Innovare
Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 6, 2014

Original Article

FREE-RADICAL SCAVENGING ACTIVITY SCREENING OF SOME INDONESIAN PLANTS

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Received: 12 Mar 2014 Revised and Accepted: 10 Apr 2014

ABSTRACT

Objective: Indonesia is well known for its biodiversities and very rich of plants species which only a small portion of the species have been investigated in detail. This study aimed to investigate free radical scavenging activity of some Indonesian plants.

Methods: Ethanol extract of leaves and stems of plants were tested for their free radical scavenging activity using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) by TLC-autography and spectrophotometry method.

Results: The TLC-autography result showed that all samples have free-radical scavenging activities. Spectrophotometry analysis results showed that the lowest IC_{50} value was ethanol extract of *Alectryon serratus* stem with IC_{50} value of 2.04 ppm, lower than vitamin C with IC_{50} value of 3.11 ppm. The highest IC_{50} value was ethanol extract of *Ochrosia akkeringae* leaves with IC_{50} value of 214.64 ppm.

Conclusion: Ethanol extract of *Alectryon serratus* stem may be potential to be developed as a medicinal drug.

Keywords: Free-radical scavenging activity, Indonesian plants, TLC-autography, Spectrophotometry.

INTRODUCTION

Free radical was molecules which have unpaired electron at the outer orbit, as a consequence, they were tend to be unstable and very reactive [1]. The unpaired electron determined the reactivity degree of free radical. Main class of free radicals generated in living organism was derived from oxygen, such as superoxide, hydroxyl, peroxyl ($RO^{2\bullet}$), alkoxyl ($RO^{\bullet}$), and hydroperoxyl ($HO^{2\bullet}$) radicals [1, 2]. They were called as reactive oxygen species (ROS). Another major radicals such as nitric oxide ($NO^{\bullet}$) and nitrogen dioxide ($^{\bullet}NO_2$) were free radicals nitrogen and were called as reactive nitrogen species (RNS). Both ROS and RNS were normal products of metabolism processes and could be beneficial or even deleterious for organism. At a low concentration, ROS and RNS defense the body from infectious agents and played roles in a number of cellular signaling systems. The over production of ROS and RNS could damage and decrease the function of cellular lipid, proteins, and DNA in biological process usually called as an oxidative stress or nitrosative stress.

Organisms have developed some mechanisms to protect their bodies from free radicals-induced oxidative or nitrosative stress. They produced antioxidant molecules to protect cells from damage which caused by free radicals [3]. At a low concentration in the body, antioxidants could protect the cells and its content like proteins, lipids, carbohydrates, and DNA significantly [4]. Plants produced some compounds such as polyphenols and flavonoid that tend to have free radical scavenging activity.

Indonesia is well known in rich biodiversities of plant species where only small portion of the species have been investigated in detail. This study aimed was to investigate free radical scavenging activity of some Indonesian plants obtained from Alas Purwo National Park at Banyuwangi, East Java. Plants which have obtained from exploration were *Garuga floribunda* (Burseraceae), *Ochrosia akkeringae* (Apocynaceae), *Tabernaemontana pandacaqui* (Apocynaceae), *Mitrephora polypyrena* (Annonaceae), *Alectryon serratus* (Sapindaceae), and *Lepisanthes rubiginosum* (Sapindaceae). There were a little studies of those species, but there were a lot of publications about another species on the same family, such as Burseraceae [5], Apocynaceae [6,7], Annonaceae [8], and Sapindaceae [9]. Thus, the main goal of this study was to explore the potential in vitro radical scavenging activity of the plants.

MATERIALS AND METHODS

Chemicals and reagents

2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Sigma Aldrich), vitamin C (Merck), ethanol and methanol pro analysis (J.T Baker), TLC silica gel 60 F254 (Merck), Spectrophotometer Shimadzu UV-1800.

Plant materials

Stems and leaves of *Garuga floribunda, Alectryon serratus, Ochrosia akkeringae, Tabernaemontana pandacaqui, Mitrephora polypyrena*, and *Lepisanthes rubiginosum* were obtained from Alas Purwo National Park at Banyuwangi, East Java. All samples were authenticated by the authority of Purwodadi Botanical Garden, Pasuruan, East Java.

Extract preparations

Twelve samples obtained from Alas Purwo National Park were powdered, and then 50 g of each powdered sample was extracted using 250 mL of ethanol 80% by ultrasonic assisted extraction, for 3x2 minutes and repeated until reached total ethanol 80% being used was 750 mL. The extracts were evaporated in rotary evaporator and then stored in an oven at 40^0C until constant weight.

Preliminary phytochemical screening

The presence of polyphenols was tested with $FeCl_3$ reagent, flavonoids with H_2SO_4 10%, and terpenoids with anisaldehyde-H_2SO_4.

DPPH free radical scavenging assay

Qualitative assay

Stock solutions 10,000 ppm of each samples were spotted on silica gel TLC (Thin layer chromatography)-plates by 5x2 µL and the plates were developed in appropriate solvent systems to resolve components of the extracts. The plates were dried at room temperature and were sprayed with 0.02% DPPH in methanol. Bleaching of DPPH by the resolved bands was observed for 30 minutes and 60 minutes and the color changes (yellow on purple background) were noted. The qualitative assay was conducted to

observe the color changes of spots. Pale yellow spot meant that sample has a radical scavenging activity.

