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Potency of Curry (*Murayya koeniigi*) and Salam (*Eugenia polyantha*) Leaves as Natural Antioxidant Sources

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Abstract: The potency of curry leaves (*Murayya koeniigi*) and salam leaves (*Eugenia polyantha*) as natural antioxidant sources was investigated. The active antioxidant compounds from curry leaves and salam leaves were extracted using three types of solvent with different polarities; water, ethanol (50%) and hexane. The total polyphenol contents of the leaves were determined using the Folin-Ciocalteu phenol reagent. Their antioxidant activities were studied using DPPH (1,1-diphenyl-2-picrylhydrazyl) free radicals scavenging and ferric reducing power measurement. The results provide evidence for potent antioxidative effect of curry leaves and salam leaves. Water extraction of curry leaves contain the highest amount of polyphenols than extraction using ethanol and hexane, while ethanol was the most efficient solvent for the salam leaves. Total polyphenol extracts had a positive correlation with antioxidant activity in both DPPH radical scavenging and ferric reducing power. In general, extracts that contain a high amount of polyphenols also exhibited high antioxidant activities. The result indicated that the polarity level of the solvent will determine extraction result and its antioxidant activity.

Key words: Curry leaves (*Murayya koeniigi*), salam leaves (*Eugenia polyantha*), antioxidant activity, total polyphenol content

INTRODUCTION

Free radicals, in the form of reactive oxygen and nitrogen species, induce oxidative damage to lipids, proteins and nucleic acids. This may lead to the development of human chronic diseases such as cancer, aging and atherosclerosis. In addition, those molecules cause lipid oxidation leading the quality deterioration in color, flavor, texture and nutritive values during food processing and storage. One effective way to prevent such oxidative damage is the use of antioxidants. Antioxidant can protect the human body from free radicals and inhibit the progress of many chronic diseases as well as inhibit the lipid oxidative damage in foods, so it can prolong the shelf life of foods, especially those rich in poly-unsaturated fats. However, it can not fix the food products that have been oxidized (Pokorny, 1991; Pokorny *et al*., 2001; Lai *et al*., 2001; Arnao *et al*., 2001; Wong *et al*., 2006; Lana and Tijskens, 2006; Lee *et al*., 2007).

The addition of synthetic antioxidants, such as propyl gallate, butylated hydroxyanisole (BHA) and butylated hydroxy toluene (BHT) has been used in the food industry to control lipid oxidation in foods. However, the use of these synthetic antioxidants has potential health risks and toxicity (Wong *et al*., 2006; Gortzi *et al*., 2006; Nor *et al*., 2008). Therefore, consumers tend to search for natural antioxidants which is considered safer because the extracts obtained from natural ingredients. This encourages researchers and food industry to search for antioxidants from natural sources to replace synthetic ones.

Vitamins A, C and E, carotenoids and phenolic compounds are antioxidants derived from the diet. Phenolic compounds, commonly occur as glycosides in plants, have proved to be more potent antioxidants than vitamin A, C and E, carotenoids (Pietta, 2000; Hung and Yen, 2002; Villano *et al*., 2005). As antioxidants, flavonoids have been reported to be able to retard lipid peroxidation, to scavenge free radicals and active oxygen, to inactivate lipoxygenase and to chelate iron ions (Yen *et al*., 1997; Proestos *et al*., 2006; Elzaawely *et al*., 2007). Their antioxidant activity depends on their chemical structure, their ability to donate hydrogen or electron and their ability to delocalised the unpaired electron within the aromatic structure (Villano *et al*., 2005).

Curry leaves (*Murayya koeniigi*) and salam leaves (*Eugenia polyantha*), often used to give a refreshing, fragrant flavour to South-East-Asian foods, are known to contain active phenolic compounds and these compounds are potential antioxidants. Wong *et al*. (2006) reported that curry leaves obtained from the market in Singapore contain high total phenol, but showed low antioxidant activity while salam leaves contain high total phenol and showed high antioxidant activity as well.

