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Research Article

TEST OF ANTIOXIDANT ACTIVITY LEAVES OF *SCAEVOLA TACCADA* (GAERTN.) ROXB. USING DPPH (1, 1-DIPHENYL-2-PICRYLHYDRAZYL)

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ABSTRACT
Free radicals can caused damage cell and tissue which lead to various disease then needed antioxidant as radicals scavenging. The objective of this study was to determine antioxidants activity leaves of *Scaevola taccada* (Gaertn.) Roxb based to DPPH (1,1-diphenyl-2-picrilhydrazil) free radicals scavenging. The study was initiated with sample extraction by maceration used ethanol then partitioned with ethyl acetate. The obtained fraction tested to antioxidant activity DPPH radicals. Testing done in five series concentration is 50 ppm, 100 ppm, 200 ppm, 300 ppm and 400 ppm, which compared to antioxidant activity vitamin C and BHT (butylatedhydroxytoluene). Activity against free radicals is measured with at a spectrophotometer UV-VIS wavelength 517 nm and calculated value ES_{50}. The result showed the value ES50 of a leaves ethyl acetate fraction of 1473 ppm while ES_{50} vitamin C and BHT of 9.054 ppm and 29.067 ppm, respectively. Therefore, obtained results showed that ethyl acetate fraction *Scaevola taccada* (Gaertn.) Roxb leaves has weak activity compared vitamin C and BHT as positive control with ES_{50} values respectively 1473.064 ppm, 9.054 ppm and 29.067 ppm.
Keywords: *Scaevola taccada* (Gaertn.) Roxb, ethyl acetate fraction, DPPH

INTRODUCTION

Oxidative metabolism in living organism produces many free radicals such as the reactive oxygen species (ROS) and reactive nitrogen species (RNS) includes free radicals such as superoxide anion radicals (O_2), hydroxyl radicals (OH^-) and non free radicals such as H_2O_2 and single oxygen (O_2) are known to cause damage to lipids, enzyms, protein and nucleic acid leading to cell or tissue injury and implicated in more than 100 diseases[1,2]. These diseases including acquired immunodeficiency syndrome, type II diabetes, stroke, arteriosclerosis, cancer, hepatic ailments and process of aging[1,3]. Antioxidants are molecules or compounds that have ability to act as free radical scavengers. Most antioxidant are electron donors and react with the free radicals with the result protect against oxidative stress and prevent damage to cell[4]. Our body is rich in endogenous antioxidant which the antioxidant can occur endogenously in body e.g.: enzymes and melatonin, or exogenously as they can obtained from dietary and natural or synthetic drugs[5]. Several endogenous antioxidant enzymes including superoxide dismutase (SOD), glutathione peroxidise (GSH-Px), glutathione reductase and catalase (CAT)[6]. Many studies have shown that antioxidant nutrient supplements, especially B-carotene, vitamin C and vitamin E, are effective in protecting the oxidation of DNA, LDL, and protein against damage by smoking *in vitro*[7,8]. A wide range of antioxidants of synthetic origin such as butylated hydroxytoluene (BHT) has been proposed for use in the treatment of various free radicals related diseases, but it has been proven that these compounds also show toxic effects like liver damage and mutagenesis[9-11]. *Scaevola taccada* (Gaertn.) Roxb has some benefit in treatment of disease. The leaves were used for indigestion, cure headache and the root is used as an antidote when fish and crabs poisonous are consumed[12]. In Philippines, the root decoction is used to treat the syphilis infections and dysentery disease. In Thailand, root and leaves are used to treatment of skin diseases. Its leaves also can be chewed to relieve coughing and malaria. Similarly in some on North Island in Papua New guinea, leaves used to treat coughing or flu[13]. Manimegalai (2012)[14] reported that the preliminary phytochemical screening of *Scaevola taccada* showed the presence of proteins, phenols, carbohydrates and glycosides. Therefore, from the description above so performed testing research antioxidants activity leaves of plant *Scaevola taccada* (Gaertn.) Roxb based on ability DPPH (1,1-Diphenyl-2-Picryl hydrazil) free radicals scavenging to increase scientific data nutritious plants as medicine.

