Phenolics Content and DPPH Free Radical Scavenging Activity of *Dalbergia latifolia* Leaf Ethanolic Extract

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Abstract

Dalbergia latifolia or Sonokeling is one of the most well-known tree species that naturally grows in Java Island, Indonesia. It produces purple streaked wood used in luxurious furniture while its leaf can be utilized as forage. The purpose of this study is to determine the bioactivity and phenol contents of *D. latifolia* leaf ethanolic extract. The antioxidant activity and anti-termite activity were measured through the DPPH method and *Neotermes bosei* activity. Furthermore, phenol contents were determined through colorimetric method and ethanolic extract constituents were identified by GC-MS. The DPPH inhibition of ethanol extract of *D. latifolia* leaf exhibited 138.20±2.14 µg/ml. While the measurement of total phenol and total flavonoid content of *D. latifolia* leaf showed a value of 192.67±9.41 mg GAE/g and 55.23±5.11 mg QE/g of dried extract sample. The termiticidal activity of ethanolic extract showed low activity. The GC-MS detection showed fatty acids as dominant compounds. The inhibition of DPPH by *D. latifolia* leaf ethanol extract in this study suggested this leaf is potent as antioxidant agents.

Keywords: Sonokeling, leaf, antifeedant, termiticidal, flavonoid, *Neotermes bosei*.

Introduction

Antioxidant is an important substance that is usually used in the food and beverages industry, animal feed industry as well as the pharmacological industry and serves to control oxidation (free radical scavengers), retard spoilage, and putative health benefits (Finley *et al*. 2011). In food and beverages and animal feed industry, an antioxidant can maintain the food quality/nutrient stability and lengthen the shelf life, meanwhile in pharmacological application, an antioxidant can prevent drug degradation that produces a lower efficacy drugs until a toxic compound generation in drugs so it might lead to some risks for the patient health (Housheh 2017; Gabrič *et al*. 2022). Due to its importance, the global demand for antioxidants is always growing and predicted to grow. For feed antioxidants only, the global consumption has grown from 120.7 million metric ton in 2017 to 131.2 million metric ton in 2020 according to FAO statistics (Anonymous 2022).

Nowadays, synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), 2,6-di-*tert*-butyl-*p*-benzoquinone (BHT-Q), propyl gallate (PG) and tert-butylhydroquinone (TBHQ) are widely used in food industry (Gülçin 2012). It is inexpensive, has higher antioxidant activity (medium to high) than the natural antioxidant, but can cause some harms (such as DNA damage, cancer induction, tumour promotion, etc) if incorrectly use or being used excessively (Gülçin 2012; Xu *et al*. 2021). Even a low concentration of BHT-Q can cause DNA damage (Liu and Mabury 2020). Recent studies focus on the low toxicity, low migration ability and more environmentally friendly synthetic antioxidants or basically pointing to the natural antioxidant (Liu and Mabury 2020). Therefore, massive exploration in finding the natural antioxidants resources that have high antioxidant activity was conducted.

Natural antioxidants such as polyphenols/phenolic compound (flavonoids, tannin, phenolic acids), terpenoids (carotenoids), ascorbic acid (vitamin C), tocopherol (vitamin E), and unsaturated fatty acids (omega 3) were known to have high antioxidant activity (Ali *et al*. 2012; Barbieri *et al*. 2015; Lv *et al*. 2015; Anwar *et al*. 2018; Wang *et al*. 2019). The polyphenol, a well-known hydrophilic antioxidant, together with their hydroxyl groups will act as inhibitors on the 2,2-diphenyl-1-picrylhydrazyl (DPPH). Moreover, it is easily found in plant tissues and its total compound (the total phenolic compound) is known to be highly correlated with antioxidant activity (Kalaycıoğlu and Erim 2016). For flavonoid (Figure 1a) form, especially its B ring, the donated protons (H) interact with the DPPH and convert it into DPPH-H (Pannala *et al*. 2001; Kongpichitchoke *et al*. 2015) in performing the antioxidant activity. Flavonoids are also found as a high anti termite substances against Coptotermes with its C-5 and C-7 hydroxyl groups in A-rings, carbonyl groups at C-4 in pyran rings, and 3-hydroxyflavones and 3-hydroxyflavanones with 3',4'- dihydroxylated B-rings (Ohmura *et al*. 2000). Those phenolic compounds were highly extracted by ethanol, methanol and their mixture (Alara *et al*. 2021). Meanwhile, terpenoid (especially their carotenoid form) and unsaturated fatty acids (see Figure 1b and 1c for chemical structure of monoterpene and fatty acid) that classified as lipophilic antioxidant are depending on their singlet oxygen quenching, hydrogen transfer, or electron transfer and also their long-chain polyunsaturated fatty acids for their antioxidant activity performance (Graßmann 2005; Richard *et al*. 2018). Terpenoid and unsaturated fatty acids were highly extracted by n-hexane and dichloromethane solvents. All those natural antioxidants with high potential development were found in some trees and non tree plants such as *Diospyros abyssinica*, *Acacia auriculiformis*, *Ficus microcarpa*, *Polyalthia cerasoides*, *Uncaria tomentosa* etc in Africa, Algeria, USA, Australia, Brazil, Bulgaria, China, India, Iran, Italy, Japan, Turkey, Poland, Portugal, Thailand, and Malaysia (Krishnaiah *et al*. 2011).

