Antitermite activity of methanol extract of lichen *Teloschistes flavicans* (Sw) Norman against *Coptotermes curvignathus*

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Abstract. Antitermite activity methanol extract of lichen *Teloschistes flavicans* (Sw) Norman was investigated in the laboratory scale. No-choice test was used as method with 5 concentrations tested of 1.25%, 2.5%, 5.0%, 6.25% and 7.5% w/v. The termiticide activity showed that the mortality of termites was increased every day as well as the concentration of the extract increased, at the concentration of 7.5% all termite dead at fourth days. Termiticide activity of methanol extract of *T*. flavicans was influenced by the GC-MS data that showed 78 chemical components. Vicanicin, methyl oleate, methyl palmitate, and patchouli alcohol was detected as major components that be expected as termiticide. *T*. flavicans might be possible as a botanical termiticide in the near future.

1. Introduction
The economic losses caused by insect attacks annually around the world reached US$ 470 billion [1]. Termites are a highly devastating insect pest, which causes damage to buildings, furniture, plants, and agricultural crops. In the present, the management of these insect pests is commonly achieved using synthetic chemicals. Some synthetic insecticides have a negative impact on the environment, animals and humans. Therefore, research has been carried out based on natural materials that are more environmentally friendly, called botanical insecticide [2].

Lichens are symbiotic associations between a filamentous fungus and algae and/or a cyanobacterium. Lichens are known as indicators of air pollution, and their adaptability in habitat enable them to produce unique naturally occurring secondary metabolites. Extracts, fractions, and isolated compounds of lichens exhibited biological activities such as antioxidant, anticancer, insecticidal, herbicidal, antibacterial, and fungicidal activities [3]. Emsen *et al.*, [4] reported that extracts of three lichens species *Lecanora muralis*, *Letharia vulpina* and *Peltigera rufescens* demonstrated insecticidal effect against adults of *Sitophilus granarius* (L.). *Parmotrema cristiferum* and *Dirinaria applanata* showed insecticide activity against *Aedes aegypti* mosquito larvae [5]. *Usnea* sp. reported to have anti-termite activity, the active compounds are atranorin and usnic acid [6]. Some compounds were isolated from lichens such as fumarprotocetraric acid, barbatic acid, vulpinic acid, diffraactaic acid, gyrophoric acid, and physodic acid was potent as insecticidal agents to prevent and control insect pests that cause damages to plants and transmit diseases [7].

*Teloschistes flavicans* (Sw) Norman was known as golden hair lichen or “Jenggot Mas” in Indonesia. *T*. flavicans exhibited anti-inflammatory, fungicide, and antibacterial activities [8-10]. Based on the literature search, there have been no studies show that antitermite activity of *T*. flavicans. In this study,
we reported the anti-termite activity of the methanol extract of *T. flavicans* against *Coptotermes curvignathus*, as well as determine its constituents by GC-MS.

2. Methods

2.1. Plant material

*Teloschistes flavicans* was collected from the trees in Rejang Lebong, Bengkulu Province, Indonesia during June 2018. Identification of the plant material was performed by Herbarium Bogoriense, Indonesian Institute of Sciences (LIPI).

2.2. Extraction

Fresh *T. flavicans* as much as 1230 g were cleaned and cut into small pieces about 0.5-1.0 cm and then macerated at room temperature using methanol (7x2 L) for 4 days. The filtrate was evaporated using a rotary evaporator to yield a methanol extract [11].

2.3. Analysis of chemical components by GC-MS

The chemical components of methanol extract of *T. flavicans* were analyzed using a gas chromatography-mass spectrometry (GC-MS) Shimadzu Qp-2010 with Rtx 5 MS column (30 m x 0.25 mm) using Helium as a carrier gas. Operating conditions at an column temperature from 70°C (5 min) to 260°C (29 min) at 5°C/min; injection temperature of 300°C. The mass spectrometer was operated in electron-ionization (EI) mode at 70 eV. The compounds were identified based on the comparison of their retention time (RT) and mass spectra of Wiley 22, and NIST 62 library data of the GC-MS system. The percentage of components was calculated by the GC peak area [12].

