Antioxidant activities of curry leaves (*Murayya koeniigi*) and salam leaves (*Eugenia polyantha*)

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Abstract. This study aimed to extract the active antioxidant compounds from curry leaves (*Murayya koeniigi*) and salam leaves (*Eugenia polyantha*) using three types of solvent; water, ethanol (50%) and hexane, and determine the total polyphenols contents, activity of free radicals scavenging using DPPH (1,1-diphenyl-2-picrylhydrazyl) and ferric reducing power of the extract of those materials. The result showed that curry leaves extracted using water contain a higher amount of polyphenols than other solvent extracts, while for the salam leaves, ethanol (50%) extracts give a higher polyphenol content than others. Total polyphenols extracts had a positive correlation with antioxidant activity in both DPPH radical scavenging and ferric reducing power. Extracts that contain a high amount of polyphenols also exhibit high antioxidant activity. The result indicated that the polarity level of the solvent will determine extraction result and its antioxidant activity.

Keywords: Curry leaves (Murayya koeniigi), salam leaves (Eugenia polyantha), antioxidant activity, total polyphenol contents.

Introduction

Lipid oxidation is a major cause of 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 inhibit the lipid oxidative damage, 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 (Wong et al., 2006; Lee et al.,2007; Sarastani et al., 2002; Pokorny, 1991).

The addition of synthetic antioxidants, such as propyl gallate, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (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; Sarastani et al., 2002). Therefore, consumers tend to search for natural antioxidants which is considered more safe 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 flavonoids are antioxidants derived from the diet. flavonoids, also called polyphenols, commonly occur as glycosides in plants (Pietta, 2000). As antioxidants, flavonoids have been reported to be able to inhibit lipid peroxidation, to scavenge free radicals and active oxygen, to inactivate lipoxygenase, and to chelate iron ions (Yen et al., 1997).

Several potential local plant products, such as curry leaves and salam leaves, are known to contain active polyphenolic 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. Nowadays, both curry leaves and salam leaves only used as vegetable and flavoring food that still have low economic values.

The purpose of this study is to extract the active antioxidant compounds from the curry leaves (Murayya koeniigi) and salam leaves (Eugenia polyantha) grown in Aceh using the solvent of 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 antioxidant activity of those plant extracts so that the active antioxidant compounds from the curry leaves (*Murayya koeniigi*) and salam leaves (*Eugenia polyantha*) can be used further as a functional food or natural antioxidants used in the food products processing.

Materials and Methods

Materials

Plant materials were 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 hr in the dark, then the mixture was centrifuged at 2000 rpm for 5 minutes. The obtained extract was filled in sealed small bottles and stored in a refrigerator at 4ºC 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 minutes (Leong and Shui, 2001). The obtained extract was filled in sealed small bottles and stored in a refrigerator at 4ºC until ready for analysis.

Total polyphenol contents determination

The total polyphenol contens of the extracts was 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 minutes 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 minutes in the dark. The absorbance of the reaction mixture was read at λ = 750 nm. The total polyphenols contents of the extract was 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 minutes 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:

Free radical scavenging activity=100 x(1 – absorbance of sample/absorbance of reference)

Reducing Power Determination

The reducing power of sample extracts was 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 50ºC 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 statistically analyzed using Analisys Of Variance (ANOVA). If the test result indicates significant differences between the treatments, it will be proceed with the advanced test Smallest Real Difference (LSD) (Sugandi and Sugianto, 1994).

Results and Discussion

Total polyphenol contents

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

The Folin-Ciocalteu phenol reagent is used to obtain a crude estimate of the amount of phenolic compounds present in the plant extracts. The result of the total polyphenol analysis (Figure 1) shows that the phenolic content of aqueous extract of curry leaves is higher than salam leaves and curry leaves extracted using other solvent, while the phenolic content of the ethanol extract of the salam leaves is higher than those of curry leaves and other solvent extract of salam leaves. Wong et al. (2006) reported a similar trend where the total polyphenol of water extract of curry leaves was also higher than those of salam leaves. This indicates that the phenolic compounds of curry leaves extract are more polar than salam leaves extract. According to Larson (1988) in Lubis et al. (2007), phenolic components known as primary antioxidants derived from plants are polar.

