Antioxidant Activity of the Extract of Tropical Rhizomes

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ABSTRACT

The objectives of the present research was to evaluate antioxidant activity of the extracts of several rhizomes, i.e., kencur (Kaempheria galanga), laos (Alpinia galangan), temulawak (Curcuma xanthorrhiza), temugiring (C. viridoflora), temuireng (C. aeruenosa), temukunci (Boesembergia pandurata), ginger (Zingiber officinalle), and turmeric (C. domestica). A certain quantity of powdered sample was extracted with acetone and with ethanol. After evaporation the extracts were dissolved in methanol at a given concentration and then assayed for radical scavenging activity by DPPH method. The extracts were then evaluated for antioxidant activity in linoleic acid system by monitoring peroxide value. The results show that among the ethanolic extracts, radical scavenging activity of ginger was the most potent, followed by temulawak, while kencur was the lowest. Compared with BHA (300µg/mL), radical scavenging activity of ethanolic extract (1%) of rhizomes were higher except temuireng and kencur. Among the acetone extracts, the highest and the lowest activity were also ginger and kencur, respectively. In linoleic acid buffer system, the trend of the activity among the extracts of rhizomes were also the same as monitored by peroxide value.

Keywords: Rhizome, radical scavenging, antioxidant activity, linoleic acid, peroxide value.

INTRODUCTION

Lipid peroxidation is recognized as one of the main factors in deterioration of fat and fat-containing foods during processing and storage, thus the addition of antioxidants is a common mean of increasing the shelf life of food products and improving the stability of fat-containing foods. In addition, in living system, lipid peroxidation is also associated with some degenerative diseases including cancer, cataract , and aging (Torel *et al.*, 1986, Halliwel and Gutteridge, 1985, Duh and Yen, 1997)). Therefore, dietary antioxidants is assumed to be effective protection against peroxidative damage in organisms.

The use of antioxidant for preserving food products is extensive in the food industry. It is estimated that antioxidants can increase the shelf life of foods by 15 – 200% (Duh and Yen, 1997). The commonly used antioxidants in food preservation are synthetic antioxidants such as butylatedhydroxy anisole (BHA), butylatedhydroxy toluene (BHT), and propyl gallate. These compounds are effective antioxidants, however, recently their use is restricted since they are suspected to give adeverse effects to human health(Miyake and Shibamoto, 1997)). Therefore, natural antioxidants are now gaining special attention .

Plants in the tropic are regarded as great resources of natural antioxidants although their distribution and structure have not been fully studied (Nakayama *et al.*, 1994). The prominent antioxidants found in plant food materials are ascorbic acid, tocopherols and carotenoids, however, plant tissues are also rich in a wide variety of phenolic compounds including flavonoids. These substances are important antioxidants found in food, however, a variety of plant food contains a large number of components including unknown biological activities. Many compounds of these groups have been reported to have a multiple functional effects including antioxidant activity (Marrinova and Yanishlieva, 1994; Gadow *et al.*, 1997; Kanner *et al.*, 1994).

Lately, the targets of antioxidant application are shifting from food ingredients to nutritional or functional purposes because some human diseases including aging and cancer are now ascribed to oxidation of cellular components (Nakayama *et al.*, 1994). It has been reported that numerous pathological conditions are produced by active oxygen radicals including superoxide anions, hydroxyl radicals and hydrogen peroxides (Halliwel and Gutteridge, 1985; Mendoza *et al.*, 1994; Minamiyama *et al,* 1994). There are some indications that not only endogenous antioxidants but also dietary antioxidants may be effective protection from peroxidative damage in living systems. Thus, recently radical scavengers have attracted special concern because they can protect the human body from radical attack which may cause many diseases (Ariga and Hamano, 1990).

Many higher plants produce important biologically active compounds that are useful in the industrial and medicinal purposes including food and beverages. The investigation of bioactive natural products has assumed a greater sense of urgency in response to the demands for human food and a good heakth (Colegate and Molyneux, 1993).