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Although the antioxidative activities of curry leaves and salam leaves were recognized by some studies, further research on the efficient type of solvent used for the extraction is required. It has been reported that the antioxidant activity of certain compounds depends on the solvent used (Van den Berg *et al*., 1999; Villano *et al*., 2005). The level of solvent polarity will determine extraction result and antioxidant activity contained in the extract. Therefore, the purpose of this study is to extract the active antioxidant compounds from the curry leaves and salam leaves grown in Indonesia, using three different types of solvent; water, ethanol and hexane and determine the total polyphenol contents, free radical scavenging activity using DPPH (1,1-diphenyl-2-picrylhydrazyl) and reducing power of extracts of those materials. Results from this study will provide information on the most efficient solvent used for extraction of antioxidant compounds from the curry leaves and salam leaves with their high activities, so that the active antioxidant compounds from those plant extracts can be used further as a functional food or natural antioxidants used in the food products processing.

MATERIALS AND METHODS

Materials: Plant materials, curry leaves (*Murayya koeniigi*) and salam leaves (*Eugenia polyantha*), were obtained fresh from Penyeurat village, Banda Aceh, Indonesia. Chemicals used were ethanol, hexane, Folin-Ciocalteu phenol reagent, gallic acid, Sodium carbonate (Na_2CO_3), potassium ferricyanide ($K_3Fe(CN)_6$), trichloroacetic acid, ferric chloride ($FeCl_3$), phosphate buffer and 1,1-diphenyl-2-picryhydrazyl free radical (DPPH).

Extraction: The samples were ground using a domestic dry blender. Extraction of samples were performed according to the method of Wong *et al.* (2006) and Leong and Shui (2001). Each sample (2.5 g) was extracted using 25 mL of water (1:10 w/v). The mixture was allowed to stand at room temperature for 1 h in the dark, then the mixture was centrifuged at 2000 rpm for 5 min. The obtained extract was filled in sealed small bottles and stored in a refrigerator at 4°C until ready for analysis (Wong *et al*., 2006).

For extraction using ethanol and hexane, each sample (2.5 g) was extracted with 25 mL of solvent (50% ethanol and hexane) (1:10 w/v). Then the mixture was stirred for 60 s using a vortex and centrifuged at 2000 rpm for 5 min (Leong and Shui, 2001). The obtained extract was filled in sealed small bottles and stored in a refrigerator at 4°C until ready for analysis.

Total polyphenol contents determination: The total polyphenol content of the plant extracts were determined using the Folin-Ciocalteu assay according to the method described by Hung and Yen (2002). A 0.1 mL extract was mixed with 0.1 mL of aquadest and 0.1 mL of Folin-Ciocalteu reagent 50%. The mixture was stirred for 3 min using a vortex and added 2 mL of Na_2CO_3 2%. Then the solution was shaken by using a vortex and allowed to stand for 30 min in the dark. The absorbance of the reaction mixture was read at 750 nm. The total polyphenol content of the extracts were expressed as mg gallic acid equivalents per g of plant material.

Antioxidant activity determination using DPPH free radical scavenging method: The DPPH free radical scavenging activity of each sample was measured using Spectrophotometer (UV-Vis 1700 Pharma Spec, Shimadzu) according to the method of Burda and Oleszek (2001), which modified. Briefly, a 0.1 mM solution of DPPH in ethanol was prepared. Each extract (1 mL) was added to 2 mL of ethanolic DPPH solution until the color of sample became purple. Then, the mixture was shaken using a vortex and left to stand at room temperature for 30 min in a dark place. Furthermore, it was stirred again using a vortex. The absorbance of the solution was measured at 517 nm. The degree of decoloration of the solution indicates the scavenging efficiency of the added substance. The free radical scavenging activity was calculated as a percentage of DPPH decoloration using the following equation:

$$\text{Free radical scavenging activity} = \frac{100 \text{ x (1-absorbance of sample)}}{\text{Absorbance of reference}}$$

Reducing power assay: The reducing power of sample extracts were assayed according to the method of Yen and Chen (1995) modified from the method of Oyaizu (1986). Sample extracts were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%). The mixture was shaken using a vortex and incubated at 50°C for 20 min. Then it was cooled. Trichloroacetic acid (2.5 mL, 10%) was added to the mixture, which was then stirred using a vortex and centrifuged at 3000 rpm for 10 min. The solution (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride ($FeCl_3$) (0.5 mL, 0.1%). Then the mixture was shaken again using a vortex and the absorbance of the solution was measured at 700 nm.

