Study of Antioxidant Activities from Antihypertension Drug Plant of the Indralaya Area

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Abstract

Ogan ethnic community in Indralaya, Ogan Ilir District, South Sumatra has been used several types of plants, i.e. Swietenia mahagoni, Averrhoa carambola, Syzygium samarangense, Musa acuminata, Nymphaea rubra, Syzygium polyanthum, and Andrographis paniculata for hypertension medicine. Hypertension is a degenerative disease caused by free radical activity in the body. The research aimed to study antioxidant activities from antihypertension drug plant. The study began with the extraction of seven types of plants using methanol as a solvent. The crude extract was tested for its activity using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. The methanol extract with the highest antioxidant activity, subsequently examined in vitro antihypertension test using the Angiotensin Converting Enzyme (ACE) method. Antioxidant test results showed the methanol extract from stem bark of S. samarangense had the highest antioxidant activity with IC50 at 83.06 μg/mL. Antihypertension test of methanol extract from stem bark of S. samarangense obtained IC50 by 61.56 μg/mL. Based on the IC50 value, stem bark of S. samarangense has potential as a source of antioxidant compounds as well as a source of antihypertension compounds.

Keywords: Syzygium samarangense, stem bark, antioxidant, antihypertension

INTRODUCTION

The research for bioactive compounds from traditional medicinal plants was growing, along with the number of studies in the field of ethnobotany in various ethnicities, especially in Indonesia [1,2,3]. The results showed that many plants have been used...
by the community for the treatment of various diseases but have not been supported by adequate scientific information [4]. Besides, currently many herbal products were sold freely to treat various diseases. These herbal products were in great demand by the public, arguing that they were cheaper and more efficient than modern medicine [5,6,7].

The efficacy of a plant as a traditional medicine was related to secondary metabolite compounds contained in the plant extract which includes terpenoids, steroids, flavonoids, phenolic, and alkaloids [8,9]. These secondary metabolites have varied pharmacological activities as antimicrobial, anti-inflammatory, antioxidant, cytotoxic, antidiabetic, antihypertension, antitumor, anticancer and others [10,11,12].

The results of a survey of Ogan ethnic population in Indralaya, Ogan Ilir District, and South Sumatra found several types of plants that have been used for the treatment of hypertension; there are Swietenia mahagoni, Averrhoa carambola, Syzygium samarangense, Musa acuminata, Nyphea rubra, Syzygium polyanthum, and Andrographis paniculata [13]. The use of several kind from antihypertension drug plant were expected by related of antioxidant activities found in plants.

Hypertension is a condition of systolic blood pressure of more than 140 mmHg and diastolic blood pressure of more than 90 mmHg [14]. The role of antioxidant compounds in reducing blood pressure for hypertension patients was through inhibition of enzymes an increase occurred of blood pressure [15,16]. In vitro, the test method used for testing antihypertension activity was the Angiotensin Converting Enzyme (ACE) method [17]. Antioxidants from bioactive compounds can inhibit the formation of Angiotensin II from Angiotensin I which was catalyzed by the enzyme Angiotensin Converting Enzyme (ACE) [18,19]. Angiotensin II that was formed stimulate aldosterone so that the body performs sodium absorption and potassium excretion [20,21]. Further analysis was recommended to prove antioxidant compounds as candidates for hypertension drugs to obtain good scientific information.

**MATERIALS AND METHODS**

**Materials and Instrumentation**

The fresh of medicinal plants, which were leave, stem bark and fruit of Swietenia mahagoni, leave and stem bark of Averrhoa carambola, leave and stem bark of Syzygium samarangense, rind and flower of Musa acuminata, bark and bulbs of Nyphea rubra, leave of Syzygium polyanthum, and leave of Andrographis paniculata. The medicinal plants were collected in Indralaya area, Ogan Ilir district, South Sumatra. Identification carried out at the Botany Laboratory, University of Sriwijaya. The measurement of antioxidant activity was carried out at the Joint Basic Laboratory, Faculty of Mathematics and Natural Science, University of Sriwijaya. The measurement of antihypertension activity conducted at the Research Laboratory of the Department of Chemistry, Faculty of Mathematics and Natural Science, University of Sriwijaya. Materials needed in isolation: technical methanol and Kiesel gel 60 GF254 TLC plate. Antioxidant activity testing reagents: methanol p.a., 1,1-diphenyl-2-picrylhydrazyl (DPPH), and ascorbic acid. Reagent testing for antihypertension activity: borate buffer pH 8.3, BSA, DMSO, HCl, Hippuryl-L-Histidyl-L-Leucine substrate (HHL), ACE enzymes, captopril, phosphate buffer pH 7. The instrument in this study used spectrophotometer UV-Vis Beckman DU-700.

