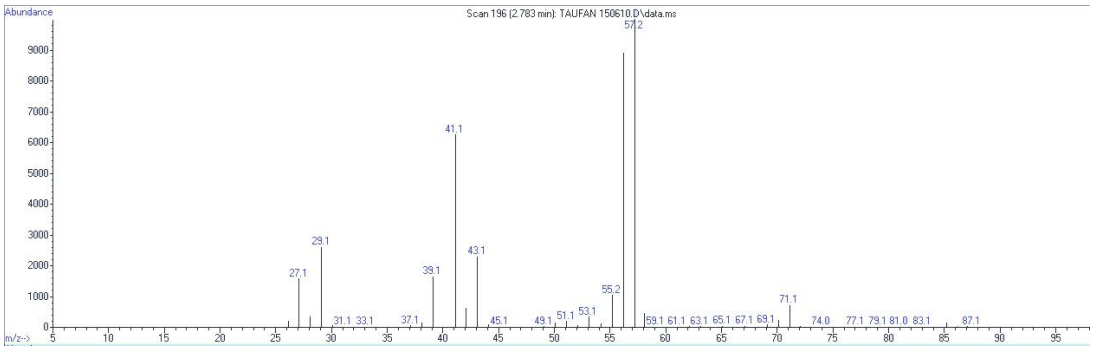

Figure 10. Mass spectra of isolated compounds of fraction $B_{1.3}$

To show the structure of bonding of isolated compound of fraction $B_{1.3}$, a DBE (Double Bond Equivalent) calculation is performed as follows:

$$\text{DBE} = \text{Amount of C atoms} - \frac{Amount\ of\ H\ atoms}{2} + \frac{Amount\ of\ N\ atoms}{2} + 1$$

$$\text{DBE} = 5 - \frac{13}{2} + \frac{1}{2} + 1 = 0$$

The DBE result is implied that there are no double or cyclic bonds on the structures. Besides, through MS spectra data, compounds undergo fragmentation by releasing the N-H group at m / e = 71.1 and also breaking the C-H bond at m / e = 57.2.

3.6. *Structural Elucidations*

The compound of fraction $B_{1.3}$ that shown in **Figure 9**, was isolated from vetiver grass-root (*Vetiveria zizanioides* Stapf). Through GC-MS analysis, it was obtained MS spectrum showing a molecular ion peak at $M^+ = 57.2$ (100%) as a base peak. Other analyses included FTIR and TLC assay is acquired N-H group, C-H group of methyl and methylene corresponding to FTIR spectra as well as an R_f value of 0.27 (eluent of n-hexane 100 %), 0.59 (eluent of n-hexane: DCM 95 %), and 0.91 (eluent of n-hexane: acetone 70 %), respectively. All these analyses were also indicated the molecular weight of 87.1 g/mol which is the molecular formula $C_5H_{13}N$, suggesting it is a precursor terpenoid because of C<10 as a single form isoprene unit. Therefore, based on investigated that the proposed structure for fraction $B_{1.3}$ is a 2-methyl butane-1-amine with the elucidation information.

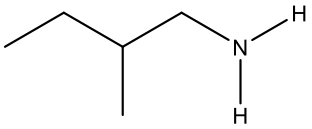

Figure 11. The proposed structure of 2-methyl butane-1-amine

4. Conclusions

In conclusion, the bioactivity of the isolated compound from the vetiver grass-root indicates that the compound is effective as a repellent against termites because of AI value = -0.039 with parameter AI value ≤ -0.2 is categorized as a repellent. The elucidation structure also demonstrates that the molecular weight is 87.1 g/mol, which is the molecular formula $C_5H_{13}N$, suggesting it is a precursor terpenoid because of C<10 as a single form isoprene unit. Therefore, the proposed structure for fraction $B_{1.3}$ is a 2-methyl butane-1-amine with the elucidation information.

Conflicts of interest

"There are no conflicts to declare."

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