Statistical analysis: The research was conducted using randomized complete block design with two treatments (the source of natural antioxidant and the type of solvent for the sample extraction) and three replications. The obtained data were then subjected to analysis of variance using the general linear model (GLM) procedure of SAS and means were compared at $p = 0.05$ and $p = 0.01$ using the Least Significant Difference Test (LSD).

RESULTS

Total polyphenol contents: The Folin-Ciocalteu phenol reagent was used to obtain a crude estimate of the amount of phenolic compounds present in the plant extracts and the results were expressed as gallic acid equivalents. The total polyphenol content of the curry leaves and salam leaves extracts was significantly ($p<0.01$) affected by the treatments. The result of the total polyphenol analysis (Fig. 1) showed that the phenolic content of aqueous extract of curry leaves (54.99 mg gallic acid equivalent per g plant material) was higher than that of salam leaves and curry leaves extracted using other solvent, while the phenolic content of the ethanol extract of the salam leaves (55.31 mg gallic acid equivalent per g plant material) was higher than that of curry leaves and other solvent extract of salam leaves. Both curry leaves and salam leaves extracted using hexane contain the lowest amount of phenolic compounds (7.35 and 4.89 mg gallic acid equivalent per g plant material, respectively).

Antioxidant activity

DPPH free radical scavenging activity: Treatments had significant ($p<0.01$) effect on the DPPH free radical scavenging activity of curry leaves and salam leaves (Fig. 2). Figure 2 indicated that the type of solvent gave different antioxidant activity of extracts. Aqueous extracts of curry leaves and salam leaves showed higher antioxidant activities (84.02 and 84.83%, respectively) and significantly different than the extracts using ethanol and hexane solvent. Similar to the results obtained for the total polyphenol content assay, hexane extracts of curry leaves and salam leaves exhibited the lowest antioxidant activities.

Reducing power: The reducing power of the curry leaves and salam leaves extracts was significantly ($p<0.01$) influenced by the treatments. The reducing power of curry leaves and salam leaves are shown in Fig. 3. As shown in Fig. 3, aqueous extracts of curry leaves had higher reducing power than those of ethanol and hexane extracts, whereas for the salam leaves, ethanol extracts exhibited a higher reducing power than those of other extracts.

DISCUSSION

Phenolic compounds in the sample extracts were determined using Folin-Ciocalteu reagent. This method is reported reproducible, quick, inexpensive and particularly helpful if it works with numerous or small scale samples (Cicco *et al*., 2009).

In this study, extraction was performed using three kinds of solvent with different polarities, to obtain every active component in the curry leaves and salam leaves, which are polar, semipolar and non-polar, as potential antioxidant compounds. The level of polarity will determine extraction result and antioxidant activity contained in the extract.

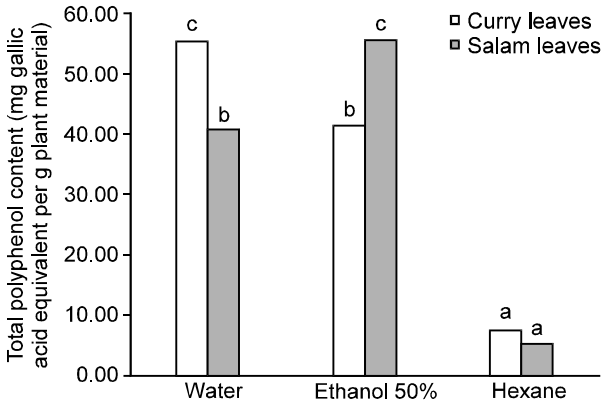

Fig. 1: Interaction effect of natural antioxidant extracts and solvent types on total polyphenol content (values followed by the same letter indicate no significant differences)

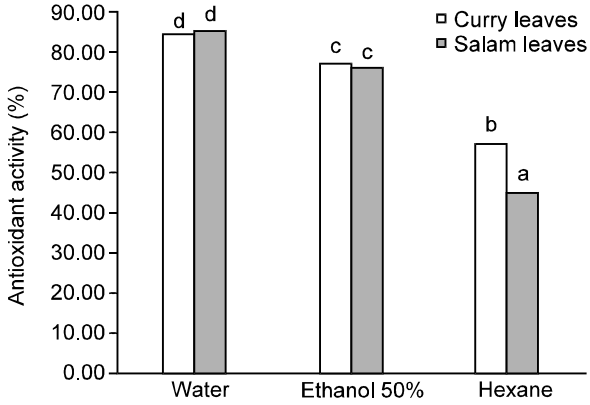

Fig. 2: Interaction effect of natural antioxidant extracts and solvent types on antioxidant activity (values followed by the same letter indicate no significant differences)

The highest amount of total polyphenol content of curry leaves was obtained from extraction using water, while the highest phenolic content of the salam leaves was obtained from ethanol extraction. Similarly, Wong *et al*. (2006) reported that the total polyphenol of water extract of curry leaves was also higher than those of salam leaves. This indicated that the phenolic compounds of curry leaves extract were more polar than salam leaves extract.

