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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-picryllhydrazyl* (DPPH) method. The methanol extract with the highest antioxidant activity, subsequently examines *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 IC_{50} at 83.06 µg/mL. Antihypertension test of methanol extract from stem bark of *S. samarangense* obtained IC_{50} by 61.56 µg/mL. Based on the IC_{50} 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

Abstrak (Indonesian)

Penduduk etnis Ogan di Kecamatan Indralaya, Ogan Ilir, Sumatera Selatan telah menggunakan beberapa jenis tumbuhan yaitu mahoni *(Swietenia mahagoni)*, belimbing manis *(Averrhoa carambola)*, jambu air *(Syzygium samarangense)*, jantung pisang *(Musa acuminata)*, teratai *(Nymphaea rubra)*, daun salam *(Syzygium polyanthum)* and sambiloto *(Andrographis paniculata)* untuk pengobatan hipertensi. Hipertensi adalah penyakit degeneratif yang disebabkan oleh aktivitas radikal bebas dalam tubuh. Penelitian ini bertujuan untuk mempelajari aktivitas antioksidan dari tumbuhan obat antihipertensi. Ekstrak ketujuh jenis tumbuhan diperoleh dengan menggunakan pelarut metanol. Masing-masing ekstrak diuji aktivitas antioksidan dengan metode 1,1-difenil-2-pikrillhidrazil (DPPH). Ekstrak metanol yang memiliki aktivitas antioksidan paling tinggi, selanjutnya dilakukan uji antihipertensi secara *in vitro* menggunakan metode *Angiotensin Converting Enzyme* (ACE). Hasil uji antioksidan memperlihatkan bahwa ekstrak metanol dari kulit batang *S. samarangense* memiliki aktivitas antioksidan tertinggi dengan IC_{50} sebesar 83,06 µg/mL. Uji antihipertensi terhadap ekstrak metanol kulit batang *S. samarangense* diperoleh nilai IC_{50} sebesar 61,56 µg/mL. Berdasarkan data IC_{50} tersebut, memperlihatkan bahwa kulit batang *S. samarangense* memiliki potensi sebagai sumber senyawa antioksidan sekaligus sumber senyawa antihipertensi.

Keywords: Syzygium samarangense, kulit batang, antioksidan, antihipertensi

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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*, *Nymphaea 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 *Switenia mahagoni*, leave and stem bark of *Averrhoa carambola*, leave and stem bark of *Syzygium samarangense*, rind and flower of *Musa acuminate*, bark and bulbs of *Nymphaea 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 Kiessel 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 λ_{max} 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 inhibiton 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:

$$\%\ inhibition = \frac{abs\ blank - abs\ sample}{abs\ blank} x\ 100\ \% \quad (1)$$

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 37ºC. 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 37ºC. 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:

$$\%\ ACE\ inhibition = \frac{(A-B)-(C-D)}{(A-B)} x\ 100\ \% \qquad (2)$$

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.

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

Concentration (µg/mL)	Leave of *Switenia mahagoni*	Leave of *Syzygium samarangense*	Flower of *Musa acuminata*	Bark of *Nymphaea rubra*	Bulbs of *Nymphaea rubra*	Rind of *Musa acuminata*	Fruit of *Switenia 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)	Leave of *Averrhoa carambola*	Leave of *Syzygium polyanthum*	Stem bark of *Switenia mahagoni*	Stem bark of *Averrhoa carambola)*	Leave of *Andrographis paniculata*	Stem bark of *Syzygium samarangense*	Ascorbic acid
DPPH	0.953	0.953	0.953	0.928	0.928	0.928	0.953
62.5	0.897	0.633	0.556	0.672	0.814	0.912	0.314
125	0.865	0.439	0.402	0.353	0.715	0.532	0.254
250	0.701	0.104	0.132	0.124	0.451	0.232	0.208
500	0.456	0.089	0.131	0.117	0.318	0.099	0.040
1000	0.087	0.089	0.130	0.114	0.099	0.062	0.034

The data shown in Table 1-3 shows that *Syzygium samarangense*, *Switenia mahagoni*, and *Averrhoa carambola* provide high antioxidant activity. Based on the IC_{50} 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 (IC_{50}<200 µg/mL), moderate (IC_{50} 200-1000 µg/mL), and weak (IC_{50}>1000 µg/mL) [25]. The IC_{50} 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 2. % inhibition value of parts from medicinal plants in testing antioxidant activity by DPPH method

Concentration (µg/mL)	Leave of *Switenia mahagoni*	Leave of *Syzygium samarangense*	Flower of *Musa acuminata*	Bark of *Nymphaea rubra*	Bulbs of *Nymphaea rubra*	Rind of *Musa acuminata*	Fruit of *Switenia 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 *Switenia 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. IC_{50} values of parts of medicinal plants in testing antioxidant activity by DPPH method

Test Sample	**IC_{50} (µg/mL)**
Leave of *Switenia mahagoni* (LSM)	125.55
Leave of *Syzygium samarangense* (LSS)	124.3
Flower of *Musa acuminata* (FMA)	19.913
Bark of *Nymphaea rubra* (BaNR)	5.189
Bulbs of *Nymphaea rubra* (BuNR)	2.091
Rind of *Musa acuminata* (RMA)	3.469
Fruit of *Switenia mahagoni* (FSM)	185.74
Test Sample	**IC_{50} (µg/mL)**
Leave of *Averrhoa carambola* (LAC)	529.76
Leave of *Syzygium polyanthum* (LSP)	107.79
Stem bark of *Switenia mahagoni* (SSM)	83.89
Stem bark of *Averrhoa carambola* (SBC)	85.10
Leave of *Andrographis paniculata* (LAP)	285.40
Stem bark of *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 given 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.