MATERIALS AND METHODS

Material Test

Leaves of *Scaevola taccada* (Gaertn.) Roxb. taken from the district Pinrang, South Sulawesi. Chemicals. Distilled demineralized water, BHT (Butylatedhydroxytoluene), leaves (*Scaevola taccada* (Gaertn.) Roxb.), ethanol, ethyl acetate, DPPH (1,1-Diphenyl-2-Picryl Hydrazil), methanol absolut, n-hexane and vitamin C.

Extraction

Leaves of *Scaevola taccada* (Gaertn.) Roxb was dried and pulverized. Leaves as 300 g was maceration method using ethanol 70 % solvent and keep it in seven days in a closed container and sheltered from direct sunlight while stirring periodically. After that done filtration and remaceration as three times. The filtrate obtained was concentrated using a rotary evaporator until obtained thick ethanol extract.

Partition

Ethanol extract obtained taken as many 5 g and extracted with ethyl acetate solvents by liquid-liquid partition and evaporated until obtained of ethyl acetate fraction.

Determination of DPPH (1-1-diphenyl 2-picryl hydrazyl) radical scavenging activity: Preliminary Test

The sample was made on TLC plates then elusion with n-hexane : ethyl acetate. TLC plates sprayed by using DPPH solvents then silenced for 30 minutes. Observed the colour change that occurs from purple to yellow.

Measurement of antioxidant

The testing of ethyl acetate fraction leaves *Scaevola taccada* by making solution stock 500 ppm is weighed 50 mg of extract made in five concentration is 50, 100, 200, 300 dan 400 ppm. For the positive control i.e. each vitamin C and BHT made in concentrations of 100 ppm by weighing 10 mg and made with a concentration 2, 3, 4 and 5 ppm for vitamin C while concentrations of 2, 4 and 6 ppm for BHT. The quantitative measurement of radical scavenging was determined 200 µl sample solution from various concentration was added 2.0 ml DPPH 0.4 mM and added with absolute methanol until 10 ml volumes. The mixtures were homogenous and left at room temperature for 30 minutes and then absorbance measured at spectrophotometer UV-VIS wavelength of 517 nm. The procedure done in triplo[15-17]. Percentage free radical scavenging activities were calculated:

% free radical scavenging activity

$$\% \ SA = (A_C - A_A) \times 100$$

Value ES_{50} calculated with using a linear regression equation, the concentration of the samples as the x axis and the y axis as inhibition %. From equation: y = a + bx can be calculate ES_{50} value using:

$$ES_{50} = \frac{(50 - a)}{b}$$

RESULTS

The ethanol extract *Scaevola taccada* (Gaertn.) Roxb was partitioned with using ethyl acetate solvent obtained 230 mg. The ethyl acetate fraction was tested of antioxidant activity by reduction DPPH. Tables 1 and 2 describes the result of antioxidant activity by DPPH free radical scavenging has a weak antioxidant with ES_{50} value is 1473.064 ppm compared BHT and vitamin C with ES_{50} value 9.054 ppm and 29.067 ppm.

DISCUSSION

Antioxidants have been defined as compounds that protected and prevented damage cell or tissue injury by free radicals. Oxidative metabolism produce many free radicals such as reactive oxygen or nitrogen oxygen. Several of oxidative stress are measured in various disease and these include total oxidant capacity as well as H_2O_2, a product of partial reduction of molecular oxygen, thiobarbituric acid reactive substances (TBARS), malondialdehyde (MDA), 8-isoprostone for lipid peroxidation[18,6] and 8-hydroxy-2-deoxyguanosine for DNA oxidation[19]. *Scaevola taccada* (Gaertn.) Roxb it is plant that estimated contain antioxidants, which contains some chemicals include alkaloid, phenolic and saponin. *Scaevola taccada* extracted by maceration and the results partitioned using as 300 ml ethyl acetate and obtained ethyl acetate fraction as 230 mg, the fraction obtained will the tested antioxidant activities qualitatively and quantitatively. Qualitatively, a number of ethyl acetate fractions *Scaevola taccada* (Gaertn.) Roxb dissolved by using ethyl acetate until obtained appropriate solubility and continued with made on TLC plates, elusion with n-hexane : ethyl acetate eluen (3:7), then observed spectro on uv 254 nm, 366 nm and DPPH spraying. Results TLC can be seen Figure 1. A compound of antioxidant will react with DPPH free radicals by mechanisms of donations hydrogen atoms which causing the occurance of the change of DPPH colour of purple to yellow in TLC plates, measured at wavelength 517 nm. Free radical scavenging causes electrons to be paired and causing disappearance colors are comparable to the quantities of electrons taken. Plant *Scaevola taccada* (Gaertn.) Roxb it is contain some compound, which contains protein, carbohydrate, phenols and glycoside. Phenolic are defined as a class of polyphenol and antioxidant activity. This activity is attributed to their donating hydrogen ability. Indeed, the phenolic groups of flavanoids serve as a source of a readily available "H" atoms such that the subsequent radicals produced can be delocolized over the flavanoid structure[20]. Results of measurements of antioxidant absorbance ethyl acetate fraction *Scaevola taccada* (Gaertn.) Roxb by using DPPH characterized with decrease absorbance from ethyl acetate fraction of leaves compared to vitamin C and BHT on Tables 1 and 2.