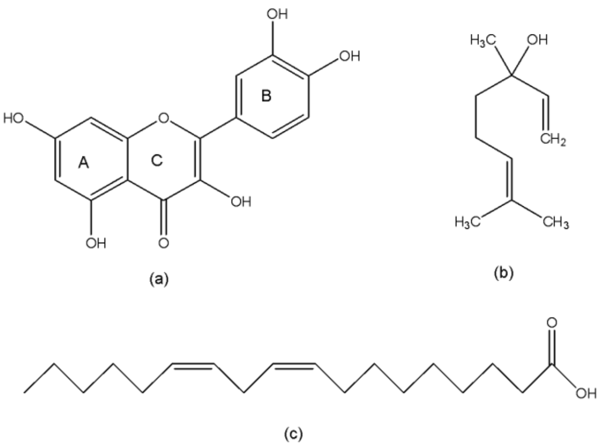

Figure 1. Chemical structure of quercetin (a), linalool (b), and linoleic acid (c).

Each country or each area has its own resource potency of the natural antioxidant that is influenced by the geography, climate, local preference, local wisdom, government policy etc. One of trees that is naturally grown and widely cultivated in Indonesia is *Dalbergia latifolia* Roxb, commonly known as Sonokeling. It is classified as the Fabaceae family and widespread in Indonesia and India natively and in Kenya, Malaysia, Myanmar, Nepal, Nigeria, Philippines, Sri Lanka and Vietnam exoticly (Sukhadiya *et al*. 2020). The wood from this species is utilized as furniture materials and the wood is categorized as fancy wood, meanwhile the leaves are used as forage and in Yogyakarta Indonesia, this species is easily found in community forest in Bantul and Gunungkidul Regency (Santoso *et al*. 2021; Jariyah and Wahyuningrum 2008). This tree might have a high potency of the natural antioxidant resources as other trees in the same genus show the potency. Several *Dalbergia* genus leaf had been studied as natural antioxidant resources and showed a potential results such as *D. katangensis* (Valentin *et al*. 2020), *D. saxatilis* (Koma and Fakunle 2014), *D. odorifera* (Ma *et al*., 2020), *D. sissoo* (Rijhwani and Bharty 2016), *D. paniculata* (Ganga *et al*. 2012), *D. ecastaphyllum* (Lucas *et al*. 2020), and *D. brasiliensis* (Dalarmi *et al*. 2017) in various extractions.

The natural antioxidant of *Dalbergia latifolia* Roxb from its wood and its bark has already been studied in our previous study and shows potential results (Khalid *et al*. 2011; Masendra *et al*. 2020). But the utilization of wood as the natural antioxidant resources is constrained by other wood uses (i.e. furniture and home furnishings). On the other hand, some studies showed that the antioxidant level of leaf is higher than bark (Katekhaye and Kale 2012; Krishna and Nair A 2010). Therefore, the leaf of *Dalbergia latifolia* might be a potential antioxidant resource as the other Dalbergia genus leaf also shows the potency. However, the information of *D. latifolia* leaf extract and its bioactivity is still limited. Due to the natural antioxidant exploration and the demand of its fulfilment, the information of antioxidant activity and phenol contents of *D. latifolia* leaf extract was investigated in this study. The anti-termite activity of the *D. latifolia* leaf extract was also investigated as the leaf might have high phenolic compounds. The ethanolic extracts were used based on the ethanol ability in dissolving the phenolic compounds as well as its renewable and non-poisonous solvent that have a low risk for acute toxicity in pharmaceutical manufacturing processes (Araújo *et al*. 2019).

Materials and Methods

Chemicals and Reagents

Gallic acid, DPPH, and quercetin were purchased from Sigma-Aldrich (Germany), while tectoquinone (2- methyl anthraquinone, 25753-31) was purchased from Kanto Chemical, Japan.

