Antioxidant Activity of *Syzygium polynthum* Extracts

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ABSTRACT

Antioxidant activities of Syzygium polyanthum *leaves extracts (methanol, ethyl acetate, dichloromethane and n-hexane) were evaluated by using DPPH (2,2-diphenyl-2-picrylhydrazyl) and ABTS (2,2'-azinobis (3-ethylbenzothiazole-6-sulfonic acid) methods. The methanol extract showed the highest antioxidant activity of DPPH assay among extract with IC_{50} value of 44.35 μg/mL. In addition, methanol extract also showed the highest antioxidant activity of ABTS assay among extracts with IC_{50} value of 17.69 μg/mL. This study indicated that the methanol extract of* S. polyanthum *leaves is potential as antioxidant.*

Keywords: *antioxidant; DPPH; ABTS;* Syzygium polyanthum*; salam leaves*

ABSTRAK

Aktivitas antioksidan ekstrak daun Syzygium polyanthum *telah dievaluasi menggunakan metode DPPH (2,2-diphenyl-2-picrylhydrazyl) dan ABTS (2,2'-azinobis (3-ethylbenzothiazole-6-sulfonic acid). Ekstrak metanol menunjukkan aktivitas antioksidan yang paling besar dibandingkan ekstrak lain melalui uji DPPH dengan nilai IC_{50} 44,35 μg/mL. Selain itu ekstrak metanol juga menunjukkan aktivitas antioksidan yang paling tinggi dibandingkan ekstrak lain melalui uji ABTS dengan nilai IC_{50} 17,69 μg/mL. Hasil penelitian ini menunjukkan bahwa ekstrak metanol daun* S. polyanthum *berpotensi sebagai antioksidan.*

Kata Kunci: *antioksidan; DPPH; ABTS;* Syzygium polyanthum*; daun salam*

INTRODUCTION

Role of free radical agents damage to cell and tissues occupies the most important position in the body metabolism. Free radical reactions occur due to the oxidation reaction of stable compound become unstable and also reactive compound. These reactive species can react with other compound in the body and causing tissue damage which will lead to disease such as cancer, alzheimer disease, cardiac reperfusion and abnormalities. These free radicals such as peroxide, hydroperoxide or lipid peroxyl may oxidize nucleic acids, proteins, lipids, DNA, and can initiate the degenerative disease [1]. Physical and chemical factors such as heavy metals, heating, radiation, dyes and preservatives play an important role in the occurrence of excessive oxidation reactions [2].

Antioxidants are chemical compounds that can neutralize free radical agents. These compounds work by donating electron to achieve of stable form, thus inhibit the oxidative mechanism that lead to degenerative disease. Antioxidant compounds can include natural and synthetic compounds. Synthetic antioxidant has some side effect and become carcinogenic agents [1]. Therefore, many studies are developing antioxidant compounds from natural materials. Most of the antioxidant compounds were obtained from plant such as vitamin C, vitamin E, carotenoids and phenolic acid. Various classes of compounds with wide of physical and chemical properties were isolated, such as gallic acid have strong antioxidant activity [3].

Indonesian biodiversity is one of important asset in the utilization of chemical plants. *S. polyanthum* which commonly known as *salam* leaves, are usually used to seasoning because of its rich aroma. Salam leaves has been widely used in Indonesian traditional medicines. It is also known to be effective for keeping the health because it has a wide range of bioactivity such as antihypertensive [4], antimicrobial [5], and antidiarrheal [6].

The previous studies on plant of Myrtaceae family found that phenolic and flavonoid such as gallic acid, eugenol, kaempferol and quercetin, contributed to antioxidant activity. Methanol extract of *S. aromaticum* exhibited high potential of antioxidant activity with IC_{50} value of 11.43 μg/mL [7], while methanol extract of

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S. Malaccense had IC_{50} value of 16.65 µg/mL [8]. These Syzygium genus studies showed great potential source of antioxidant due to many phenolic and flavonoid constituents. These findings suggested the possibility of *S. polyanthum* posses high antioxidant.

