Screening of antioxidant and antidiabetic activity from fruit bark, stem bark and leaves of *Gyrinops versteegii* (Gilg.) Domke

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**Abstract.** Agarwood is a commodity that has many benefits for the community, one of which is as medicine. *Gyrinops versteegii* (Gilg.) Domke, as one of plant producing agarwood and an endemic plant in West Nusa Tenggara, has less information of biological activity. The present study aims to investigate the biological activities from *G. versteegii*, particularly for anti-oxidants and anti-diabetic activities and their cytotoxicity. The 1,1-diphenyl-2-picrylhydrazyl (DPPH)-radical scavenging activity assay was used to determine the anti-oxidant activity, whereas the α-glucosidase inhibition assay was conducted to determine the anti-diabetic activity. The cytotoxicity test used brine shrimp lethality test (BSLT) and phytochemical analysis were also conducted. The highest anti-oxidant and antidiabetic activity was found in the methanol extract of leaves from infected *G. versteegii* with IC₅₀ values of 32.89±2.7 and 55.01±2.4 μg/mL, respectively. The cytotoxicity test showed high toxicity with the LC₅₀ value of 1.09 μg/mL. Therefore, their high activities showed that those extracts are potent for antioxidant and anti-diabetic agent.

1. Introduction

Agarwood is a commodity that has many benefits and high economic value. Recently, much attention has been paid to its resins and oil productions. Several technique on agarwood production have been developed. An inoculation technique using fungi such as *Fusarium spp.* are widely used nowadays. Some of them using drill or Agar-Wit to induce the fungi [1-3]. A modified inoculation techniques were also reported on fungal inoculation using a porous nail system and implant technique [4]. Many studies have successfully explored other benefits that can be derived from agarwood-producing plants. *Aquilaria sinensis* are also broadly used as a main ingredient in several health foods including tea, honey and flavour [5]. The phytochemical and antioxidant activity of *A. malaccensis* were studied [6]. The ethanolic extract of *A. sinensis* leaves was reported to have analgesic, anti-inflammatory, and nitrite scavenging activities [7,8]. The essential oil extract from *A. crassna* was a safe material and suggested as a potential anti-tumor candidate [9].

Moreover, *A. sinensis* have been widely explored for their chemical constituents. Isolation compounds from healthy stem of *A. sinensis* successfully confirmed Aquilarin A, a new benzenoid derivative, balanophonin and lariciresinol, which the last two exhibited cytotoxicity against SGC-7901.
and SMMC-7721 cell lines [10]. Other study was also isolated flavonoids including apigenin 7,4 dimethylether, genkwan, quercetin, and kaempferol from wild A. sinensis [5]. Moreover, from methanolic extract of the leaves of A. sinensis were reported the isolation of five new benzophenone glycosides, aquilarinenesides A-E [11]. However, the literatures mostly studied on Agaropsis agarwood from Gyrinops versteegii, another plant producing agarwood, are less informed.

G. versteegii (Gilg.) Domke is mostly distributed in the eastern part of Indonesia [12]. It is an endemic plant in West Nusa Tenggara. However, less reports studied on its biological activities compared with its resinosum heartwood production. The antioxidant assays have been studied from G. versteegii extracts [13]. Furthermore, the antioxidant and cytotoxic activities of the chloroform extract of G. versteegii leaves against Hela cell lines have been reported [14]. Moreover, our previous research reported that agarwood from G. versteegii potent for antioxidant and antidiabetic agents [15]. Nevertheless, the antidiabetic activity and cytotoxicity of different parts of G. versteegii are less studied. Therefore, this study aims to investigate the biological activity from different parts of G. versteegii for their antioxidant and antidiabetic activities as well as their cytotoxicity.

2. Materials and Methods

2.1. Materials

2.1.1. Plant materials

There are 2 types of G. versteegii trees used in this study: health trees and fungi-infected trees. The fruit barks, stem barks and leaves of G. versteegii were collected from each type of trees. G. versteegii were collected from Genggelang, Lombok Utara District, West Nusa Tenggara Barat Province. All samples were dried and then mashed into powder (40 mesh) before extraction. Each sample was extracted using methanol solvent. All specimens were deposited in Laboratory of Bioassay, Non-Timber Forest Product of Research and Development Agency, Mataram.