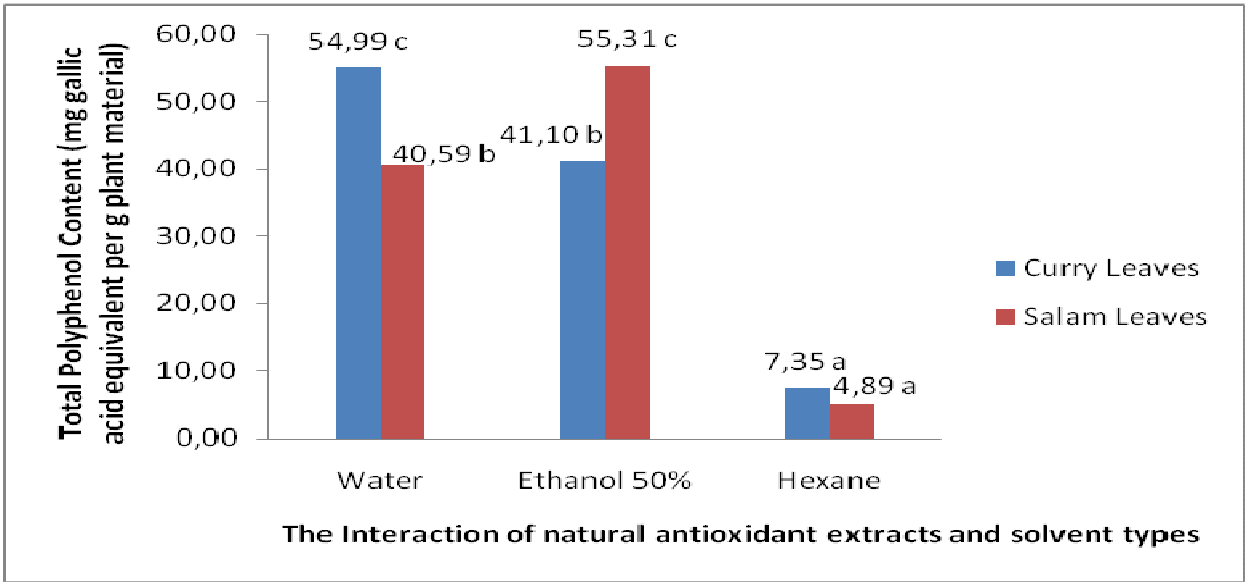

Figure 1. The effect of natural antioxidant extracts and solvent types interaction on total polyphenol content (values followed by the same letter indicate no significant differences)

Antioxidant activity

DPPH free radical scavenging activity

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 (wong et al.,2006). 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; Nenadi dan Tsimidou, 2002)

The DPPH free radical scavenging activity of curry leaves and salam leaves are shown in Figure 2. It indicates that the type of solvent gave a different antioxidant activity of extracts. Aqueous extracts of curry leaves and salam leaves showed higher antioxidant activity and significantly different than the extract using ethanol and hexane solvent. According to Chang et al. (1997), the polarity will determine the extraction result and antioxidant activity contained in the extract. Both the water extract and the ethanol extract were not significantly different for each source of raw materials, except the hexane extracts of curry leaves has antioxidant activity higher than the salam leaves.