**Methods**

**Extraction**

The sample was chopped, dried in the air at room temperature to a constant weight and grinded to form a fine powder. The sample macerated using methanol for 1x24 hours. A maceration was done three times and the methanol extract was concentrated with a rotary evaporator, to obtain crude methanol extract. The all of methanol extract was tested for antioxidant activity by the DDPH method.

**Antioxidant activity with the DPPH method**

All of methanol extract was tested for antioxidant activity through 0.2 mL of various concentrations of sample solution (1000, 500, 250, 125, 62.5 μg/mL) was added 3.8 mL of DPPH 0.05 mM solution. The mixture of solutions was homogenized and left for 30 minutes in a dark place. The absorbance was measured by a UV-Vis spectrophotometer at λmax 517 nm. Standard antioxidant solutions used ascorbic acid, which was measured by the same treatment as the sample. The antioxidant activity of the sample was determined by the amount of DPPH radical absorbance through calculation of the percentage inhibition of DPPH [22].

The antioxidant activity of each sample was determined by the percentage of free radical inhibition (percent inhibition) which can be calculated with the following formulation:

\[
\text{% inhibition} = \frac{abs\ \text{blank} - abs\ \text{sample}}{abs\ \text{blank}} \times 100 \% \quad (1)
\]

**Antihypertension activity test using ACE method**

In vitro measurement of antihypertension activity in this study was based on inhibition of the ACE
enzyme by the sample [23,24]. The filtrate 80 μL methanol extract (sample), captopril (sample control), borate buffer (blank and blank control) was added 50 μL of Hippuryl-L-Histidyl-L-Leucine (HHL) substrate (2.5 mM HHL in 0.05 M sodium borate buffer, containing 0.15 M NaCl, at pH 8.3) and added 15 μL BSA, then the mixture was incubated for 5 minutes at 37°C. Sample and blank was added 100 μL ACE, sample control and blank control was added 100 μL distilled water. The mixture was incubated for 30 minutes at 37°C. The reaction was stopped by the addition of 250 μL 0.5 M HCl, then vortex for 5 minutes. The mixture was added 1.5 mL ethyl acetate to extract the formed hippuric acid. The mixture was then centrifuged at a speed of 10000 rpm for 10 minutes. The top layer was taken as much as 800 μL and dried in an oven at 95 °C for 75 minutes. The formed hippuric acid was dissolved into 1000 μL aqua-bides. Absorbance of hipuric acid was measured at λmax 228 nm using UV-Vis spectrophotometer. Therefore, absorbance data was obtained blank (A), control blank (B), sample (C) and blank control (D). ACE inhibitor activity is calculated in percent form with the formula:

\[ \% \text{ACE inhibition} = \frac{(A-B)-(C-D)}{A-B} \times 100 \% \]  

Note:
A= absorbance blank (HHL substrate + ACE enzyme)
B= absorbance blank control (HHL substrate + aquadest)
C= absorbance of sample (HHL substrate + sample + ACE enzyme)
D= absorbance of sample control (HHL substrate + captopril + aquadest)

RESULTS AND DISCUSSION

The methanol extracts of the seven plant by parts used in Ogan people as a hypertension drug were tested for their antioxidant activity as shown in Table 1-3.

The data shown in Table 1-3 shows that Syzygium samarangense, Swietenia mahagoni, and Averrhoa carambola provide high antioxidant activity. Based on the IC₅₀ value, the methanol extract of S. samarangense stem bark was the highest antioxidant activity then all of extracts. The antioxidant activity from extract was categorized strong (IC₅₀<200 μg/mL), moderate (IC₅₀ 200-1000 μg/mL), and weak (IC₅₀>1000 μg/mL) [25]. The IC₅₀ from stem bark of S. samarangense was 83.06 μg/mL and categorized as strong.

It was also shown that the graph of the S. samarangense stem bark approaches the graph of ascorbic acid (Figure 1). Based on the graph, stem bark of S. samarangense has very high of potential antioxidant. At the same concentration (250 μg/mL), stem bark of S. samarangense and ascorbic acid have been the same of percent inhibition value at the 80 % inhibition.