2.1.2. Chemicals

Quercetin dehydrate, dimethylsulfoxide (DMSO), p-nitrophenyl-D-glucopyranoside, α-glucosidase, 1,1-diphenyl-2-picrylhydrazl (DPPH), bovine serum albumin, and quercetin as a control were purchased from Wako Chemical Co. Ltd. (Osaka, Japan). All solvents were purchased from Wako Chemical Co. Ltd. (Osaka, Japan) and distilled prior to use.

2.2. Methods

2.2.1. Extraction

The fruit barks, stem barks and leaves were dried by avoiding direct sunlight, mashed into powder and stored at room temperature before extracting. The dried material was extracted for 48 hours at room temperature with methanol solvent. The extraction was repeated in twice. The methanol extract was concentrated with a rotary evaporator under reduced pressure. The extracts were dried and prepared for the biological activity assays.

2.2.2. DPPH-radical scavenging assay

Antioxidant activity of the extracts was estimated using the DPPH radical method described by Sukito et al [15]. Approximately 0.1 mL methanolic solution containing 2 to 4 mg of extracts was mixed with 2 mL of methanol, and a methanolic solution of 1,1-diphenyl-2-picrylhydrazl (DPPH) (1mM, 0.5 mL) was then added. The mixture was stirred for 15 seconds, and then left to stand at room temperature for 30 minutes. The absorbance of this solution was then read at 517 nm. Percentage of antioxidant activity was calculated using the following equation:

\[
\text{% Antioxidant activity (AA) = } \left( \frac{A_0 - A_1}{A_0} \right) \times 100
\]

where, A0 is the absorbance of the control and A1 is the absorbance of the extract or standard. Assays were carried out in triplicate and the results were expressed as the mean ± standard deviation. Quercetin was used as standard.

2.2.3. α-Glucosidase inhibitory activity assay

Antidiabetic activity of the extracts was determined by α-glucosidase inhibition assay described by Sukito and Tachibana [16]. The reaction mixture consisted of 250 μL of 5 mM p-nitrophenyl-D-glucopyranoside and 1 mL of 100 mM phosphate buffer (pH 7.0) and was added to a flask containing 250 μL of the sample dissolved in DMSO at various concentrations. The reaction mixture was pre-incubated for 5 min at 37°C and the reaction was then initiated by adding 250 μL α-glucosidase, and
the incubation was continued for 15 minutes. The reaction was stopped by adding 1 mL of 200 mM Na₂CO₃. The activity of α-glucosidase was determined by measuring the release of p-nitrophenol at UV-VIS max of 400 nm. The percentage of inhibitory activity was calculated using the following equation:

\[
\% \text{ inhibition} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100
\]

where, \(A_0\) is the absorbance of the control and \(A_1\) is the absorbance of the extract or standard. The 50% inhibition concentration (IC₅₀) of each compound was determined from the graph of percentage α-glucosidase inhibitory activity against sample concentrations. Assays were performed in triplicate and the results were expressed as mean values ± standard deviations. Quercetin was used as the positive control.

2.2.4. Citotoxicity test
Cytoxicity test was conducted using Brine Shrimp Lethality Test (BSLT) method described by Sukito et al. [16]. In this experiment, the cytotoxicity of each sample is calculated at various concentrations of 10, 100, 250, 500, 750 and 1000 µg/mL in seawater containing 1% DMSO (v/v). Three replications were used for each concentration. A parallel series of tests with the standard potassium dichromate solution (LC₅₀=20-40 µg/mL) the blank control was always included. After 24 hours, survivors were counted, by dissection microscope, and percentage of mortality (% M) of each dose was calculated as compared with control. Cytotoxicity was considered the significant if the LC₅₀ value is less than 20-30 µg/mL.

2.2.5. Phytochemical Analisys
The extract was tested for the phytochemical characteristics according to standard methods described by Sukito et al [15] as below:

*Test for saponin:* About 5 mL of the extract was boiled in 20 mL of distilled water in a water bath and filtered. 10 mL of the filtrate was mixed with 5 mL of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion, which confirms a positive presence of saponin.

*Test for alkaloid:* Various methanol and acetone extracts of the samples were acidified by adding few drops of Dragendorff’s reagent (Potassium bismuth iodide). Appearance of orange red precipitate indicated presence of alkaloids.

*Test for flavonoid:* 3 mL of 1% Aluminium chloride solution were added to 5 mL of each extract. A yellow coloration was observed indicating the presence of flavonoids. 5 mL of dilute ammonia solution were added to the above mixture followed by addition of concentrated H₂SO₄. A yellow coloration disappeared on standing. The yellow coloration, which disappeared on standing, indicates a positive test for flavonoids.

