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Determination of potential level of Indonesian rhizomes as an antioxidant based on phenolic compound and antioxidant activity

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Abstract. This study was aimed to determine the potential level of rhizomes from Indonesia as an antioxidant. The potential level of rhizome was determined based on the total phenolic compound and antioxidant activity. The phenolic compound was determined using the Folin-Ciocalteu method, antioxidant activity was determined by the DPPH (1-diphenyl-2-picrylhydrazyl) method expressed in IC₅₀. Potential level was calculated by comparing the value of total phenolic compound and antioxidant activity. The results showed that red ginger (*Zingiber officinale* var *Rubrum*) had the highest phenolic and flavonoid content. Therefore that red ginger, *Zingiber officinale* var *Rubrum* is the most potential level as an antioxidant followed by *Curcuma longa*, *Curcuma zedoaria*, *Curcuma zanthorrhiza*, and *Curcuma aeruginosa* Roxb.

1. Introduction

Plants such as herbs have long been used in traditional medicine in various cultures throughout the world. Rhizome is one of the traditional medicinal plants that have been used in Indonesia for treating diabetes, high blood pressure, cancer, and many other illnesses. In Indonesia, many of rhizomes are often commonly provide as a medicinal traditional to health-promoting effects to relieve certain illnesses such as nausea, motion sickness, stomach-ache, asthma, diarrhoea, digestive disorder, vomiting, rheumatism, swelling, common cold and cough. Many phytochemicals of these rhizomes have been reported to have interesting biological activities include antifungal, antioxidant, and anti-inflammatory activities.

Free radicals or reactive oxygen species (ROS) during metabolism and other activities beyond the antioxidant capacity of a biological system gives rise to oxidative stress [1]. Antioxidants are substances or compounds that inhibit oxidation. Antioxidants are having scavenging power of free radicals produced inside the human body which are responsible for many of the metabolic disorders such as atherosclerosis, arthritis, cancer, and gastrointestinal disorders. [2]. In oxidation processes happening in biochemical reactions inside the human body, free radicals are produced and enzymes such as superoxidase dismutase, catalases and hydroperoxides act as natural antioxidants against them [3]. When antioxidants are consumed or fortified in food, they further slowdown the oxidation processes by fixing the stray electrons which are responsible for causing oxidative stress leading to cellular damage resulting in aging and various diseases such as cancer, diabetics, rheumatoid arthritis, cardiovascular diseases, chronic inflammation, and stroke [4].

Phenolic compounds from plants belong to a class of bioactive components with antioxidant activities [5-7]. The antioxidant activity of plants has been demonstrated in many recent studies [8,9]. Flavonoids represent one of the most studied classes of phenolic compounds containing carbohydrate units important for their biological activities [10]. Flavonoids exhibit a wide range of biological effects



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(antibacterial, antiviral, anti-inflammatory and anti-allergic) by reducing lowdensity lipoproteins in plasma, inhibiting platelet aggregation, scavenging free radicals, and preventing cell proliferation [11].

Ginger contains a component of flavonoids which can increase prostaglandin which is a defensive factor of the stomach. In addition, ginger also contains acetone and methanol can protect the stomach by reducing stomach acid and prevent irritation of the gastric mucosa. Curcuma rhizome contains active compounds including terpenoids, alkaloids, flavonoids, phenols and curcuminoids which can function as antioxidants and antimicrobials. Turmeric as an anticholesterol, anti-cancer, antimutagenic, white turmeric functions as an antioxidant, anti-inflammatory, anticancer, antibacterial and immunomodulatory. The objective of this work was to estimate the total phenolic and flavonoid content from five rhizome from Indonesia and to determined the level of potential plant species of rhizomes as an antioxidant agent.

2. Materials and Methods

2.1. Plant Material

Fresh rhizomes of *Zingiber officinale* var Rubrum, *Curcuma longa*, *Curcuma zedoaria*, *Curcuma Curcuma zanthorrhiza*, and *Curcuma aeruginosa* Roxb were purchased from Bogor, Indonesia (table 1). The rhizomes were washed, sliced and dried in a hot air oven at 50°C for 72 h.

Table 1. Botanical name, common name and traditional uses.