The increasing restriction of the use of synthetic antioxidants such as BHA, BHT and TBHQ in foods has increased the interest in natural antioxidants, including those present in spices, and during the last decades spice extracts have been marketted as antioxidants for use in the food industry (Madsen *et al.*, 1996). In Indonesia, many plant roots (rhizomes) are commonly used spices and also as traditional medicines ("jamu"). The constituents of these materials are not only beneficial for flavoring of foods but some of them have bioactivities such as antioxidative and antiinflammatory effects. Rhizomes that have been well studied for their antioxidant activity are ginger and turmeric (Majeed *et al.*, 1992, Kikuzaki and Nakatani, 1994). However, in Indonesia there are many other tropical rhizomes that are extensively used as spices and ingredients of traditional medicine. The objective of the present research was, therefore, to evaluate the antioxidant activity of the extracts of several rhizomes, *i.e.*, kencur (*Kaempheria galanga*), laos or "lengkuas" (*Alpinia galangan*), temulawak (*Curcuma xanthorrhiza*), temugiring (*C. viridoflora*), temuireng (*C. aeruginosa*), temukunci (*Boesembergia pandurata*), ginger (*Zingiber offinalle*), and turmeric (*C. domestica*). The specific objectives were to examine radical scavenging activity of the rhizome extracts on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals and to evaluate the activity on the inhibition of peroxidation of linoleic acid in buffer system as monitired by peroxide value.

MATERIALS AND METHODS

Materials

Fresh rhizomes, *i.e.*, kencur, laos, temulawak, temugiring, temuireng, temukunci, ginger, and turmeric were purchased from a local market in Yogyakarta. The maturity of the rhizomes was that of the commonly used for spices in household preparation. After selection for their soundness, the rhizomes were peeled, slices by stainless steel, and dried in a cabinet drier (<50°C) until dryness. The dried materials were ground in a blender and sieved to become a 40 mesh powder. The yield of powder from rhizomes sample was recorded.

Chemicals of 1,1-diphhenyl-2-picrylhydrazyl, linoleic acid, and ammonium thiocyanate were purchased from Sigma Co. St Louis, Mo, USA. Solvents and other chemicals were laboratory grade.

Preparation of extracts

A certain quantity of powdered samples was extracted with ethanol and with acetone according to Masuda and Jitou (1994). Briefly, a 50 g of sample in a 500mL Erlenmeyer flask was added a 150mL ethanol or acetone. The mixture was stirred for 15 minutes at room temperature. After filtration through a Whatman filter paper No. 2 the filtrate was evaporated in a rotary vacuum evaporator (<50°C) until dryness. The crude extract was dissolved in methanol at a given concentration, stored under N_2 gas in a brown bottle at -30°C until use. The amount of crude extract was measured as yield.

Assay for radical scavenging activity

This assay was performed after Yen and Chen (1995). A 1.0mL of weighed sample in methanol (1%) was added to a 4.0mL of 1mM 1,1-diphenyl picryl hydrazyl (DPPH) solution. The mixture was mixed and then measured the absorbances at 517nm at two-minute intervals. The decrease of absorbance indicates the extent of radical scavenging activity of the samples. Butylatedhydroxy anisole (BHA, 300µg/mL) was as a reference standard antioxidant.

Evaluation in linoleic acid system

Evaluation of antioxidant activity in linoleic acid system was conducted by the method of Osawa and Namiki (1981) modified by Kikuzaki and Nakatani (1994). Basically, a mixture of 4.0mL of 1% sample solution in 99.5% ethanol, 4.1mL of 2.5% linoleic acid in 99.5% ethanol, 8.0mL of 0.05M phosphate buffer pH 7.0, and 3.9mL of water were placed in a vial with screw cap and then placed in an oven at 40°C in the dark. Peroxide value (POV) of the solution was monitored every 24 hours. The determination of POV was conducted by ferrythiocyanate (FTC) method as described by Masuda and Jitou (1994). Principally, to a 0.1mL of the linoleic acid system is added 9.7mL of 75% ethanol and 0.1mL of 30% ammonium thiocyanate. Precisely 3 minutes after addition of 0.1mL 0.02M ferrous chloride in 3.5% HCl to the reaction mixture, the absorbance of red color was read at 500nm on a spectrophotometer.

RESULTS AND DISCUSSION

Yield of powder and extract

Rhizome powder was prepared from fresh materials that have been dried in a cabinet drier. Table 1 shows the yield of powder and its moisture content.