Since natural antioxidant source are still needed, antioxidant activity of *S. polyanthum* extracts have been evaluated. The aim of this study was to determine the antioxidant activity of some extracts of *S. Polyanthum.* The antioxidant activity of various extract of *S. polyanthum* will be determined by using DPPH and ABTS methods.

EXPERIMENTAL SECTION

Materials

Salam leaves (*Syzygium polyanthum*) that used in this research were obtained from Probolinggo, East Java, Indonesia. Solvent (methanol, ethanol, ethyl acetate, dichloromethane, dimethylsulfoxide and *n*-hexane) were analytical grade. DPPH (2,2-diphenyl-2-picrylhydrazyl; Sigma-Aldrich, Steinheim, Germany), ABTS (2,2'-azinobis(3-ethyl benzothiazoline-6-sulfonic acid), $K_2S_2O_8$ (potassium peroxydisulfate), Trolox (6-hydroxy-2,5,7,8- tetramethylchroman-2- carboxylic acid; Sigma Aldrich) was used as antioxidant standard.

Instrumentation

Incubator EYELA SLI-400 used to process incubation of sample. The reaction was monitored by spectrophotometer (UV Jasco V-530, Japan).

Procedure

Salam leaves extracts

Each of dried *salam* leaves (25 g) were extracted with MeOH, ethyl acetate, dichloromethane and hexane (200 mL for 24 h) at room temperature. The extracts were filtered through filter paper then concentrated with a rotary evaporator under pressure to give crude extracts.

DPPH assay

DPPH activity was assayed by method described by Brand Williams [9], modified by Dudonne et al. [10] with minor modification. Each of crude extracts (10 mg) was dissolved in 1 mL methanol. The reaction mixture was consisted of 1 mL DPPH solution $6x10^{-5}$ M and 33 µL of methanol solution of crude extract. After 20 min incubation for 37 °C, absorbance of the reaction mixture was measured at 515 nm by spectrophotometer (UV Jasco V-530, Japan) to give *As* value. Blank sample with 33 µL of methanol in DPPH solution was prepared and measured at same wavelength (*Ab*). The experiment was carried out in triplicate. Antioxidant activity was calculated using the following formula:

$$\text{Antioxidant activity (\%)} = \frac{A_b - A_s}{A_b} x100\% \quad (1)$$

ABTS assay

ABTS activity was assayed by method described by Pellegrini et al. [11]. Working solution was made from 5 mL of 7 mM ABTS and 88 µL of 140 mM potassium peroxydisulfate ($K_2S_2O_8$). The resulting mixture was allowed to stand at room temperature for 12-16 h to yield a dark blue solution. The mixture was added by 99.5% ethanol to gave an absorbance of 0.7±0.02 at 734 nm. Each of crude extracts (10 mg) was dissolved in 1 mL DMSO. The reaction mixture was consisted of 1 mL working solution and 10 µL of crude extract and shaken for 10 sec. After 4 min incubation at 30 °C, the absorbance of the reaction mixture was measured at 734 nm by spectrophotometer (UV Jasco V-530, Japan) to give *As*. Ethanol 99.5% was used as a blank and measured at same wavelength (*Ab*). The experiment was carried out in triplicate and ABTS activity was calculated using formula 1.

RESULT AND DISCUSSION

Salam Leaves Extract

The extraction process was performed by using methanol, ethyl acetate, dichloromethane and *n*-hexane which provide varying extraction yield (1.75, 1.40, 0.52, and 0.33 g), respectively. The highest extraction yield for various extract was obtained when methanol used as solvent. The results show that extraction yields depend on polarity of the sample. The component in *S. polyanthum* leaves expected to be polar when extracted with methanol. This result indicated that most compounds in *S. polyanthum* leaves were soluble in polar solvent.

DPPH and ABTS Assay

Antioxidant activity of four extracts *S. polyanthum* based on DPPH assay at a concentration of 159.73 µg/mL were presented in Fig. 1. The percentage of antioxidant activity of various extracts; MeOH, ethyl acetate, dichloromethane, and hexane were 84.9, 79.9, 52.7, and 47.5%, respectively. Trolox as a positive control have antioxidant activity of 94.4%. The methanol extract had the highest activity among the other leaves extract, due to these extract may contain many phenolic compounds that contributed of antioxidant activity.