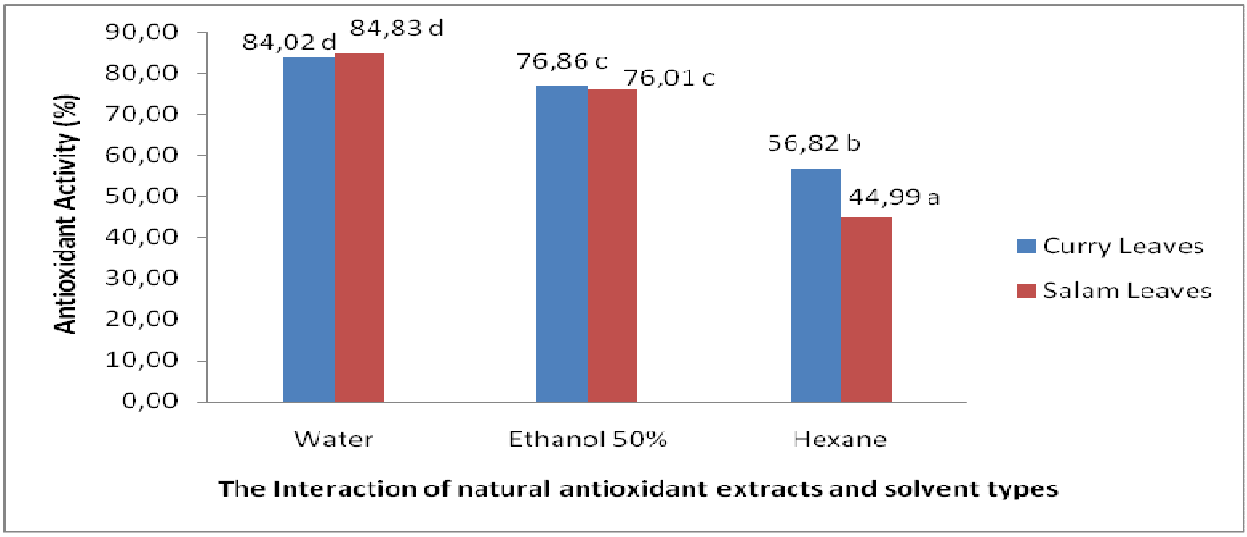

Figure 2. The effect of natural antioxidant extracts and solvent types interaction on antioxidant activity (values followed by the same letter indicate n o significant differences)

Reducing power

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 (Lai et al., 2001; Yen and Chen, 1995).

As shown in Figure 3, Aqueous extracts of Curry leaves has higher reducing power than ethanol and hexane extracts, whereas for the salam leaves, ethanol extracts exhibit a higher reducing power than other extracts. 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.

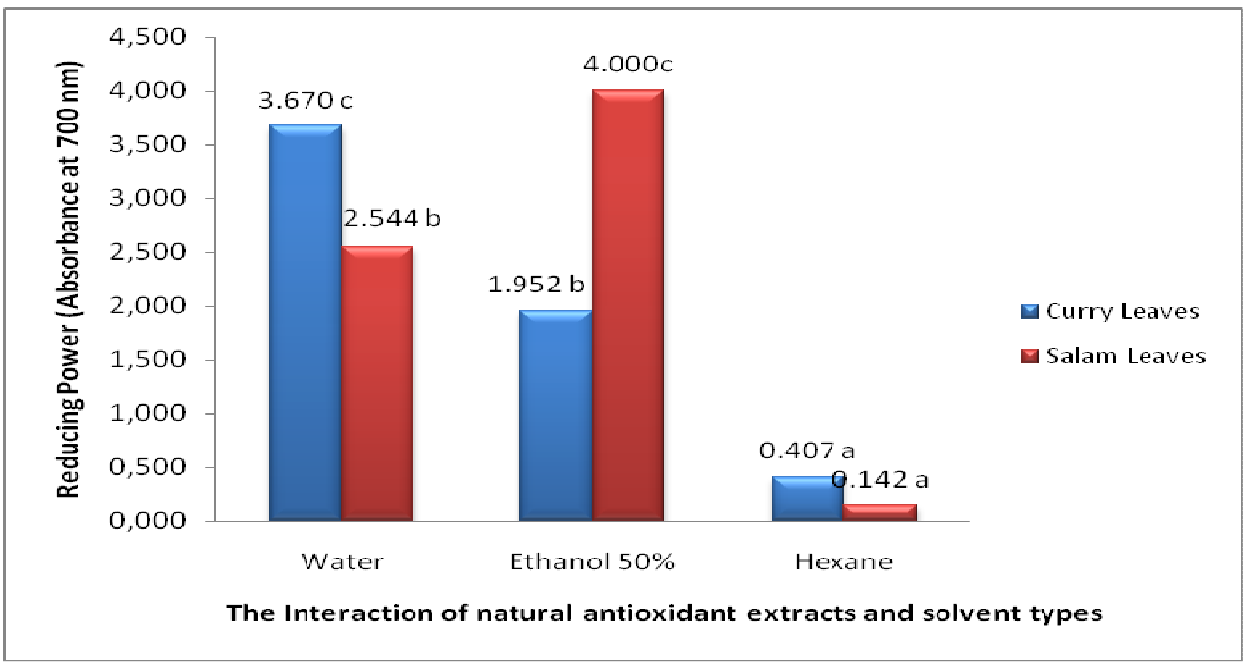

Figure 3. The effect of natural antioxidant extracts and solvent types interaction on reducing power (values followed by the same letter indicate no significant differences)

Conclusions

The result showed that aqueous extracts of curry leaves provide a higher amount of polyphenols and antioxidant activity in both DPPH radical scavenging and ferric reducing power than other solvent extracts, while for the salam leaves, ethanol (50%) extracts give 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. 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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