

Screening of Medicinal Plant for Total Flavonoid and Antioxidant Activity in South Kalimantan of Indonesian

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Abstract—The present study was undertaken to find the antioxidant value of certain medicinal plants in South Kalimantan in Indonesian. Antioxidants have been reported to prevent oxidative damage caused by free radical and can be used in cardiovascular and anti-inflammatory diseases. The amount of total flavanoids and radical scavenging activity has been studied. Major amount of flavonoid were determined in *kasturi* fruit followed by leaf of *kelakai*, stem of *gerunggang*, and root *pasak bumi*. However, *kelakai*, *kasturi*, *pasak bumi*, and *gerunggang* extracts potent of antioxidant by chelating effect on ferrous ions, hydroxyl radical scavenging, and hydrogen peroxide scavenging.

Index Terms—Indonesian medicinal plant, total flavonoid, antioxidant activity

I. INTRODUCTION

Reactive oxygen species are formed during normal cellular metabolism, but when present in high concentration they become toxic. Mammalian cells possess intracellular defenses such as superoxide dismutase, catalase or glutathione peroxidase, in order to protect the cells against excessive levels of free radicals. Exogenous addition of compounds such as vitamins (A, E, β -carotene), minerals (selenium, zinc), or proteins (transferrin, ceruloplasmin, albumin) can provide additional protection [1]. These natural antioxidants or other compounds that can neutralize free radicals may be of central importance in the prevention of vascular diseases, some forms of cancer [2], [3] and oxidative stress responsible for DNA, protein and membrane damage. External antioxidants, like antioxidants extracted from plants, can be administrated in order to combat those radicals [4]- [6]. Current research into free radicals has confirmed that foods rich in antioxidants play an essential role in the prevention of cardiovascular diseases, cancers and neurodegenerative diseases. Therefore, much attention has been focused on the use of natural antioxidants to protect the damage of free radicals. Epidemiological studies have shown that many medicinal plant such as

kelakai, *kasturi*, *pasak bumi*, and *gerunggang* might protect the human body against damage by ROS. The consumption of natural antioxidant was reported to have potential health benefits. [1]-[4].

South Kalimantan of Indonesian, as a tropical district, shows on amazing diversity of plants species. Some of them have been long used as traditional medicines. Many of them were reported to have various desirable activities [7], [8]. In South Kalimantan, the *kelakai*, *kasturi*, *pasak bumi*, and *gerunggang* has few uses. However, on the basis of empirical studies, the *kelakai* is used by the Kenyah Dayak communities for the treatment of anemia, fever, and cutaneous disorders. In spite of this, few scientific studies have confirmed this empirical evidence.

Besides, phenolic compounds and flavonoids are also widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, antiinflammatory, anticarcinogenic etc. [5]. As crude extracts of herbs and spices and other plant materials, rich in phenolics are increasing interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. While, flavonoids are a group of polyphenolic compounds with known properties, which include free radical scavenging, inhibition of hydrolytic and oxidative can enzymes and anti-inflammatory action [6].

In the present study, we investigate total flavonoid and antioxidant activity of *kelakai*, *kasturi*, *pasak bumi*, and *gerunggang* that were commonly found in the South Kalimantan of Indonesia.

II. DETERMINATION OF TOTAL FLAVONOID AND ANTIOXIDANT ACTIVITY

A. Chemical and Materials

1mM FeCl_3 , 2 mM FeCl_2 , 1mM 1,10- phenanthroline, 0.2 M phosphate buffer (pH 7.8), 0.17 M H_2O_2 , ascorbic acid, NaNO_2 , AlCl_3 , and quercetin were from Sigma. All other reagents were of the highest quality grade available.

B. Plant Material

Kelakai, *kasturi*, *pasak bumi*, and *gerunggang* ferns were collected in June 2009 from Gambut subdistrict, South Kalimantan. Species identification was performed by the Department of Biology, Pharmacy Study Program, Faculty of Mathematics and Natural Sciences (FMIPA), Lambung Mangkurat University. The active principles in the *kelakai*, *kasturi*, *pasak bumi*, and *gerunggang* plants were extracted

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by maceration. The botanical names, local names, and parts used are presented Table I.