2.4. Termiticidal activity

The termites were obtained from the trees that were attacked by *Coptotermes curvignathus* Holmgren in field around the University of Bengkulu main campus. A no-choice test was employed for evaluating termiticidal activity. The diameter of the filter paper used is 90 mm (Whatman no.3), and before it is used the filter paper was dried in an oven at 60°C to a constant weight. *T. flavicans* methanol extract was dissolved with CHCl₃, to get the test concentration of 1.25%, 2.5%, 5.0%, 6.25% and 7.5% w/v. Each concentration of the test was dropped as much as 1 mL on filter paper feed, and the papers were dried overnight at room temperature and 1 hour in a vacuum desiccator. The control (0.0%) was given the same treatment as the test group but without the extract of *T. flavicans* (CHCl₃ only). Subsequently, each dried paper was put into a Petri dish (90 mm in diameter × 20 mm in height), and then twenty-two active termites (20 workers and 2 soldiers) from an adult of *C. curvignathus* were added to each Petri. Petri dishes were closed and stored in a dark place at room temperature for 14 days. Water and humidity in Petri dishes were controlled every day. The number of dead termites was counted daily. Four replications were performed for each sample. The termicidal activity was evaluated from the mortality (%) average using the following equations [13]:

\[
\text{Termite Mortality (\%)} = \frac{\text{Number of Dead Termites}}{\text{Total Number of Test Termites}} \times 100\%
\]

3. Results and discussion

3.1. Extraction

Fresh *T. flavican* was macerated using methanol as a solvent and was obtained 95.32 g extract with a yield of 7.75%. The yield of *T. flavicans* methanol extract is quite high where the used of methanol as solvent was able to extract the chemical components of *T. flavicans* thallus.
3.2. **GC-MS analysis**

The chemical constituents of *T. flavicans* methanol extract were characterized by 78 components, and 64 components were identified. The GC-MS analysis has shown the presence of different phytochemical compounds in the methanolic extract of *T. flavicans* classified into fatty acids, long-chain hydrocarbons, depsidone, sesquiterpenes, phenols, phytosterols, ethers, esters, quinones, and furan. GC chromatogram of *T. flavicans* methanol extract can be seen in Figure 1, and its compounds are listed in Table 1.

![Figure 1. The Chromatogram GC of methanol extract Teloschistes flavicans.](image)

Based on Figure 1, the methanol extract of *T. flavicans* contained 78 compounds, the largest peak area is peak number 77 (18.34% peak area) was identified as Vicanicin from depsidone group. The other peak with % area above 5% is peak number 41 (10.12%), 35 (7.91%), and 23 (6.12%) was identified as methyl oleate, methyl palmitate, and patchouli alcohol (Figure 2). The majority compounds of methanol extract *T. flavicans* consist of fatty acids and long-chain hydrocarbons, the results of this study are in accordance with the report of Reis et al. [14]. Vicanicin has also been isolated by Neelakantan et al. from *T. flavicans* [15].

3.3. **Termiticide activity of methanol extract of Teloschistes flavicans against Coptotermes curvignathus**

We evaluated the antitermite activity crude MeOH extract of *T. flavicans* thallus against *C. curvignathus*, the result was shown in Figure 3. Termite mortality increased with increasing extract concentrations. Interestingly, mortality increased significantly starting on the fourth day for all concentration tested. At the highest concentration tested (7.5% w/v) caused all termite dead (100% mortality) only until the fourth day, while the concentrations of 6.25% and 5.0% similarly caused 100% termite mortality on the fifth day, whereas the concentration tested of 2.5% exhibited 100% termite mortality on the seventh day. Exciting, at the lowest concentration of 1.25%, caused 80.7% of termite dead on the seventh day. The concentration of 1.25% slightly out of the termite mortality pattern of methanol extract of *T. flavicans* as a whole, termite mortality was higher than the concentrations of 2.5%, and 5.0% on the first day until the third day, and on the seventh day (80.7%) until 14th day the termite mortality was constant. In Figure 3, it can also be seen that the termite mortality rate of all concentrations tested was higher than the negative control.
### Table 1. The Chemical compounds of methanol extract Teloschistes flavicans