Leaf Maceration

Fresh leaf of *Dalbergia latifolia* (30 g) from a ten-year-old tree (collected in Bantul, Yogyakarta, Indonesia) was macerated with ethanol at room temperature for three days. The solution was then filtered and evaporated through a rotary evaporator. The dried extract was weighed and compared to the fresh initial sample (in percentage). In addition, the leaf maceration was conducted in one replication.

Total Phenolic Content

Total phenolic content was measured with the Folin-Ciocalteu method, referring to Diouf *et al*. (2009). As much as

0.5 ml of *D. latifolia* ethanolic extract (1000 ppm) was reacted with 2.5 ml of Folin-Ciocalteu reagent. The reaction was then left to react for 2 min at room temperature and then 2 ml of sodium carbonate was mixed (7.5% aqueous). Then, the solution was left to react at room temperature for 30 min. The absorbance of the sample was read at 765 nm and the results were expressed as gallic acid equivalents (mg GAE/g based on dry extract weight).

Total Flavonoid Content

The total flavonoid assay was conducted according to Brighente *et al*. (2007). A 2 ml of sample extract (1mg/ml) was mixed with 2% of $AlCl_3.6H_2O$ solution in methanol. The reaction was allowed for 60 min at 20 °C before measuring the absorbance. The absorbance of the mixture was then measured at 415 nm using a spectrophotometer. The results were expressed as quercetin equivalents (mg QE/g) of the sample extract.

Antioxidant Activity

The antioxidant measurement was conducted through literature (Baba and Malik 2015). The DPPH inhibition was measured through the response of 0.1 ml sample with 0.1 mM DPPH. To assess antioxidant activity with IC_{50} value, the sample was measured in different concentrations, and each sample was measured in three replications. In addition, the standard of quercetin is used for comparison as a positive control. The antioxidant activity is calculated through below equation (1):

Antioxidant activity (%) = (Ao-A1)/Ao

(1)

Where Ao is the absorbance of blank and A1 is the sample absorbance.

Anti-termite Activity

In this experiment, a glass with diameter of 7 cm and height of 10 cm, containing sand was used as a place for the termite test. Paper discs with a diameter of 8 mm (Whatman International) containing 5 % (w/w) sample extract (dissolved in methanol) were prepared. The paper discs that contain ethanolic extract of *D. latifolia leaf*, tectoquinone (positive control), and methanol (negative control) were made in three replications. The paper disc was dried in the oven at 60 °C to evaporate the methanol solvent. Twenty worker termites (*Neotermes bosei*) were introduced into the glass with the condition of the termite environment: relative humidity of 70-80% at room temperature. After 21 days the paper discs were taken out, dried and the mass loss was observed. To measure the termiticidal activity, the number of dead termites was also calculated. For negative controls, the starvation sample also was made without feeding.

Gas Chromatography-Mass Spectra (GC-MS)

The 1 µl of ethanolic extract solution with 1000 µg/ml concentration was directly injected into the GC-MS (GCMS-QP 2010, Shimadzu, Japan). The GC column was Rtx-5MS capillary column (30 m x 0.25 mm I.D. and 0.25 µm; GL Sciences, Tokyo, Japan). The detection temperature was 285 °C, the column temperature from 70 °C (2 min) to 290 °C at 5 °C/min, and the injection temperature of 200 °C. The acquisition mass was from 50 to 500 atomic mass units and helium was the gas carrier. The mass spectra from the NIST library were used for comparison with the mass spectra of the sample and the quantification of the compound was calculated with the peak relative method.

Results and Discussions

Yield Extractive and Polyphenols

The extractive content of *D. latifolia* leaf was 2.792 g or 9.27% based on the fresh leaf. The higher extractive content of *D. latifolia* leaf shows that alcohol is one of the good solvents to dissolve the leaf extract. The average total phenols and total flavonoids of *D. latifolia* leaf ethanolic extract are 192.67 and 55.23, respectively (Table 1). This total phenol is 12 times higher than *D. odorifera* leaf ethanolic extract (16± 0.15 mg GAE/g) (Ma *et al*. 2020). Compared to the bark ethanolic extract (210±1.56 mg GAE/g), the total phenol of the leaf from this study is slightly lower. But the total flavonoid of the leaf is slightly higher than the bark (46±3.61 mg GAE/g) (Khalid *et al*. 2011). This suggests that the utilization of *D. latifolia* for pharmacological material might preferably come from leaf due to its faster growth than bark, especially when the Indonesian climate supports the growth all year around.