Table 1: Results absorbance measurement, percentage DPPH scavenging and ES50 value of ethyl acetate fraction leaves *Scaevola taccada* (Gaertn.) Roxb.

Sample	Concentration (ppm)	Absorbance	% DPPH Scavengging	ES_{50} (ppm)	ES_{50}
Replication I	50	0.826	5.708	1467.151	1473.064
	100	0.771	11.986		
	200	0.745	14.954		
	300	0.734	16.210		
	400	0.723	17.466		
Replication II	50	0.825	5.822	1476.020	
	100	0.771	11.986		
	200	0.745	14.954		
	300	0.734	16.210		
	400	0.723	17.466		
Replication III	50	0.825	5.822	1476.020	
	100	0.771	11.986		
	200	0.745	14.954		
	300	0.734	16.210		
	400	0.723	17.466		

Table 2: Results absorbance measurement, percentage DPPH scavenging and ES_{50} value of Vitamin C dan BHT

Sample	concentration (ppm)	Absorbance	% DPPH scavenging	ES_{50} (ppm)	ES_{50}
Vitamin C	2	0.747	14.726	9.054	9.054
	3	0.713	18.607		
	4	0.655	25.228		
	5	0.619	29.338		
BHT	2	0.540	38.356	29.067	29.067
	4	0.531	39.384		
	6	0.525	40.068		

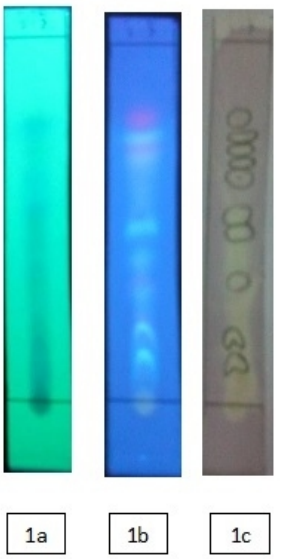

Figure 1: TLC profiles of fraction ethyl acetate leaves of *Scaevola taccada* (Gaertn.) Roxb with n-hexane : ethyl acetate (3:7) eluen shows 1a) visible on UV 254 nm, 1b) visible on UV 366 nm and 1c) visible spraying with DPPH reagent

One parameter has been introduced recently for interpretation of the results estimation DPPH radical scavenging activity is the "efficient concentration" or ES_{50} value (otherwise called the IC_{50} value). This is defined as the concentration of substrate that cause 50 % loss of the DPPH activity[21]. This parameter was apparently introduced by Brand-Williams and his colleagues[22,23]. Antioxidant activitiy indicated by value ES_{50} and a compound said as very strong antioxidant if value ES_{50} less than 200 ppm. Quantitatively, the fractions tested antioxidant activity and obtained a ES_{50} value of 1473.064 ppm. The value of antioxidant activity are obtained compared to the antioxidant activity vitamin C and BHT. From the results obtained that the ES50 value of vitamin C and BHT is 9.054 ppm and 29.067 ppm smaller than the value ES_{50} of the ethyl acetate fraction *Scaevola taccada* (Gaertn.) Roxb. So, based on the results research shown that the ES50 value of ethyl acetate fraction leaves of *Scaevola taccada* (Gaertn.) Roxb has weak antioxidant activity compared with vitamin C and BHT as a positive control.

CONCLUSION

Based of results the research can be concluded that ethyl acetate fraction *Scaevola taccada* (Gaertn.) Roxb has a weak antioxidant activity compared to vitamin C and BHT with ES_{50} values respectively 1473.064 ppm, 9.054 ppm and 29.067 ppm.

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