| No | Name                                      | RT (s) | Area % | No | Name                                      | RT (s) | Area % |
|----|-------------------------------------------|--------|--------|----|-------------------------------------------|--------|--------|
| 1  | n-Dodecane                                | 10.39  | 0.63   | 40 | Not Identified                            | 35.77  | 0.28   |
| 2  | Not Identified                            | 11.75  | 0.26   | 41 | Methyl oleate                             | 37.42  | 0.12   |
| 3  | 1-Methyl-1-(2-methyl-2-propenyl) cyclopentane | 15.04  | 0.31   | 42 | Hexadecane                                | 37.67  | 0.73   |
| 4  | Not Identified                            | 17.46  | 0.70   | 43 | Methyl stearate                           | 37.86  | 1.49   |
| 5  | Decane, 2,3,5,8-tetramethyl               | 17.71  | 0.38   | 44 | Oleic acid                                | 38.29  | 3.21   |
| 6  | 4-Methyl-1-undecene                       | 17.89  | 0.29   | 45 | Stearic acid                              | 38.73  | 1.47   |
| 7  | Tridecane                                 | 18.85  | 2.41   | 46 | Octadecyl vinyl ether                     | 39.09  | 1.00   |
| 8  | Eugenol                                   | 20.04  | 2.32   | 47 | cis-6-Octadecenoic acid methyl ester      | 39.47  | 0.25   |
| 9  | β-Gurjunene                               | 20.93  | 0.25   | 48 | Cholestan-4,5-epoxy-,(4α,5α)-             | 39.89  | 0.30   |
| 10 | β-Caryophyllene epoxide                   | 22.79  | 0.30   | 49 | Not Identified                            | 40.14  | 0.36   |
| 11 | α-Patchouline                             | 23.12  | 0.45   | 50 | Heptadecane                               | 41.00  | 0.54   |
| 12 | Not Identified                            | 23.50  | 0.85   | 51 | Linoleoyl chloride                        | 41.20  | 0.61   |
| 13 | 9-Heptadecanone                           | 23.77  | 0.44   | 52 | Tetraatriocanone                          | 41.48  | 1.01   |
| 14 | Alloaromadendrene                         | 24.17  | 0.94   | 53 | Icosylcylohexane                          | 41.87  | 0.48   |
| 15 | Eugenol acetate                           | 24.42  | 0.17   | 54 | 14-Methyl-5α-cholest-8-en-3-one           | 41.97  | 0.50   |
| 16 | Not Identified                            | 24.68  | 0.61   | 55 | Docosane                                  | 42.20  | 0.81   |
| 17 | 9-Heptadecane-4,6-diyne-8-ol             | 24.87  | 0.23   | 56 | 2-Methyl-octadecane                       | 42.74  | 0.53   |
| 18 | cis-3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol | 25.51  | 0.36   | 57 | (2-Dodecyl-1-yl)-succinic anhydride        | 42.92  | 0.27   |
| 19 | 2,2,3-Trimethyl-4-oxo-cyclopentane acetic acid 1,2-Methylenedioxy-5,6-dimethoxy-4-allylbenzene | 26.27  | 0.52   | 58 | 9-Octadecenoic acid methyl ester          | 43.23  | 0.33   |
| 20 | Not Identified                            | 27.09  | 0.22   | 59 | Nonane,3,3,6,9,9-hexamethy                | 43.59  | 0.41   |
| 21 | Not Identified                            | 27.38  | 0.20   | 60 | Heptacosane                               | 44.40  | 0.76   |
| 22 | (+)-Ledol                                 | 28.16  | 1.23   | 61 | 2,6,11,15-Tetramethyl-heptadecane         | 44.98  | 1.20   |
| 23 | Patchouli Alcohol                         | 28.64  | 6.12   | 62 | Methiocarb sulfone                        | 45.33  | 0.94   |
| 24 | 2,6,11-Trimethyldecane                    | 29.09  | 0.36   | 63 | Not Identified                            | 45.63  | 0.54   |
| 25 | 2-Hydroxy-3-(isopropyl)-6-methylocyclohex-2-en-1-one | 29.50  | 1.53   | 64 | Eicosane                                  | 46.00  | 0.46   |
| 26 | Not Identified                            | 29.76  | 0.50   | 65 | 2,3,5-Trimethyl-phenol                    | 46.43  | 1.08   |
| 27 | Not Identified                            | 29.99  | 0.22   | 66 | cis-2-Methyl-5-n-propenylfurra            | 46.96  | 0.41   |
| 28 | 1-Chloro-octadecane                       | 30.26  | 0.64   | 67 | 1,8-Dihydroxy-3-methoxy-6-methyl-9,10-anthraquinone | 47.28  | 0.56   |
| 29 | Atratic acid                              | 31.18  | 0.96   | 68 | Tetracosane                               | 47.54  | 0.58   |
| 30 | Isopropyl myristate                       | 31.64  | 0.21   | 69 | Methyl tetracosanoate                     | 48.02  | 0.44   |
| 31 | 1,2-Epoxyhexadecane                      | 31.91  | 1.37   | 70 | Nonacosane                                | 48.16  | 0.85   |
| 32 | Not Identified                            | 32.43  | 0.26   | 71 | Henecosane                                | 48.79  | 0.54   |
| 33 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol    | 32.83  | 0.72   | 72 | Not Identified                            | 49.04  | 0.52   |
| 34 | n-Nonadecane                              | 33.42  | 1.54   | 73 | Chloroxylenol                             | 49.36  | 3.74   |
| 35 | Methyl palmitate                          | 33.91  | 7.91   | 74 | Ergosta-5,7-dien-3-ol, (3.beta)-         | 50.12  | 0.34   |
| 36 | Not Identified                            | 34.33  | 0.63   | 75 | Octacosane                                | 50.46  | 0.37   |
| 37 | Not Identified                            | 34.47  | 0.25   | 76 | Heptacosanoic acid, methyl ester          | 50.94  | 1.17   |
| 38 | Palmitic acid                             | 34.91  | 4.27   | 77 | Vicanicin                                 | 52.24  | 18.34  |
| 39 | Ethyl palmitate                           | 35.22  | 0.69   | 78 | Hexacosane, 9-octyl-                      | 54.38  | 0.19   |
Figure 2. The structure main components of methanol extract of Teloschistes flavicans.