Table 1. Total phenol, total flavonoid, and antioxidant activity of *D. latifolia* leaf ethanolic extract

Parameter	Value
Total phenol (mg GAE/g)	192.67± 9.41
Total flavonoid (mg QE/g)	55.23± 5.11
DPPH scavenging activity IC50 (µg/ml)	138.20 ± 2

Antioxidant and Anti-termite Activity

The antioxidant activity (IC_{50}) of the *D. latifolia* leaf extract against DPPH radical is shown in Table 1. Compared to the positive control (IC_{50} of quercetin: 47 µg/ml), the *D. latifolia* leaf extract also showed 3 times lower inhibition of DPPH. According to Molyneux (2004), the antioxidant activity of *D. latifolia* leaf ethanolic extract is categorized as moderate activity. In comparison, the resulting activity of antioxidants is stronger than the bark of *D. latifolia* (170 µg/ml) (Khalid *et al*. 2011).

The termiticidal activity of *D. latifolia* leaf ethanolic extract is shown in Figure 2-3. On the no choice antifeedant assay, the paper disc with ethanolic extract was 100% eaten by *N. bosei* which has the same value as the negative control.

The mortality number (Figure 2) of *D. latifolia* leaf ethanolic extract also was similar to that of positive control and lower than that of tectoquinone. The similarity of the mortality rate with the positive control indicates that the toxicity of the samples came from the MeOH solvent instead of the ethanolic extract. These results showed that *D. latifolia* leaf ethanolic extract did not exhibit any antifeedant nor toxicity to *N. bosei* termite. Compared to a similar study, the result differed from *Cibotium barometz* ethanolic leaf extract which was able to exhibit both antioxidant and anti-termite activity (Musman *et al*. 2019). Increasing extract concentration on the paper discs might increase its effectiveness on the termite.

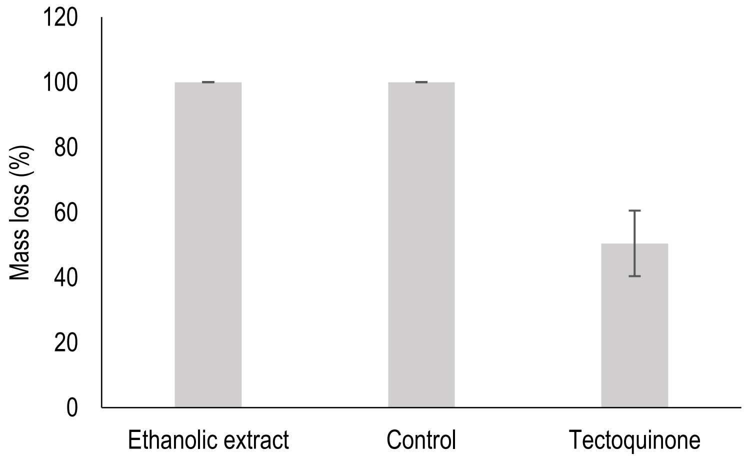

Figure 2. The mass loss of paper disc attacked by *N. bosei.*

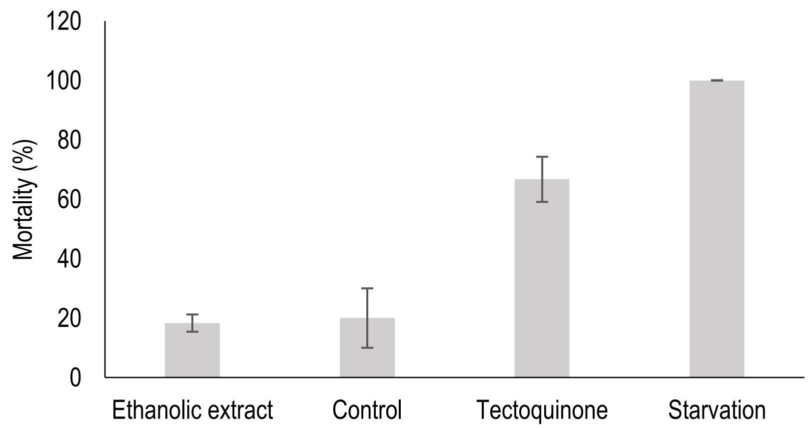

Figure 3. The mortality rate of *N. bosei* against ethanolic extract of *D. latifolia* leaf.