Quantitative assay

Solution of DPPH 0.004% in methanol was measured for its absorbance at 497 nm, 517 nm, and 537 nm using spectrophotometer. Afterwards, a diluted concentration series of extract solutions were pipetted 300 µL and were mixed with 2,700 µL DPPH 0.004% solution. After 30 minutes from mixing time, the mixed solutions absorbance were measured and repeated again after 60 minutes. The absorbance was calculated using formula

$$A = A_{517} - \frac{A_{497} + A_{537}}{2}$$

and radical scavenging activity was calculated as % reduction of DPPH's absorbance using formula

$$\% \text{ reduction} = \left\{1 - \frac{A1}{A0}\right\} \times 100\%$$

Which A0 was the absorbance of DPPH 0.004% solution and A1 was the absorbance of the mixed solutions. All data then were processed by linear regression equation and IC_{50} value was calculated. Vitamin C was used as a reference. The quantitative assay was carried out to determine radical scavenging activity which expressed by IC_{50} value.

RESULTS

Preliminary phytochemical screening

Qualitative test of the sample extracts were performed to detect the presence of various phytochemicals including polyphenols, flavonoids, and terpenoids. The result was showed in Table 1.

Phytochemical screening of the plant samples revealed some differences in the phytochemical constituents of the plants tested. All the samples tested were positive for polyphenols and terpenoids while only *Garuga floribunda* stem and *Tabernaemontana pandacaqui* leaves were negative for flavonoids.

Table 1: Preliminary phytochemical screening results of some Indonesian plants

Plant extract	Terpenoid	Flavonoid	Polyphenol
Garuga floribunda stem	+	-	+
Garuga floribunda leaves	+	+	+
Ochrosia akkeringae stem	+	+	+
Ochrosia akkeringae leaves	+	+	+
Tabernaemontana pandacaqui stem	+	-	+
Tabernaemontana pandacaqui leaves	+	+	+
Mitrephora polypyrena stem	+	+	+
Mitrephora polypyrena leaves	+	+	+
Lepisanthes rubiginosum stem	+	+	+
Lepisanthes rubiginosum leaves	+	+	+
Alectryon serrratus stem	+	+	+
Alectryon serratus leaves	+	+	+

DPPH free radical scavenging assay

The DPPH test showed the ability of the samples as a free radical scavenger. DPPH was a free radical and gave a strong absorption band at 517 nm in the visible region of the electromagnetic radiation. It has a deep violet color. This absorption diminished as the electron was paired off resulting in decolorization with respect to the number of electrons taken up and the color changes to a pale yellow. The TLC-autography result showed that all samples have free-radical scavenging activity in various strength determined by intensity of yellow color shown at the TLC plate as showed on Figure 1.

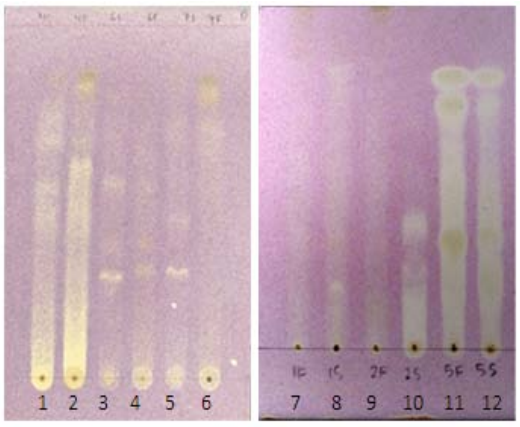
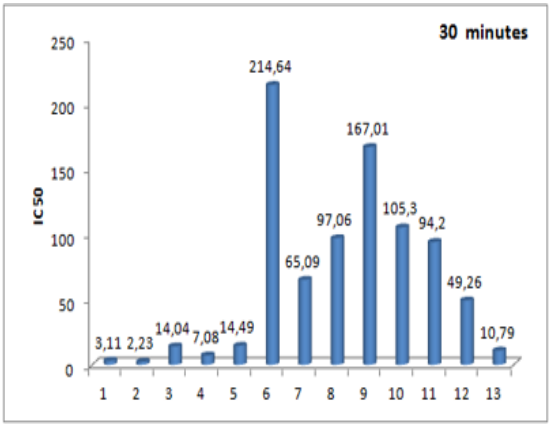


Fig. 1: TLC-autography result after 30 minutes sprayed by DPPH 0,2%. 1,2: *Garuga floribunda* stem, leaves; 3,4: *Ochrosia akkeringae* stem, leaves; 5,6: *Tabernaemontana pandacaqui* stem, leaves; 7,8: *Mitrephora polypyrena* leaves, stem; 9,10: *Lepisanthes rubiginosum* leaves, stem; 11,12: *Alectryon serratus* leaves, stem.