In general, extracts that contain a high amount of polyphenols also show high antioxidant activity. Ethanol extract of salam leaves which exhibited high antioxidant activities also had high total polyphenol content. Aqueous extract of curry leaves were exception because they had high total polyphenol content but did not show high DPPH free radical scavenging activity. It could be due to the presence of compounds not reactive to DPPH. Antioxidant compounds such as polyphenols may be

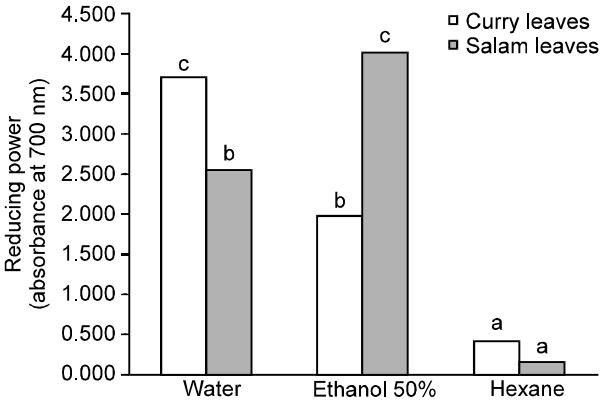

Fig. 3: Interaction effect of natural antioxidant extracts and solvent types on reducing power (values followed by the same letter indicate no significant differences)

more efficient reducing agents for ferric iron but some may not scavenge DPPH free radicals as efficiently because of stearic hindrance (Wong *et al*., 2006). The antioxidant activity of phenolic compounds also depends on their chemical structure, their ability to donate hydrogen or electron and their ability to delocalised the unpaired electron within the aromatic structure (Villano *et al*., 2005).

The determination of DPPH free radical scavenging activity is based on the reduction of DPPH radicals in ethanol which causes an absorbance drop at 515 nm. The color of solution changes from purple to yellow. This change occurs when DPPH was captured by antioxidants which remove H atoms to form a stable DPPH-H (Frankel, 1998; Nenadis and Tsimidou, 2002; Wong *et al*., 2006). The results imply the antioxidative activity of curry leaves and salam leaves may be attributed to its proton-donating ability.

In the reducing power determination, the reductant (antioxidant) in the sample will reduce Fe^{3+} ions (potassium ferricyanide complex ($K_3Fe(CN)_6$) to the ions Fe^{2+} (ferrous form). Therefore, Fe $^{2+}$ can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increased absorbance indicated an increase in reducing power (Yen and Chen, 1995; Lai *et al*., 2001).

These results correlated positively with the total polyphenol content. High content of total polyphenols showed a high reducing power of curry leaves and salam leaves extracts. The results reveal that both curry leaves and salam leaves are electron donors and could react with free radicals, convert them to more stable products and terminate radical chain reaction.

Conclusions: The result showed that both curry leaves and salam leaves are very potent as natural antioxidant sources that interact with a wide range of species directly responsible for oxidative damage. Aqueous extracts of curry leaves contained higher amount of polyphenols and antioxidant activities in both DPPH radical scavenging and ferric reducing power than other solvent extracts, while for the salam leaves, ethanol (50%) extracts gave a higher polyphenol content and reducing power than others. Total polyphenols extracts had a positive correlation with antioxidant activity in both DPPH radical scavenging and ferric reducing power. The antioxidative activity of curry leaves and salam leaves may be attributed to their proton-donating ability. Moreover, they can also act as electron donors that could react with free radicals, convert them to more stable products and terminate radical chain reaction. The components responsible for the antioxidative activity of curry leaves and salam leaves are currently unclear. Therefore, further research is required to isolate and identify the antioxidative components in curry leaves and salam leaves. It is also necessary to test the heat stability and its application in the food system.

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