C. Total Flavonoid Content

Flavonoids were determined using Aluminum chloride colorimetric method [9]. The calibration curve was made by preparing quercetin solutions at different concentrations. Each experiment was carried out in triplicate and results averaged expressed as mean $\pm$ SD.

D. Chelating Effect on Ferrous Ions

The chelating effect of ferrous ions was estimated by the method of Hung-Ju Chou *et al.* [10]. The absorbance of the mixture was measured at 562 nm. Chelating effect was calculated using the equation: $(1 - \text{absorbance of sample} / \text{absorbance of control}) \times 100$. Each experiment was carried out in triplicate and results averaged expressed as mean $\pm$ SD.

E. Hydroxyl Radical Scavenging Activity

The scavenging activity for hydroxyl radicals was measured with Fenton reaction [11]. The absorbance of the mixture at 560nm was measured with a spectrophotometer. Hydroxyl radical scavenging activity was calculated using the equation: $(1 - \text{absorbance of sample} / \text{absorbance of control}) \times 100$. Each experiment was carried out in triplicate and results averaged expressed as mean $\pm$ SD.

F. Hydrogen Peroxide Scavenging Activity

The hydrogen peroxide scavenging was determined according to the method of Ruch *et al.* [12]. The absorbance value of the reaction mixture was recorded at 230 nm. Hydrogen peroxide scavenging activity was calculated using the equation: $(1 - \text{absorbance of sample} / \text{absorbance of control}) \times 100$. Each experiment was carried out in triplicate and results averaged expressed as mean $\pm$ SD.

TABLE I: CHARACTERISTICS OF THE USED MEDICINAL PLANTS

Botanical names	Local names	Parts used
<i>Stenochlaena palustris</i>	kelakai (KL)	leave
<i>Mangifera casturi</i>	kasturi (KT)	fruit
<i>Eurycoma longifolia</i> Jack	pasak bumi (PB)	root
<i>Cratoxylon arborescens</i> Blume	gerunggang (GR)	stem

III. RESULTS AND DISCUSSION

A. Total Flavonoid

The total flavonoid concentrations equivalents were used for the measurements of water extract concentrations. Based on this study, we proposed that the potent free radical-scavenging and antioxidative activity of medicinal plant might result from its high contents of flavonoid type compounds. Flavonoids are regarded as one of the most widespread groups of natural constituents found in plants. The results are shown in Fig. 1. All the tested fractions had high flavonoid content; kasturi had the highest (30 $\mu\text{g/mL}$ of quercetin equivalent, QE) and gerunggang had the lowest content (3.8 $\mu\text{g/mL}$ of QE). Flavonoids are known to show antioxidant activity having considerable effects on human

nutrition and health. The mechanism of flavonoid action is based on scavenging or chelating process.

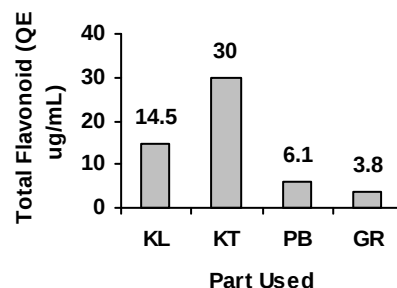


Fig. 1. Total flavonoid content in different plants extract (KL: Kelakai; KT: kesturi; PB: pasak bumi, and GR: gerunggang)

B. Chelating Effect on Ferrous Ions

The ferrous ion chelating activities of *kelakai*, *kasturi*, *pasak bumi*, and *gerunggang* extracts are shown in Fig. 2. The metal scavenging effect of these samples decreased in the order of kasturi > gerunggang > kelakai > ascorbic acid > pasak bumi. Metal chelating capacity was significant, since it reduced the concentration of the catalysing transition metal in lipid peroxidation. It was reported that chelating agents are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ion.