Figure 3. Termiticide activity of methanol extract of Teloschistes flavicans against Coptotermes curvignathus.

The termiticide activity of T. flavicans methanol extract might be due to the presence of active compounds it contains. GC-MS profiling the methanol extract of T. flavicans showed the presence of the active compounds. Among than 78 chemical components, 4 of which are the major compounds namely vicanicin, methyl oleate, methyl palmitate, and patchouli alcohol. Fatty acids and long-chain hydrocarbons compounds are the largest compounds of methanol extract T. flavicans, followed by the depsidon and sesquiterpene compounds.

Zhu et al. [16] reported that patchouli alcohol has toxic and repellent activity against Coptotermes formosanus Shiraki by causing damage to the tissue of the termite inside exoskeleton. Methyl palmitate demonstrated acaricidal activity against Tetranychus cinnabarinus [17]. Eugenol (peak no. 8) with concentration of 2.32% is thought to have also contributed to the termiticide activity of the methanol extract of T. flavicans, where Chang and Cheng [18] reported that eugenol has strong termiticide activity with the ability to kill 100% of termites C. formosanus after 1 day at a dose of 1 mg/g filter paper feed. Vicanicin is the largest chemical component of T. flavicans methanol extract but has not been reported its antitermite or insect repellent activity. Vicanicin has been reported as an anticancer against prostate cancer cells [19]. The results of this study provide a new contribution to the role of the vicanicin compound as an antitermite, but further research is needed to evaluate the effect of the vicanicin compound singly as an antitermite either termiticide or antifeedant. The other depsidone compounds usnic, fumarprotocetraric and barbatic acids demonstrated their potential as an insecticide against termite Nasutitermes corniger [20].
4. Conclusion

The methanol extract of *T. flavicans* exhibited termiticidal effect to worker and soldier of *Coptotermes curvignathus* due to the presence of vicaincin, patchouli alcohol, eugenol, methyl oleate, and methyl palmitate compounds. It is expected that *T. flavicans* can play a role as bio-insecticides, especially termiticide in the future. Further studies are needed for discovering new bio-antitermite compounds from lichens and investigation their action mechanisms.

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