Botanical name	Common name	Traditional uses	References
<i>Zingiber officinale</i>	Red ginger	Arthritis, pneumonia, tuberculosis, toothache, cough, bronchitis, diarrhea, headaches	[12]
<i>Curcuma longa</i>	Tumeric	Hematologic, flatulence, infection	[13]
<i>Curcuma zedoaria</i>	White tumeric	Carminative, blood purifier, infection, fever, and heart disease	[14]
<i>Curcuma xanthorrhiza</i>	Ginger	Stomach disorders, diarrhea, fever, liver damage	[15]
<i>Curcuma aeruginosa</i> Roxb	Black tumeric	Cough, asthma, stomach disorders, intestinal, antihelminthic	[16]

2.2. Solvent and chemicals

All solvents used were of analytical grade. Folin-Ciocalteu reagent, sodium carbonate, trichloroacetic acid, ethanol (EtOH), ascorbic acid, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), gallic acid, and sodium chloride were purchased from Sigma-aldrich (Germany).

2.3. Extraction and yield of samples

The equal amount of different rhizomes powder (20g) was extracted with EtOH (200mL) for 2 days at room temperature (25°C) with continuous magnetic stirring to prevent oxidation by air and shielding from sunlight. The extraction was carried out so that the components were completely extracted and not oxidized. Then the solutions were filtered through double filter paper (Whatman™ No. 1). Fresh EtOH was added into the used plant material and the process was repeated three times. The filtered solutions were dried on a rotary evaporator under reduced pressure at 40°C. The yield of all extracts was recorded and kept in the refrigerator at 4°C for experimental analyses.

2.4. Total phenolic contents

Total phenolic content was estimated as gallic acid equivalents (GAE) per gram of dried plant extract, according to the Folin-Ciocalteu phenol reagent method [17]. First, a standard curve was generated using gallic acid as a standard. Different concentrations of gallic acid were prepared in 80% methanol, and

their absorbance values were measured at 765 nm. For sample measurement, 0.5 mL (1/10 dilution) of Folin-Ciocalteu phenol reagent and 1000 mL of distilled water were added to 100 µL of plant extract. The solutions were mixed and incubated at room temperature for 1 min. After 1 min, samples were combined with 1500 mL of 20% sodium carbonate (Na_2CO_3) solution, mixed, and incubated for an additional 120 minutes. Absorbance at 765 nm was measured. Data presented are average values of four measurements for each sample.

2.5. Total flavonoid contents

Total flavonoid contents (TFC) were estimated according to the method [18]. Quercetin was used to make a calibration curve. 100µL of samples (1000µg/mL) were put in the microplate and 100µL of 2% AlCl_3 was added. The reaction was mixed and stand at room temperature for 15min. The absorbance of the mixture was measured at 430 nm by using a Spectrophotometer. The TFC was expressed as quercetin equivalents in mg per g extract. The experiment was carried out in triplicate.

2.6. DPPH radical scavenging assay

The DPPH radical scavenging assay was estimated according to the procedure [19]. 40µL of DPPH and 80µL of sodium acetate buffer (0.1M, pH=5.5) were added into 80µL samples at different concentrations (10, 25 and 50µg/mL). The mixture solution was incubated at room temperature in the dark for 30min. The absorbance was measured at 517nm by using a Spectrophotometer (Winooski, USA). Trolox was used as positive control in this assay. The % DPPH radical scavenging activity was calculated using the formula:

$$\% \text{ scavenging capacity } (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100 \quad (1)$$

where control is the absorbance of control without test sample and sample is the absorbance of the sample. The experiment was carried out in triplicate. The % radical scavenging activity was plotted against the corresponding extract concentration to obtain the IC_{50} value.

2.7. Determination of potential level

Determination of potential level was calculated based on phenolic content and antioxidant activity using the formula :

$$\text{Potential level} = \text{total phenolic content} / \text{antioxidant activity} \quad (2)$$

where antioxidant activity assumed has a same density of extract. Rhizomes that have the highest potential value are expressed as the most potential plants to overcome digestive disorders.

3. Results and discussion

3.1. Total Phenolic and flavonoid content

Total phenolic and flavonoid of rhizomes extract are shown in Table 2. The amount of total phenol was determined with the Folin-Ciocalteu reagent. Gallic acid was used as a standard compound and the total phenols were expressed mg GAE/g extract. Zingiber officinale had the greatest levels of phenolic content (400.20 mg GAE/g extract) whereas Curcuma aeruginosa had the lowest level of phenolic (51.49 mg GAE/g extract). This value from this research is significantly lower in compared to research of [20] that polyphenolic content in ginger extract nearly 2 times (871 mg GAE/g ginger extract) higher than our sample. Composition and quantity of the phenolic are vary significantly according to different intrinsic and extrinsic factors, including plant genetics and cultivars, soil and growing conditions, maturity state and harvest conditions. Extracted process also has a significant effect on the composition and properties of the final extract.