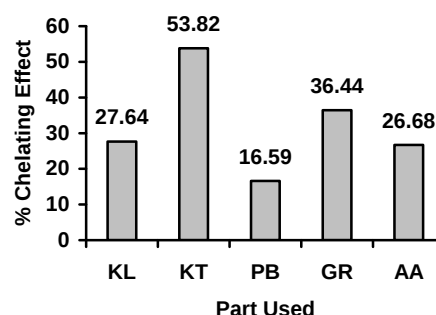


Fig. 2. Chelating effect on ferrous ions in different plants extract (KL: Kelakai; KT: kesturi; PB: pasak bumi, GR: gerunggang; and AA: ascorbic acid)

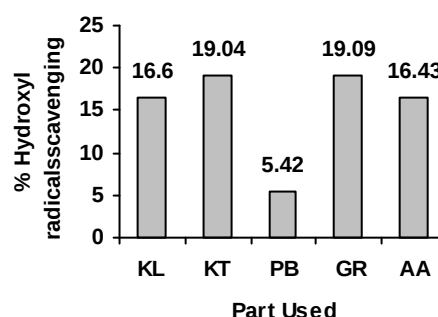


Fig. 3. Hydroxyl radical scavenging in different plants extract (KL: Kelakai; KT: kesturi; PB: pasak bumi, GR: gerunggang; and AA: ascorbic acid)

C. Hydroxyl Radical Scavenging Activity

The hydroxyl radical can damage virtually all types of macromolecules: carbohydrates, nucleic acids (mutations), lipids (lipid peroxidation) and amino acids (e.g. conversion of Phe to m-Tyrosine and o-Tyrosine). The hydroxyl radical

has a very short *in vivo* half-life of approximately 10^{-9} seconds and a high reactivity. This makes it a very dangerous compound to the organism. Unlike superoxide, which can be detoxified by superoxide dismutase, the hydroxyl radical cannot be eliminated by an enzymatic reaction. Mechanisms for scavenging peroxy radicals for the protection of cellular structures includes dietary antioxidants such as flavonoid and vitamin C. The hydroxyl radical scavenging activity of the various extracts was investigated (Fig. 3).

The hydroxyl radical scavenging of these samples decreased in the order of *kasturi* $\approx$ *gerunggang* > *kelakai* $\approx$ ascorbic acid > *pasak bumi*.

D. Hydrogen Peroxide Scavenging

Hydrogen peroxide scavenging activity are shown in Fig. 4. Hydrogen peroxide can be formed *in vivo* by many oxidase enzymes such as superoxide dismutase. It can cross membranes and may slowly oxidize a number of compounds. The hydrogen peroxide scavenging effect of 75 $\mu\text{g/mL}$ concentration of *kelakai*, *kasturi*, *pasak bumi*, and *gerunggang* extracts decreased in the order of ascorbic acid $\approx$ *kelakai* > *gerunggang* > *kasturi* > *pasak bumi*. Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cells because it may give rise to hydroxyl radicals in the cells. Addition of hydrogen peroxide to cells in culture can lead to transition metal ion-dependent OH radical mediated oxidative DNA damage. Levels of hydrogen peroxide at or below about 20–50 mg seem to have limited cytotoxicity to many cell types. Thus, removing hydrogen peroxide as well as superoxide anion is very important for protection of food systems.

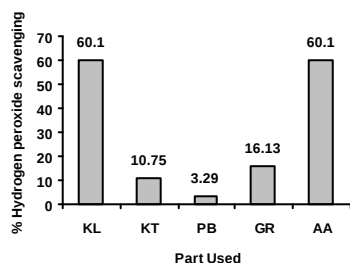


Fig. 4. Hydrogen peroxide scavenging in different plants extract (KL: Kelakai; KT: kasturi; PB: pasak bumi; GR: gerunggang; and AA: ascorbic acid)

IV. CONCLUSIONS

In the present study, the antioxidant activity of four traditionally used medicinal plants grown around the Sout

Kalimantan of Indonesian was evaluated. The results of the present study suggest that tested plant materials have moderate to potent antioxidant activity and/or free radical scavenging activity. Total flavonoid were decreased in the order of *kasturi* > *kelakai* > *gerunggang* > *pasak bumi*.

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