Table 1. Absorbance values of parts of medicinal plants in testing antioxidant activity by DPPH method

| Concentration (μg/mL) | Leaf of Swietenia mahagoni | Leave of Syzygium samarangense | Flower of Musa acuminata | Bark of Nymphaea rubra | Bulbs of Nymphaea rubra | Rind of Musa acuminata | Fruit of Swietenia mahagoni |
|----------------------|-----------------------------|-------------------------------|--------------------------|------------------------|-------------------------|------------------------|----------------------------|
| DPPH                 | 0.953                       | 0.928                         | 0.928                    | 0.928                  | 0.953                   | 0.953                  | 0.953                      |
| 62.5                 | 0.702                       | 0.664                         | 0.924                    | 0.915                  | 0.917                   | 0.921                  | 0.644                      |
| 125                  | 0.415                       | 0.420                         | 0.924                    | 0.912                  | 0.901                   | 0.903                  | 0.521                      |
| 250                  | 0.078                       | 0.128                         | 0.920                    | 0.899                  | 0.867                   | 0.877                  | 0.412                      |
| 500                  | 0.064                       | 0.067                         | 0.821                    | 0.892                  | 0.817                   | 0.855                  | 0.092                      |
| 1000                 | 0.054                       | 0.055                         | 0.819                    | 0.892                  | 0.811                   | 0.853                  | 0.063                      |

| Concentration (μg/mL) | Leaf of Averrhoa carambola | Leave of Syzygium polyanthum | Stem bark of Swietenia mahagoni | Stem bark of Andrographis paniculata | Stem bark of Syzygium samarangense | Ascorbic acid |
|----------------------|-----------------------------|-------------------------------|----------------------------------|------------------------------------|----------------------------------|---------------|
| DPPH                 | 0.953                       | 0.953                         | 0.953                            | 0.928                              | 0.928                            | 0.953         |
| 62.5                 | 0.897                       | 0.633                         | 0.556                            | 0.672                              | 0.814                            | 0.912         |
| 125                  | 0.865                       | 0.439                         | 0.402                            | 0.353                              | 0.715                            | 0.532         |
| 250                  | 0.701                       | 0.104                         | 0.132                            | 0.124                              | 0.451                            | 0.232         |
| 500                  | 0.456                       | 0.089                         | 0.131                            | 0.117                              | 0.318                            | 0.099         |
| 1000                 | 0.087                       | 0.089                         | 0.130                            | 0.114                              | 0.099                            | 0.062         |

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Table 2. % inhibition value of parts from medicinal plants in testing antioxidant activity by DPPH method

| Concentration (μg/mL) | Leave of *Swietenia mahagoni* | Leave of *Syzygium samarangense* | Flower of *Musa acuminata* | Bark of *Nymphaea rubra* | Bulbs of *Nymphaea rubra* | Rind of *Musa acuminata* | Fruit of *Swietenia mahagoni* |
|-----------------------|-----------------------------|---------------------------------|-----------------------------|---------------------------|--------------------------|--------------------------|--------------------------------|
| 62.5                  | 24.35                       | 28.45                           | 0.431                       | 1.4                       | 1.18                     | 3.45                     | 32.42                         |
| 125                   | 55.28                       | 54.74                           | 0.431                       | 1.72                      | 2.91                     | 5.39                     | 45.33                         |
| 250                   | 91.59                       | 86.21                           | 0.86                        | 3.12                      | 6.57                     | 8.19                     | 56.77                         |
| 500                   | 93.1                        | 92.78                           | 11.53                       | 3.88                      | 11.96                    | 10.56                    | 90.33                         |
| 1000                  | 94.18                       | 94.07                           | 11.73                       | 3.88                      | 12.61                    | 12.61                    | 93.39                         |

| Concentration (μg/mL) | Leave of *Averrhoa carambola* | Leave of *Syzygium polyanthum* | Stem bark of *Swietenia mahagoni* | Stem bark of *Averrhoa carambola* | Leave of *Andrographis paniculata* | Stem bark of *Syzygium samarangense* |
|-----------------------|-------------------------------|---------------------------------|----------------------------------|----------------------------------|-----------------------------------|-----------------------------------|
| 62.5                  | 5.876                         | 36.51                           | 44.68                            | 39.02                            | 21.28                             | 10.59                            |
| 125                   | 9.23                          | 55.97                           | 60                               | 67.97                            | 30.85                             | 47.84                            |
| 250                   | 26.44                         | 89.57                           | 86.86                            | 88.75                            | 56.38                             | 77.25                            |
| 500                   | 52.15                         | 91.07                           | 86.96                            | 89.38                            | 69.24                             | 90.29                            |
| 1000                  | 90.87                         | 91.07                           | 87.06                            | 89.65                            | 90.42                             | 93.92                            |