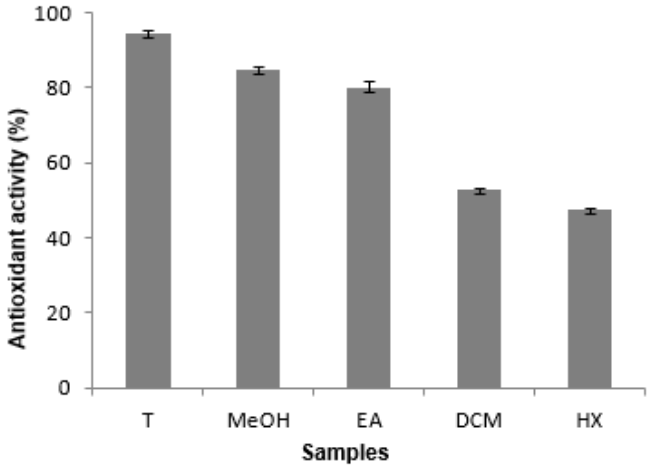

Fig 1. DPPH Scavenging activity of *S. polyanthum* extracts at a concentration of 159.73 μg/mL, MeOH, methanol extract; EA, ethyl acetate extract; DCM, dichloromethane extract; Hx, hexane extract and T, trolox (positive control). Each column represents the mean ± SD, n = 3

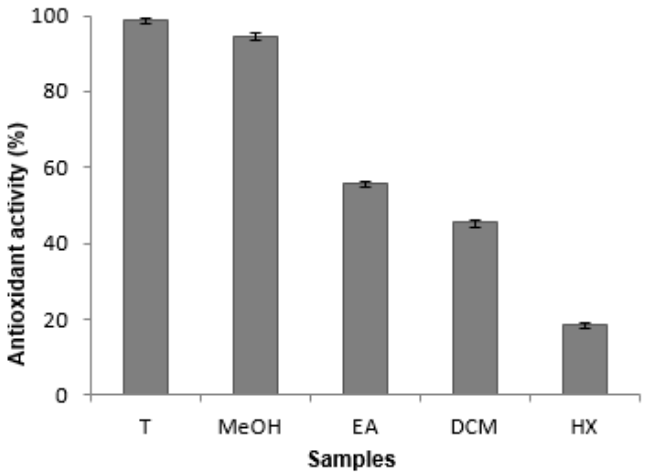

Fig 3. ABTS Scavenging activity of *S. polyanthum* extracts at a concentration of 45.9 μg/mL, MeOH, methanol extract; EA, ethyl acetate extract; DCM, dichloromethane extract; Hx, hexane extract and T, trolox (positive control). Each column represents the mean ± SD, n = 3