The amount of flavonoid content was determined with the Quercetin reagent. Quercetin was used as a standard compound and the total flavonoid were expressed as mg QE/g extract. Similar trend with total phenolic that was observed in total flavonoid. The maximum flavonoid content was found in the

ethanol extract of *Zingiber officinale* (400.20 mg GAE/g extract) and the minimum also was found of *Curcuma aeruginosa* (33.36 mg QE/g extract).

Table 2. Total phenolic and flavonoid content of rhizomes.

Sample	Total phenolic content (mg GAE/g extract)	Total Flavonoid content (mg QE/g extract)	Antioxidant Activity, IC ₅₀
<i>Zingiber officinale</i>	400.20 ± 0.1	268.20 ± 0.02	3.96 ± 0.02
<i>Curcuma longa</i>	285.00 ± 0.5	79.36 ± 0.01	24.06 ± 0.05
<i>Curcuma zedoaria</i>	195.10 ± 0.3	58.69 ± 0.03	40.00 ± 0.03
<i>Curcuma xanthorrhiza</i>	139.16 ± 0.2	101.60 ± 0.05	167.03 ± 0.04
<i>Curcuma aeruginosa</i> Roxb	51.49 ± 0.6	33.36 ± 0.02	406.52 ± 0.02

3.2. Antioxidant activity

In this research, the antioxidant activity of ethanol extracts of rhizomes were investigated by using DPPH radical scavenging assay. The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolourize in the presence of antioxidants. The colour of DPPH radical will turn to yellow from purple when the odd electron of DPPH radical becomes paired with hydrogen from antioxidant compound. DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition brought by various additives. A freshly prepared DPPH solution exhibits a deep purple colour generally disappears when antioxidants present in the medium. Thus the antioxidants present in the extract can quench DPPH free radicals by donating hydrogen atom or by electron transfer and convert them to the colourless product (2,2-diphenyl-1-picrylhydrazyl) or a substituted analogous hydrazine, resulting in a decreasing absorbance at the 517 nm. The antioxidants present in the rhizome extract can acts as radical scavengers may protect the cells against various diseases such as cancer, neurodegenerative and digestive disorder.

The result of antioxidant activity from five rhizomes extract are tabulated in Table 2. A low IC₅₀ value indicates a strong ability to scavenge free radicals. *Z. officinale* exhibited the highest antioxidant activity both assays, with IC₅₀ values of 3.96 µg/mL (DPPH) and the lowest antioxidant activity was *Curcuma aeruginosa* Roxb (406.52 µg/mL).

The relationship between antioxidant activity and total phenolic content was found strong correlation. It has been reported that phenolic compounds are responsible for antioxidant activity due to their capacity by donating electrons or a hydrogen atom to free radical. The high value of total phenol in *Z. officinale* with low antioxidant activity showed that *Z. officinale* had high antioxidant activity.

3.3. Determination of potential level

Determination of potential level of rhizomes as an antioxidant agent calculated by comparing phenolic content with antioxidant activity. The rhizome that has a highest value showed the most potential rhizome as antioxidant agent. The result of this research presented in table 3.

Table 3. Potential level of rhizomes as an agent antioxidant.

Sample	Total phenolic content (mg GAE/g extract)	Antioxidant Activity, IC ₅₀	Potential level
<i>Zingiber officinale</i>	400.20 ± 0.1	3.96 ± 0.02	101.06
<i>Curcuma longa</i>	285.00 ± 0.5	24.06 ± 0.05	11.85
<i>Curcuma zedoaria</i>	195.10 ± 0.3	40.00 ± 0.03	4.87
<i>Curcuma xanthorrhiza</i>	139.16 ± 0.2	167.03 ± 0.04	0.83
<i>Curcuma aeruginosa</i> Roxb	51.49 ± 0.6	406.52 ± 0.02	0.13

Table 3 presented that *Z. officinale* has a highest potential level followed by *C. longa*, *C. zedoaria*, *C. xanthorrhiza* and the lowest is *C. aeruginosa*. Based on this research known that *Z. officinale* is rhizome that is most potential as an agent of antioxidant. It is known that the antioxidant activity of plant extracts

containing polyphenol components is due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals. The ginger extract showed antioxidant effect in inhibiting DPPH radical, IC₅₀ was 3.96 µg/ml.

4. Conclusion

In the present study showed that red ginger (*Zingiber officinale* var Rubrum) had the highest phenolic and flavonoids content so *Zingiber officinale* var Rubrum is the most potential level as an antioxidant, followed by *Curcuma longa*, *Curcuma zedoaria*, *Curcuma zanthorrhiza*, and *Curcuma aeruginosa* Roxb.

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