Table 1. Yield of rhizome powder and its moisture content

Sample	Moisture of fresh rhizome (%)	Yield of powder (%)	Moisture of powder (%)
Ginger (*Zingiber offinalle*)	82.14	10.37	11.75
Turmeric (*Curcuma domestica*)	91.28	14.84	10.76
Kencur (*Kaempheria galanga*)	79.84	12.95	12.57
Laos (*Alpinia galangan*)	71.77	17.44	12.89
Temulawak (*C. xantho rrhiza*)	83.47	22.35	15.83
Temugiring (*C. viridoflora*)	85.02	12.55	11.53
Temuireng (*C. aeruginosa*)	78.44	24.33	12.98
Temukunci (*Boesembergia pandurata*)	65.17	22.93	9.08

The rhizomes used for sample materials in the present experiment were fresh materials with maturity as commonly used for spices, thus the moisture content was remarkable high. After being dried, ground and sieved the yield of powder varried. The yield of temuireng was the highest (24.33%), followed by temukunci and temulawak, and the lowest was ginger (10.37%). The variation of powder yield is related with the tissue structure of the rhizomes that influences the extent of size reduction after grinding. The more fibrous of the tissue, the more difficult in size reduction, thus in consequence with lower yield of powder. Since the drying process of rhizomes was performed by a cabinet drier in a similar condition, the moisture content of sample powder were not considerable different, ranged from 9.08 to 12.89%, except that of temulawak that its moisture content was 15.83%. Moisture content of the powders is mainly influenced by moisture content of the fresh rhizome, the tissue structure, and the efficiency of the drying process.

Yield of extracts

Solvents used for extraction of antioxidants from the rhizomes were ethanol and acetone. The yield of ethanolic and acetone extracts from rhizome is presented in Table 2. As shown in the table, the yield of ethanolic and acetone extracts were approximately 1.75% and 1.50% fresh materials, respectively.

The yield of ethanolic extract from ginger was the lowest, those from temulawak and temukunci were the highest. Similar result was obtained that the yield of acetone extract from ginger was the lowest, those from temulawak and temukunci were the highest (Table 2). The yield of extracts of the same solvent at similar condition of extraction is dependent on the chemical composition of the rhizomes. Ethanol and acetone are organic solvents that are commonly used for extraction of bioactive substances from biological materials. They have been utilized for extraction of curcuminoids from ginger and other rhizomes (Kikuzaki and Nakatani, 1994). Due to relatively high polarity, the solvents do not only extract the curcuminoids and phenolic compounds but also possibly extract sugars and other polar compounds.

Table 2. Yield of ethanolic and acetone extracts of rhizomes

Sample	Ethanolic extract (% fresh material)	Acetone extract (% fresh material)
Ginger (*Zingiber offinalle*)	1.04	0.86
Turmeric (*Curcuma domestica*)	2.12	1.69
Kencur (*Kaempheria galanga*)	1.41	1.37
Laos (*Alpinia galangan*)	1.50	0.89
Temulawak (*C. xantho rrhiza*)	2.38	2.19
Temugiring (*C. viridoflora*)	1.12	1.05
Temuireng (*C. aeruginosa*)	1.48	1.51
Temukunci (*Boesembergia pandurata*)	2.92	2.97

Radical scavenging activity

The extracts of rhizomes were dissolved in methanol at the same concentrations and each was evaluated for radical scavenging activity using 1,1-diphenyl-2-picryl hydrazyl (DPPH) method according to Yen and Chen (1995). Figure 1 and Figure 2 show radical scavenging activity of the ethanolic and acetone extracts of rhizomes, respectively. The degree of radical scavenging activity is indicated by the decrease in absorbance of 517nm of the DPPH solution that monitored at two-minute intervals. The higher decrease of absorbance, the higher activity of the extract.

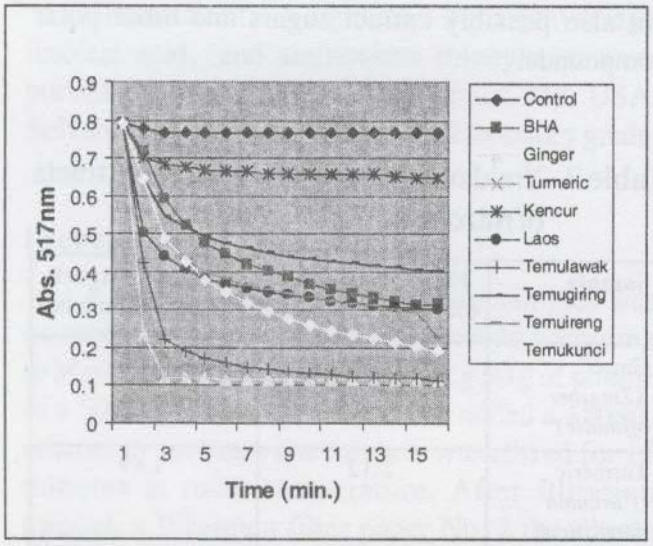

Figure 1. Radical scavenging activity of ethanolic extracts of rhizomes as assayed by DPPH method. Ginger (*Zingiber offinalle*), turmeric (*Curcuma domestica*), kencur (*Kaempheria galanga*), laos (*Alpinia galangan*), temulawak (*C. xanthorrhiza*), temugiring (*C. viridoflora*), temuireng (*C. aeruginosa*), and temukunci (*Boesembergia pandurata*).