IC_{50} 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 *Syzigium 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.

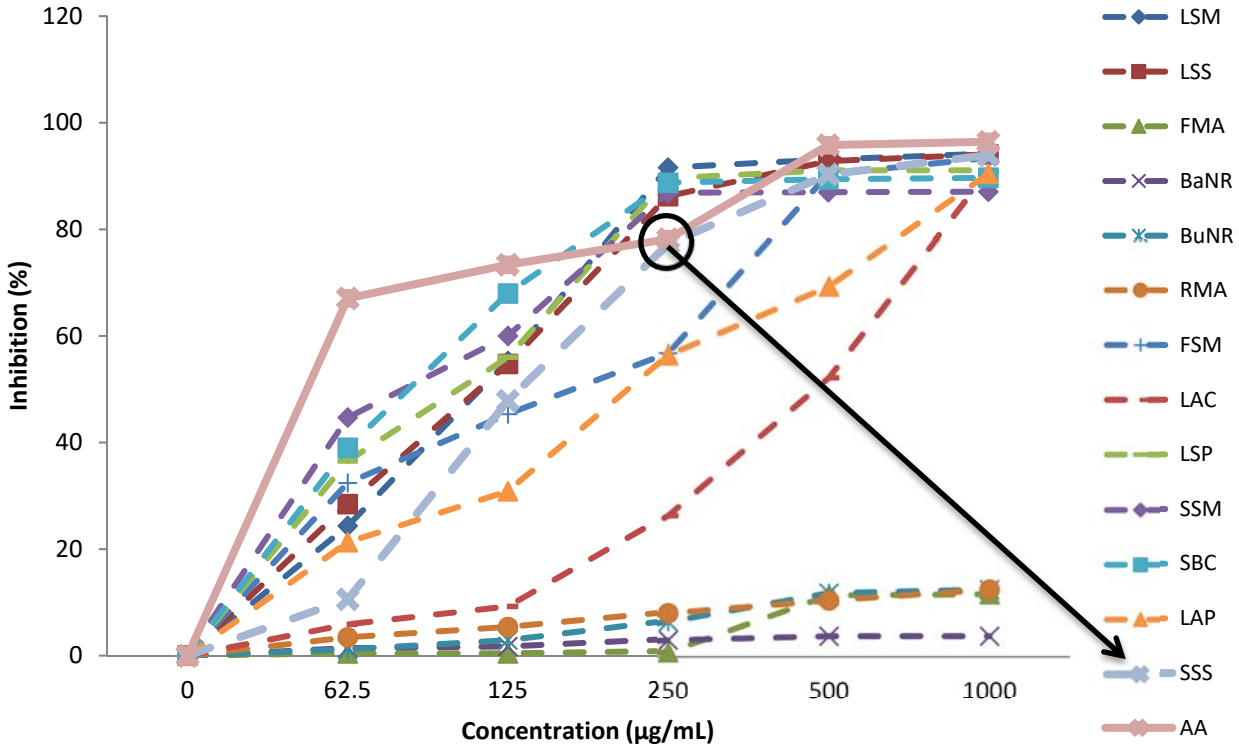

Figure 1. The graph between percentage of inhibition and concentration

Table 4. Value of absorbance and % ACE inhibition

Concentration (µg/mL)	Type	Methanol extract SSS		Captopril	
		A	I (%)	A	I (%)
6.25	Blank	0.405		0.405	
	Sample	0.321	20.78	0.198	51.20
12.5	Blank	0.402		0.402	
	Sample	0.298	25.83	0.174	56.66
25	Blank	0.383		0.383	
	Sample	0.227	40.76	0.157	58.94
50	Blank	0.374		0.378	
	Sample	0.207	44.61	0.137	63.83
100	Blank	0.373		0.371	
	Sample	0.126	66.19	0.002	99.43

Note: A: Absorbance I: Inhibition

Table 5. IC_{50} ACE values of methanol extract SSS and captopril

Test Sample	IC_{50}(µg/mL)
Methanol extract SSS	61.56
Captopril	0.06

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.

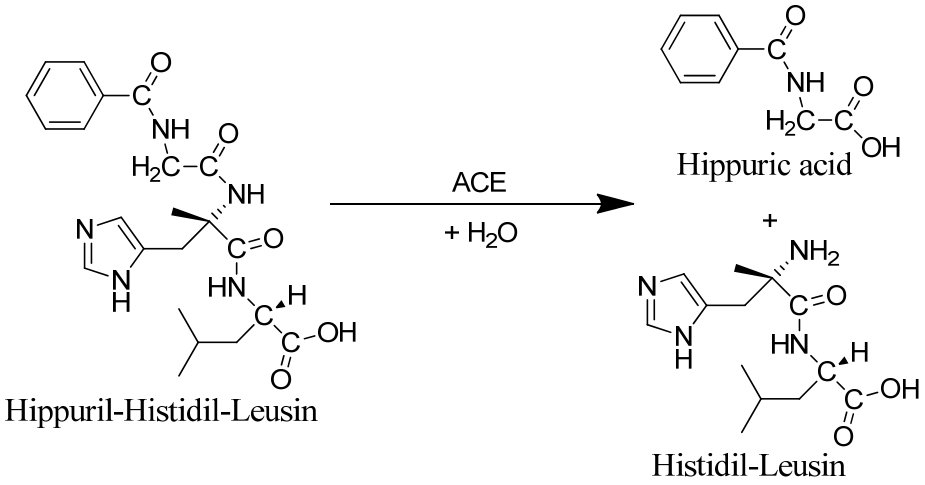

Figure 2. Hydrolysis of HHL by ACE to produce hippuric acid and HL

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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