3. Results

3.1. DPPH radical scavenging assay
The antioxidant activity of each methanol extract and quercetin as positive control were evaluated based on their DPPH-radical scavenging activities. To determine the IC₅₀ value of anti-oxidant activity for each extract, further testing was carried out at several concentrations and analysed using a regression equation to obtain the results as shown in Table 1 below.

| Explant type          | IC₅₀ (µg/mL) |
|-----------------------|-------------|
| Fruit barks A         | 43.61±2.5   |
| Fruit barks B         | 41.90±3.3   |
| Stem barks A          | 57.71±2.2   |
| Stem barks B          | 50.27±1.9   |
| Leaves A              | 33.17±2.3   |
| Leaves B              | 32.89±2.7   |
| Quercetin             | 8.59±0.3    |

Note: A: tree without artificial inoculation using fungi; B: tree with artificial inoculation using fungi.
Extracts with the highest scavenging activity were leaves extracts in both of trees with and without inoculating fungi, with the IC\textsubscript{50} value of 32.89±2.7 and 33.17±2.3 μg/mL, respectively. However, none of the extracts of G. verstegii were found to be more active than the standard (quercetin, IC\textsubscript{50} value of 8.59±0.3 μg/mL) for DPPH scavenging activity.

3.2 \(\alpha\)-Glucosidase inhibition assay
The inhibition of \(\alpha\)-glucosidase inhibitory activity was monitored on each methanol extract. The 50% inhibition concentration (IC\textsubscript{50}) of each extract was determined from the graph of percentage \(\alpha\)-glucosidase inhibitory activity against sample concentration as shown on Table 2.

**Table 2.** IC\textsubscript{50} value of inhibitory activity of the enzyme \(\alpha\)-glucosidase methanol extract of fruit barks, stem barks and leaves of G. versteegii

| Explant type  | IC\textsubscript{50} (μg/mL) |
|--------------|----------------------------|
| Fruit barks A | 97.03±3.3                 |
| Fruit barks B | 95.14±2.8                 |
| Stem barks A  | 116.43±5.4                |
| Stem barks B  | 112.67±4.3                |
| Leaves A      | 55.63±3.1                 |
| Leaves B      | 55.01±2.4                 |
| Quercetin     | 5.34±0.2                  |

Note: A: tree without artificial inoculation using fungi; B: tree with artificial inoculation using fungi

The leaves extracts of G. verstegii collected from tree with and without artificial inoculating fungi were shown the highest \(\alpha\)-glucosidase inhibitory activity with the IC\textsubscript{50} value of 55.01±2.4 and 55.63±3.1 μg/mL, respectively.

3.3 Phytochemical screening
The quantitative phytochemical screening was carried out to determine the total phenolic content (TPC), total flavonoid content (TFC), and total saponin/triterpenoid content (TSC) as shown in Table 3.

**Table 3.** Quantitative results of phytochemical screening on methanol extracts of fruit barks, stem barks and leaves of G. versteegii

| Explant type  | Content (% or mg/100 g dried extract) |
|--------------|---------------------------------------|
|              | TPC        | TFC         | TSC      |
| Fruit barks A| 8.28       | 13.12       | 0.99     |
| Fruit barks B| 8.56       | 13.15       | 0.91     |
| Stem barks A | 9.71       | 12.13       | 0.61     |
| Stem barks B | 9.57       | 12.35       | 0.50     |
| Leaves A     | 14.93      | 18.75       | 7.81     |
| Leaves B     | 15.18      | 18.90       | 7.95     |

Note: A: tree without artificial inoculation using fungi; B: tree with artificial inoculation using fungi

The phytochemical screening of methanol extract of different part of G. versteegii revealed the presence of some secondary metabolites such as alkaloid, flavonoid and saponin contents in all extract. Qualitative analysis showed that saponin and tannin were detected in stem barks however absent in leaves and fruit barks (data not displayed in this paper).