Among the ethanolic extracts, radical scavenging activity of ginger was the highest and kencur was the lowest. Conpared to BHA (300µg/mL) radical scavenging activity of the ethanolic extracts (1%) of rhizomes were higher except that of temuireng and kencur (Figure 1). Radical scavenging activity of the ethanolic extracts decreased in the order of ginger > turmeric = temulawak > temukunci > temuireng = laos > BHA > temugiring > kencur. Those of acetone extracts decreased in the order of ginger > turmeric = temulawak > temukunci > BHA = temuireng > temugiring = laos > kencur (Figure 2).

1,1-Diphhenyl-2-picrylhydrazyl is a stable free radical usually used for measuring antioxidant activity of substances, this is considered since a most interesting role of antioxidants, both biologically and technologically it may be supposed, is their interaction with oxidative free radicals. Radical scavenging activity of the extracts may be associated with curcuminoids naturally occurring in the rhizomes (Majeed *et al.*, 1995). The properties of curcuminoids in preventing build up of tissue-injuring free radicals, particularly those responsible for cardiovascular disease lipid

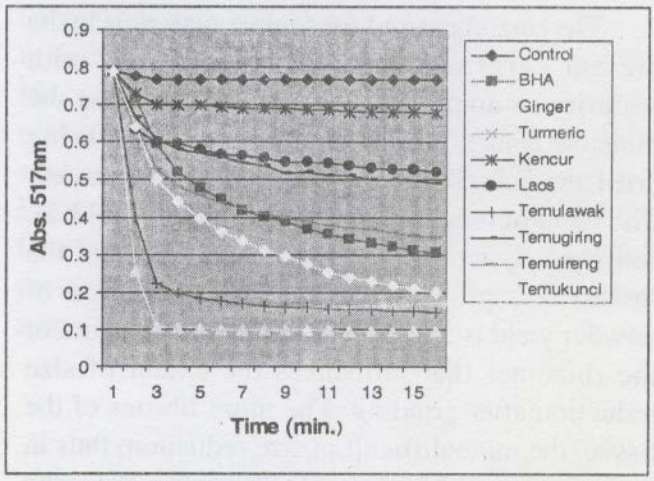

Figure 2. Radical scavenging activity of acetone extracts of rhizomes as assayed by DPPH method. Ginger (*Zingiber offinalle*), turmeric (*Curcuma domestica*), kencur (*Kaempheria galanga*), laos (*Alpinia galangan*), temulawak (*C. xanthorrhiza*), temugiring (*C. viridoflora*), temuireng (*C. aeruginosa*), and temukunci (*Boesembergia pandurata*).

peroxides, are among better known antioxidant properties of these compounds. Ethanolic extracts and acetone extracts have a similar in activity, this possibly due to the similarity of the rhizome constituent extracted. Generally, the yield of ethanolic extracts were slightly higher than that of acetone extracts (Table 2). As shown in Figure 1 and Figure 2, temulawak – the rhizome commonly used as a traditional healthy drink, also had a considerable high radical scavenging activity compared with that of ginger. This result is in agreement with the investigation of Masuda and Jitou (1994) that antioxidant activity of temulawak extract is remarkable high.