Furthermore, the samples were measured their IC_{50} value by spectrophotometry method. IC_{50} value was the concentration of substrate that caused 50% loss of the DPPH color/activity[10]. As a reference, vitamin C was measured for its IC_{50} value as well.

The result from spectrophotometry measurement showed that IC_{50} value of vitamin C were 3.11 ppm at 30 minutes and 3.13 ppm at 60 minutes. The lowest IC_{50} value of the samples was ethanol extract of *Alectryon serratus* leaves with IC_{50} value of 2.23 ppm at 30 minutes and 1.96 ppm at 60 minutes, which was lower than vitamin C. The highest IC_{50} value was ethanol extract of *Ochrosia akkeringae* leaves with IC_{50} value of 214.64 ppm at 30 minutes and 189.25 ppm at 60 minutes. The complete result was showed at Figure 2.

The lower IC_{50} value indicated the higher free radical scavenging activity of the sample, plant with low IC_{50} value may be potential to be developed as a medicinal drug.



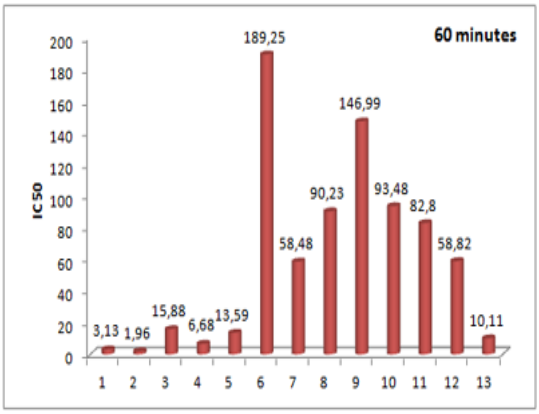


Fig. 2: IC_{50} of all samples at 30 minutes (left) and at 60 minutes (right). 1:Vitamin C; 2:*Alectryon serratus* leaves; 3:*Alectryon serratus* stem; 4:*Garuga floribunda* leaves; 5:*Garuga floribunda* stem; 6:*Ochrosia akkeringae* leaves; 7:*Ochrosia akkeringae* stem; 8:*Tabernaemontana pandacaqui* leaves; 9:*Tabernaemontana pandacaqui* stem; 10:*Mitrephora polypyrena* leaves; 11:*Mitrephora polypyrena* stem; 12:*Lepisanthes rubiginosum* leaves; 13:*Lepisanthes rubiginosum* stem.

DISCUSSION

Organisms have developed some mechanisms to protect their bodies from free radicals-induced oxidative or nitrosative stress. Human produced endogenous antioxidants such as *superoxide dismutase* (SOD), *glutathione peroxidase* (GPx), *catalase* (CAT), and *glutathione* (GSH) [2]. The imbalanced between those radicals and antioxidants defense system may lead to the damage of macromolecules such as DNA, carbohydrates, and protein that is suspected playing roles in pathological processes of various diseases.

Plants have been the basis of traditional medicines in Indonesia and throughout the world for thousands years and continued to provide new remedies to human. Plants contain wide range of chemicals constituents such as vitamins (C, E, carotenoids, etc.), flavonoids (flavones, isoflavones, flavonones, anthocyanins and catechins), polyphenols (ellagic acid, gallic acid and tannins) which exhibit potent antioxidant activities [4], and also some triterpenoids possess antioxidant activity [10]. Thus, plants were potential sources of antioxidant.

Phytochemical screening revealed some differences in polyphenols, flavonoids, and terpenoids constituents of the samples. The differences in phytochemical constituents lead to different radical scavenging activity that was proven by TLC-autography test results. Comparison between TLC plates from phytochemical screening with TLC-autography plates showed that polyphenol and flavonoid compounds were attributed for major radical scavenging activity concluded from the resemblance of spot pattern between those compounds with major bleaching spot pattern from TLC-autography plate. All samples then tested by spectrophotometric method using DPPH to measure its IC_{50} value. The decreased in absorbance of DPPH radical was caused by an antioxidant molecules, reaction between antioxidant and DPPH results in the scavenging of DPPH by hydrogen donation that reducing absorbance [11]. From this study, the scavenging activity of leaves and stem were found as concentration dependent and the lowest IC_{50} value was ethanol extract of *Alectryon serratus* leaves with IC_{50} value of 1.96 ppm which result was lower than vitamin C.

There was a growing interest in the investigation of plants as a source of natural antioxidant compounds [12] since they contain a wide range of secondary metabolite which have structural diversity [13]. Ethanol extract of *Alectryon serratus* leaves and stem; *Garuga floribunda* leaves and stem; and *Lepisanthes rubiginosum* stem that exhibited low IC_{50} value may be potential to be developed as a medicinal drug. These plants would give benefits for human health by protecting body from ill induced by free radicals and preventing free radical induced diseases such as cancer and atherosclerosis. Further research will be conducted to obtain the substance responsible for antioxidant activity of extracts.

ACKNOWLEDGEMENT

The authors acknowledge Universitas Airlangga, Japan International Cooperation Agency (JICA), and Science and Technology Research Partnership for Sustainable Development (SATREPS) in supporting this research.

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