3.3 Cytotoxicity test
The cytotoxicity test in this study used the BSLT method with shrimp larvae (Artemia salina Leach.) as a bio-indicator. The result of BSLT on the crude fruit barks, stem barks and leaves methanol extract were shown on Table 4.
Table 4. Value LC$_{50}$ of Brine Shrimp Lethality Test of fruit bark, stem bark and leaves of G. versteegii methanol extract

| Explant type | LC$_{50}$ ($\mu$g/mL) |
|--------------|-----------------------|
| Fruit barks A | 11.01±0.7             |
| Fruit barks B | 10.35±0.4             |
| Stem barks A  | 3.55±0.5              |
| Stem barks B  | 2.63±0.3              |
| Leaves A      | 1.71±0.3              |
| Leaves B      | 1.53±0.4              |

Note: A: tree without artificial inoculation using fungi; B: tree with artificial inoculation using fungi

In the BSLT for LC$_{50}$ values, we found that leaves extract from both tree with and without inoculating fungi exhibited most active, which obtained LC$_{50}$ values of 1.53±0.4 and 1.71±0.3 $\mu$g/mL, respectively.

4. Discussion

The antioxidant (DPPH scavenging), $\alpha$-glucosidase inhibition and cytotoxic activities are associated with phytochemical compounds. We found that the methanol extract of leaves was the most active as a radical scavenger and $\alpha$-glucosidase inhibitor than other extracts of different G. versteegii explant. However, their activities showed lower activity than quercetin as positive control. For the cytotoxic activity according to Kowalska [17], when assessing the toxicity of plant extract with BSLT, a LC$_{50}$ value is less than 1000 $\mu$g/mL is considered to be bioactive. Therefore, we found that the samples analysed in this study were most active for biochemical and pharmacological activities. Table 4 showed that leaves extract (A and B) have the highest activity in the BSLT with LC$_{50}$ value of 1.71±0.3 and 1.53±0.4 $\mu$g/mL, respectively. The result showed that leaves extract had high both radical scavenging and $\alpha$-glucosidase inhibition activities. Their activities is though to be related with the presence of phenolic, flavonoid and sapinon contents. According to Table 3, the TPC, TFC and TSC of leaves extract were higher than fruit bark and stem bark. Several study revealed the antioxidant and $\alpha$-glucosidase inhibitory activities related with the presence of phenolic, flavonoid and sapinon contents [18-22]. Triterpenoid and sapinon are reported to have antioxidant and cytotoxic activities [23,24]. The results of this study are also corroborated by our previous research that G. versteegii wood methanol extract has high DPPH radical scavenging and $\alpha$-glucosidase inhibition activities [15].

5. Conclusions

Leaves extract from G. vesteegii has potential as an antioxidant and antidiabetic agent due to their high bioactivity (IC$_{50}$ value of 32.89±2.7 and 55.01±2.4 $\mu$g/mL, respectively). The highest antioxidant value found in both of trees with and without inoculating fungi leaves extract. The high antioxidant and $\alpha$-glucosidase inhibitory activities might be due to their phenolic, flavonoid and triterpenoid/saponin containing in the extract. However, further isolation is needed to explain the bioactive compounds that have responsible to their activities.

References

[1] Faizal A, Estyanti RR, Aulianisa EN, Iriawati, Santoso E, and Turjaman M 2017 Formation of agarwood from Aquilaria malaccensis in response to inoculation of local strains of Fusarium solani Trees 31189-97

[2] Putri N, Karinasari L, Turjaman M, Wahyudi I, and Nandika D 2017 Evaluation of incense-resinous wood formation (Aquilaria malaccensis Lam.) using sonic tomography Agriculture and Natural Resources 51(2) 84-90
[3] Liu Y, Chen H, Yang Y, Zhang Z, Wei J, Meng H, Chen W, Feng J, Gan B, Chen X, Gao Z, Huang J, Chen B, and Chen H 2013 Whole-tree agarwood-inducing technique: an efficient novel technique for producing high-quality agarwood in cultivated Aquilaria sinensis Trees Molecules 18(3) 3086-106

[4] Wahyuni R and Prihantini AI 2020 The best inoculation technique applied on gyrinops versteegii tree trunk at 4 to 5 meters’ height Biosaintifika: Journal of Biology & Biology Education 12(2)

[5] Yang MX, Liang YG, Chen HR, Huang YF, Gong HG, Zhang TY, and Ito Y 2018 Isolation of flavonoids from wild Aquilaria sinensis leaves by an improved preparative high-speed counter-current chromatography apparatus Journal of Chromatographic Science 56(1) 18-24

[6] Huda AWN, Munira MAS, Fitrya SD, and Salmah M 2009 Antioxidant activity of Aquilaria malaccensis (thymelaeaceae) leaves Pharmacognosy Research 1(5) 270-73