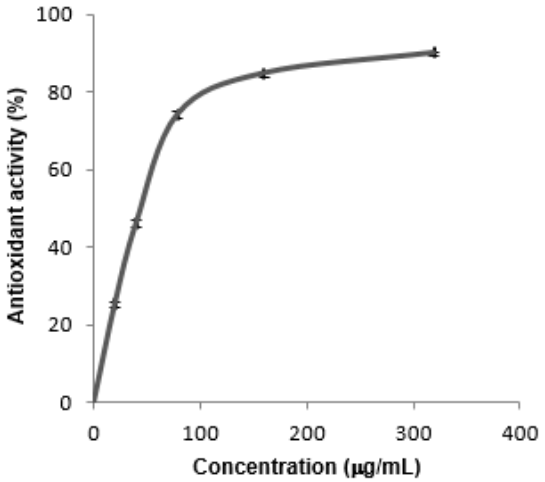

Fig 2. DPPH Scavenging activity of *S. polyanthum* methanol extract

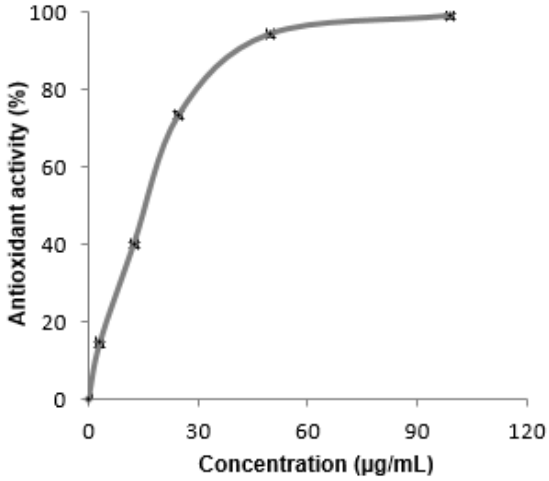

Fig 4. ABTS Scavenging activity of *S. polyanthum* methanol extract

Determination of IC_{50} value for each extracts were determine to perform the doses of extract that can reduce intensity of 50% free radical absorption. Based on the result of interpolation of Fig. 2, the IC_{50} value of *S. polyanthum* MeOH extract was 44.35 μg/mL. Trolox as positive control have higher activity than that of methanol extract with IC_{50} value of 3.09 μg/mL. Trolox is consist of aromatic ring with substitution of hydroxy and carboxylate group. The existence of these two group have an important role to determine the efficiency of compound in free radical inhibition.

DPPH (2,2-diphenyl-2-picrylhyrazyl) is a method of measuring the antioxidant activity which is widely used to test the activity of natural compounds in food and biological system [12]. DPPH is a stable free radical that interacts with antioxidant through the electron transfer. When reacting with antioxidant, DPPH radical is converted to DPPH and its color change from purple to yellow [13]. DPPH assay is one of simple and rapid test. This assay needs UV-vis spectrophotometry to determine the absorbance of compound [14].

The methanol extract of *S. polyanthum* have polar compound. Phenolic compound is one class of polar compounds that acting as antioxidants. The antioxidative activity of phenolic compounds play an important role in absorption and neutralization of free

Table 1. DPPH and ABTS radical scavenging activity of *S. polyanthum* extracts

Extracts	DPPH IC_{50} (μg/mL)	ABTS IC_{50} (μg/mL)
Hexane	136.7	124.9
Dichloromethane	126.1	53.0
Ethyl acetate	56.7	40.17
Methanol	44.35	17.69
Trolox	3.09	4.11

radicals. Phenolic compounds are classified as simple phenols, single aromatic ring with one hydroxyl group and polyphenol with two or more subunits such as flavonoids, or three or more phenol subunits, called tannin [15].

Antioxidant activity of *salam* leaves extracts are also performed by using the ABTS method. The principle of this method is the radical cation decolorization through the transfer of electrons that neutralize free radicals which are marked with a dark blue color change to yellow light [13]. ABTS is soluble in both aqueous and organic solvents. This assay can be used in multiple media to determine both hydrophilic and lipophilic antioxidant capacities of extracts. ABTS reacts rapidly with antioxidant. This assay can be used to determine effects of pH on antioxidant mechanisms and leading to the ABTS wide pH range availability [14].

The results of antioxidant activity of four extracts (MeOH, ethyl acetate, dichloromethane, and hexane) at a concentration of 45.9 μg/mL were presented in Fig. 3. The percentages of antioxidant activity were 94.3, 55.9, 45.4, and 13.0%, respectively, while trolox as a standard has antioxidant activity of 98.8%. It indicated that the methanol extract had the highest activity among the other leaves extracts (Fig. 3). ABTS scavenging activity of methanol extract of *S. polyanthum* was shown in Fig. 4. The IC_{50} value of methanol extracts is 17.69 μg/mL whereas Trolox is 4.11 μg/mL.

The various extracts of *S. polyanthum* were tested for antioxidant activity using DPPH and ABTS. Table 1 showed the IC_{50} value of DPPH from the methanol extract was important role in absorption and neutralization of free radicals.

CONCLUSION

S. polyanthum leaves are widely used in traditional medicine in South East Asia. Antioxidant activities of *S. polyanthum* leaves extract were determined by using DPPH and ABTS. The various extracts was compared with trolox which indicated that only methanol extract which more potential for further isolation process to have bioactive compounds with antioxidant activity. The methanol extract showed the highest antioxidant activity both in DPPH and ABTS assay. This finding support that methanol extract of *S. polyanthum* leaves should have compounds which can be acting as antioxidant source.

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