Fatty Acids and Alcohols

Table 2 shows the composition of ethanolic extract of *D. latifolia* leaf. The GC-MS detection by direct injection method exhibited fatty acids and alcohols in the sample. The dominant compound of fatty acid was found as 17-octadecynoic acid, palmitic acid, and linoleic acid, respectively. In the fatty alcohols, the major compound was 14-methyl-8-hexadecyn-1-ol. Other fatty alcohols in the *D. latifolia* leaf extract were phytol and pentadecadien-1-ol. Compared to previous work, these main fatty acids were different from the main types of fatty acid of *D. odorifera* leaves (palmitic acid, linoleic acid, oleic acid) (Ma *et al*. 2020) and *D. ecastaphyllum* leaves (palmitic acid and linolenic acid) (Lucas *et al.* 2020). 17-octadecynoic acid was also detected in other Magnoliopsida plants, i.e. *Momordica cymbalaria* Fenzl and this fatty acid has an anti-hypertensive effects (Gopu *et al*. 2021).

Table 2. GC-MS result of *D. latifollia* leaf ethanolic extract

No	Retention time (min)	Constituents	Percentage	Similarity index (%)
1	27.4	Methyl isohexadecanoate	3.85	95
2	28.4	Palmitic acid	19.17	94
3	30.2	9,12- Octadecenoic acid	3.51	96
4	30.2	Methyl 10-octadecenoate	2.03	96
5	30.5	Phytol	0.53	93
6	30.6	Methyl stearate	1.26	94
7	31.1	17-Octadecynoic acid	20.35	91
8	31.5	Stearic acid	4.63	92
9	31.6	Methyl 9,12-octadecadienoate	0.57	86
10	33.3	Dipalmitin	11.06	85
11	33.4	Arachidic acid	1.52	74
12	33.6	Methyl 18-methylnonadecanoate	0.91	92
13	35.8	Linoleic acid	14.99	89
14	35.9	Pentadecadien-1-ol	1.65	85
15	36.1	Dipalmitin (isomer1)	2.57	84
16	38.7	Dipalmitin (isomer 2)	1.15	84
17	39.6	Diethyl *n*-hexadecylmalonate	0.53	73
18	41.9	Isopropyl linoleate	0.64	84
19	44.8	14-Methyl-8-hexadecyn-1-ol	8.21	89
20	47.1	Palmitone	0.86	90

Attribution of Phenol Contents, Fatty Acids and Alcohols to Antioxidant and Termiticidal Activity

In this study, the free radical scavenging of ethanolic extract of *D. latifolia* leaf was categorized as moderate activity. However, the termiticidal activity was categorized as low activity. It indicates that the phytochemical screening results of *D. latifolia* leaf e.g., phenol contents, fatty acids and fatty alcohols are responsible for DPPH inhibitor rather than for anti-termite activity of *N. bosei*. In previous works, the correlation of phenol contents (total phenol and total flavonoid) and DPPH scavenging activity was observed in leafy vegetables and fruit crops (Chandra *et al*. 2014), *Lantana camara* leaf (Kumar *et al*. 2014), *Ceropegia* species (Chavan *et al*. 2013), and indigenous herbs in Indonesia (Muflihah *et al*. 2021).

In correspondence of fatty acids and alcohols, the compounds that corroborate inhibition of the DPPH is from unsaturated fatty acids and alcohols. In Table 2, the unsaturated fatty acids and alcohols were 9,12- octadecenoic acid, methyl 9,12-octadecadienoate, linoleic acid, pentadecadien-1-ol, and isopropyl linoleate. These unsaturated fatty acids and alcohols, especially the dominant compound of linoleic acid potent to act as antioxidants. This agrees with previous works that linoleic acid and conjugated linoleic acid exhibited as DPPH inhibitor (Ali *et al*. 2012; Lv *et al*. 2015). However, as the phenolic compounds were the most responsible for DPPH inhibitor, the investigation of these compounds through derivative method (GC-MS) is needed in the future works.

Conclusions

The antioxidant and termiticidal activity of *D. latifolia* leaf ethanolic extract were investigated together with their total phenol, total flavonoid, and GC-MS analysis. Observing phenol contents, the *D. latifolia* leaf contained total phenol of 192.67± 9.41 mg GAE/g and total flavonoid of 55.23± 5.11 mg QE/g. Bioactivity of *D. latifolia* leaf ethanolic extract exhibited IC_{50} antioxidant activity of 138.20± 2.14 µg/ml. The GC-MS detection on the ethanolic extract described fatty acids as dominant compounds. The ability of *D. latifolia* leaf extract to inhibit DPPH suggested that this leaf extract is a potent antioxidant. However, due to the activity of *D. latifolia* leaf ethanolic extract to *N. bosei* was in low value, the presence of phenol contents as well as fatty acids and alcohols in the *D. latifolia* leaf did not act for anti-termite activity, but acted as DPPH inhibitor. This shows that local wisdom regarding the use of *D. latifolia* leaves as animal feed has proven to be nutritious and good for livestock health.

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