[7] Zhou MH, Wang HG, Tu LP, Kou JP, and Yu BY 2008 Antinociceptive and anti-inflammatory activities of Aquilaria sinensis (Lour.) Gilg. leaves extract Journal of Ethnopharmacology 117 345-50

[8] Yang MX, Mao SS, and Chen HR 2012 Scavenging effect of different fractions extracted from leaves of Aquilaria sinensis on NO2 Journal of Beihua University 13 406-09

[9] Dahham SS, Hassan LEA, Ahamed MBK, Majid ASA, Majid AMSA, and Zulkepli NK 2016 In vivo toxicity and antitumor activity of essential oils extract from agarwood (Aquilaria crassa) BMC Complementary Medicine and Therapies 16(1) 236

[10] Wang QH, Peng K, Tan LH, Dai HF 2010 Aquilarin A a new benzenoid derivative from the fresh stem of Aquilaria sinensis Molecules 15(6) 4011-16

[11] Sun J, Wang S, Xia F, Wang KY, Chen JM, and Tu PF 2014 Five new benzophenone glycosides from the leaves of Aquilaria sinensis (Lour.) Gilg. Chinese Chemical Letters 25(12) 1573-76

[12] Lee SY, Turjaman M, and Mohamed 2018 Phylogenetic Relatedness of Several Agarwood-Producing Taxa (Thymelaeaceae) from Indonesia Tropical Life Science Research 29(2) 13-28

[13] Prihantini AI and Rizqiani KD 2019 Various antioxidant assays of agarwood extracts (Gyrinops versteegii) from West Lombok, west Nusa Tenggara, Indonesia Asian Journal of Agriculture 3(1) 1-5

[14] Nuringtyas TR, Isromarina R, Septia Y, Hidayati L, Wijayanti N, and Moeljopawiro S 2018 The antioxidant and cytotoxic activities of the chloroform extract of agarwood (Gyrinops versteegii (Gilg.) Domke) leaves on Hela cell lines AIP Conference Proceedings 2002 020067

[15] Sukito A, Darmawan S, and Turjaman M 2020 Anti-oxidant and anti-diabetes activities of agarwood extracts from Gyrinops versteegii (Gilg.) Domke and their cytotoxicity IOP Conference Series: Earth and Environmental Science 415(1) 012001

[16] Sukito A and Tachibana S 2014 Potent α-Glucosidase Inhibitors Isolated from Ginkgo biloba Leaves Pakistan Journal of Biological Sciences 17 1170-78

[17] Kowalska K 2011 Natural compounds involved in adipose tissue mass control in vitro studies Postepy higieny i medycyny doswiadczalnej 65 515-23

[18] Tofigh Z, Alipour F, Hadavinia H, Abdollahi M, Hadjiakhoondi A, and Yassa N 2014 Effective antidiabetic and antioxidant fractions of Otostegia persica extract and their constituents, Pharmaceutical Biology 52(8) 961-66

[19] Chen D, Bi D, Song YL, and Tu PF 2012 Flavonoids from the stems of Aquilaria sinensis Chin. J. Nat. Med. 10 287-91

[20] Sarian MN, Ahmed QU, Mat So‘ad SZ, Alhassan AM, Murugesu S, Perumal V, Syed Mohamad SN, Khatib A, and Latip J 2017 Antioxidant and antidiabetic effects of flavonoids A structure-activity relationship based study BioMed research international 2017
[21] Pranakhon R, Aromdee C, and Pannangpetch P 2015 Effects of iriflophenone 3-C-β-glucoside on fasting blood glucose level and glucose uptake Pharmacogn Mag. 11(82)

[22] Qi J, Lu JJ, Liu JH, and Yu BY 2009 Flavonoid and a rare benzophenone glycoside from the leaves of *Aquilaria sinensis* Chemical & Pharmaceutical Bulletin 57(2) 134-37

[23] Tapondjou LA, Nyaa LBT, Tane P, Ricciutelli M, Quassinti L, Bramucci M, Lupidi G, Ponou BK, and Barboni L 2011 Cytotoxic and antioxidant triterpene saponins from *Butyrospermum parkii* (Sapotaceae) Carbohydrate Research 346 2699-704

[24] Ponou BK, Teponno RB, Ricciutelli M, Quassinti L, Bramucci M, Lupidi G, Barboni L, and Tapondjou LA 2010 Dimeric antioxidant and cytotoxic triterpenoid saponins from *Terminalia ivorensis* A. Chev. Phytochemistry 71(17-18) 2108-15

**Authors’ Contribution**

All authors contributed equally to this work.