Antioxidant activity in linoleic acid

The rhizome extracts were evaluated for antioxidative properties in linoleic acid buffer system incubated at 40°C. The results are shown in Figure 3 and Figure 4 for ethanolic and acetone extracts, respectively. As monitored by peroxide value, the most potent antioxidant activity were extracts of ginger and temulawak, and the lowest was that of kencur. The on set of peroxidation of control (linoleic acid system without antioxidant) occurred at 20 hours of incubation but those of linoleic acid

system with extract samples occurred at 119 hours. Antioxidant activity of the ethanolic extracts in linoleic acid system decreased in the order of : ginger = laos > temulawak > BHA > turmeric > temugiring > temuireng > temukunci > kencur (Figure 3). Those of the acetone extracts deceased in the order of : temulawak = ginger = laos = temugiring > BHA = turmeric > temuireng > temukunci > kencur (Figure 4). Again, temulawak and ginger were the most potent antioxidant as also evidenced in the assay of radical scavenging activity. The antioxidant activity of the extract of temulawak may be contributed by a derivative of curcumin. Masuda and Jitou (1994) reported that 5'-methoxylated curcumin which isolated from temulawak (*Curcuma xanthorhiza*) had a slightly stronger antioxidant activity than curcumin. Antioxidant effect is the result of the capacity of the antioxidant to (*i*) inhibit the initiation of free radical process, or (*ii*) to interrupt the chain reactions in the propagation step of oxidation (Madsen *et al.*, 1996).

CONCLUSION

In conclusion, ethanolic as well as acetone extracts of all rhizomes tested exhibited radical scavenging activity in the DPPH assay with ginger and temulawak showed the highest activity. The extracts also showed antioxidant activity in the assay using linoleic acid buffer system incubated at 40°C; again it was evidenced that extracts of ginger and temulawak were the most potent. The results of this study can also provide us with some basic understanding of the functional value of the tropical rhizomes. Their content of natural antioxidants represents a major beneficial property which may improve their market value in the future.

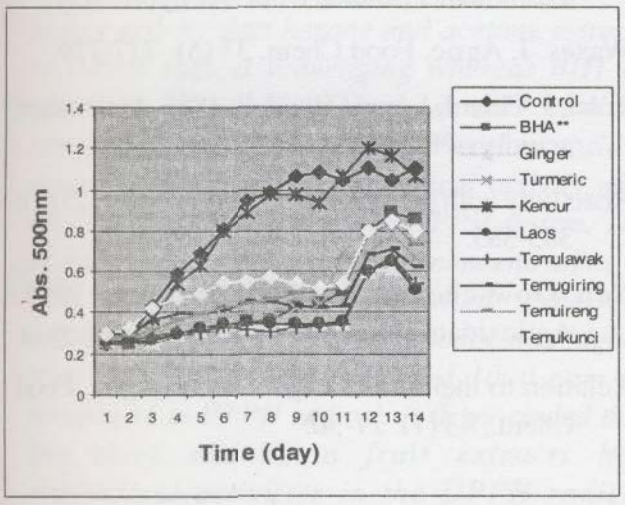

Figure 3. Peroxide value of linoleic acid with ethanolic extract of rhizomes incubated at 40°C (determined by FTC method). Ginger (*Zingiber offinalle*), turmeric (*Curcuma domestica*), kencur (*Kaempheria galanga*), laos (*Alpinia galangan*), temulawak (*C. xanthorrhiza*), temugiring (*C. viridoflora*), temuireng (*C. aeruginosa*), and temukunci (*Boesembergia pandurata*).

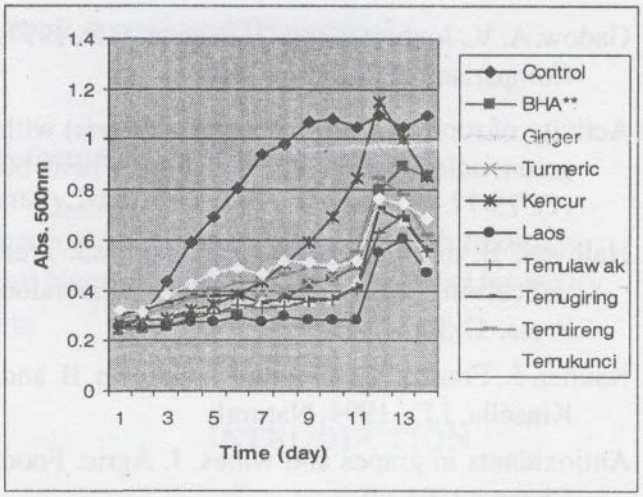

Figure 4. Peroxide value of linoleic acid with acetone extract of rhizomes incubated at 40 °C (determined by FTC method). Ginger (*Zingiber offinalle*), turmeric (*Curcuma domestica*), kencur (*Kaempheria galanga*), laos (*Alpinia galangan*), temulawak (*C. xanthorrhiza*), temugiring (*C. viridoflora*), temuireng (*C. aeruginosa*), and temukunci (*Boesembergia pandurata*).

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