Table 3. IC₅₀ values of parts of medicinal plants in testing antioxidant activity by DPPH method

| Test Sample | IC₅₀ (μg/mL) |
|-------------|--------------|
| *Swietenia mahagoni* (LSM) | 125.55 |
| *Syzygium samarangense* (LSS) | 124.3 |
| *Musa acuminata* (FMA) | 19.913 |
| *Nymphaea rubra* (BuNR) | 5.189 |
| *Nymphaea rubra* (BuNR) | 2.091 |
| *Musa acuminata* (RMA) | 3.469 |
| *Swietenia mahagoni* (FSM) | 185.74 |

| Test Sample | IC₅₀ (μg/mL) |
|-------------|--------------|
| *Averrhoa carambola* (LAC) | 529.76 |
| *Syzygium polyanthum* (LSP) | 107.79 |
| *Swietenia mahagoni* (SSM) | 83.89 |
| *Averrhoa carambola* (SBC) | 85.10 |
| *Andrographis paniculata* (LAP) | 285.40 |
| *Syzygium samarangense* (SSS) | 83.06 |
| Ascorbic acid (AA) | 22.23 |

A methanol extract from stem bark of *S. samarangense* (SSS) was carried out in vitro antihypertension test with the Angiotensin Converting Enzyme (ACE) method. ACE inhibition was one of the main classes of antihypertension drugs in reducing blood pressure [15]. ACE inhibitors (ACE-I) can inhibit converting of ACE enzyme from angiotensin I become angiotensin II to give a vasodilation effect [26]. A vasodilation effect was the wide of blood vessels to reducing blood pressure. Determination of ACE activity by Hippuryl-L-Histidyl-L-Leucine (HHL) substrate carried out of methanol extracts from stem bark of *S. samarangense* (SSS) was expressed from the percent inhibition of ACE (y) and concentration (x) in Table 4 and Table 5.

IC₅₀ ACE values from methanol extract SSS and captopril were 61.56 and 0.06 μg/mL. This shows that methanol extract stem bark of *S. samarangense* have weak potential to inhibition of ACE enzyme if compared to captopril. But, this was supported by research reports on several medicinal plants in Indonesia. The reports state that the plants have the best potential for antihypertension, like that ethanol extract from leaves *Averrhoa blimbi* L; leaves of *Morinda citrifolia* L; leaves of *Orthosiphon stamineus* Benth; leaves of *Syzygium polyanthum*; and leaves of *Solanum indicum* Linn with a percentage of inhibitor activity each of 71.48; 66.64; 55.41; 53.37; and 53.24.
μg/mL [27]. Therefore, the stem bark of *S. samarangense* methanol extract with IC$_{50}$ 61.56 μg/mL is also the best potential for antihypertension.

![Graph showing percentage inhibition vs concentration](image1.png)

**Figure 1.** The graph between percentage of inhibition and concentration.

| Concentration (μg/mL) | Type   | Methanol extract SSS | Captopril |
|----------------------|--------|----------------------|-----------|
|                      |        | A   | I (%) | A   | I (%) |
| 6.25                 | Blank  | 0.405 | 20.78 | 0.198 | 51.20 |
|                      | Sample | 0.321 | 25.83 | 0.174 | 56.66 |
| 12.5                 | Blank  | 0.383 | 38.33 | 0.178 | 63.83 |
|                      | Sample | 0.298 | 40.76 | 0.157 | 58.94 |
| 25                   | Blank  | 0.374 | 37.4 | 0.178 | 63.83 |
|                      | Sample | 0.207 | 44.61 | 0.137 | 63.83 |
| 50                   | Blank  | 0.373 | 37.3 | 0.371 | 63.83 |
|                      | Sample | 0.126 | 66.19 | 0.002 | 99.43 |

**Note:** A: Absorbance  I: Inhibition

**Table 4.** Value of absorbance and % ACE inhibition

| Test Sample       | IC$_{50}$(μg/mL) |
|-------------------|------------------|
| Methanol extract SSS | 61.56            |
| Captopril         | 0.06             |

**Table 5.** IC$_{50}$ ACE values of methanol extract SSS and captopril

The method based on the hydrolysis of the *Hippuril-Histidil-Leucine* (HHL) substrate by the ACE enzyme so that it releases hippuric acid and Histidil-Leucine (HL). The methanol extract from stem bark of *S. samarangense* thought to have a role in inhibiting the action of the ACE enzyme in the hydrolysis reaction from hippuril acid to hippuric acid [28] shown in Figure 2.
Figure 2. Hydrolysis of HHL by ACE to produce hippuric acid and HL

CONCLUSION
The seven plants that traditionally been used by the Ogan ethnic community as a cure for hypertension, the stem bark of *S. samarangense* plant have the highest antioxidant activity and potential as an antihypertension activity.

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