Antiradical Activity of Andaliman (*Zanthoxylum achanthopodium* DC) Fruit Extract

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ABSTRACT

Andaliman (Zanthoxylum achanthopo-dium DC) was evaluated as a potential source of phenolic antioxidant compounds. Phenolic compounds were obtained from andaliman fruits by sequential extraction using hexane, acetone and ethanol to obtain three separate fractions. In this study, the antioxidant properties of andaliman at 10 different concentrations and common food additives of BHT at 200 ppm and α-tocopherol at 1000 ppm were compared. The antioxidant properties of andaliman fruits extracts were evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical decoloration test. The ethanol extract consistently showed higher activity than hexane and acetone extracts in DPPH radical scavenging whereas BHT as synthetic antioxidant had weaker radical scavenging activity. The α-tocopherol as positive control showed similar antiradical activity with ethanol extract at 500 ppm in DPPH system. The addition of ethanol extract at concentration of 900 and 1000 ppm exhibited excellent antiradical activities and its efficacy was higher than that of 200 ppm BHT and 1000 ppm α-tocopherol in DPPH radical. It is concluded that the three andaliman fruit extracts had antiradical activities in the DPPH radical decoloration test.

Key words: Zanthoxylum achanthopodium DC, DPPH, natural antioxidant, antiradical activity.

INTRODUCTION

Andaliman (*Zanthoxylum acanthopodium* DC.) is one of traditional spices that grow on altitude of 1500 m above sea level. In Indonesia this spice has not been popular and only used by specific group of people in North Sumatra province. The andaliman is not been commonly cultivated by farmer and mostly found in North Sumatera, particularly at Dairi, North Tapanuli and South Tapanuli regencies. Since it offers unique flavor and is believed to impart a health benefit, therefore it is gaining popularity among consumers in the big cities in Indonesia.

The andaliman fruit is used as a spice for some special food, for example in traditional Bataks food mixed with meat and blood then marinated under acidic condition for 24 hours. As a spice, andaliman gives specific flavour, also it is used to preserve cooked meat for few days against rancidity and spoilage. In addition, andaliman fruit could mask odor of raw fish or meat.

The andaliman fruit has a strong specific odor and pungent taste like fresh citrus with bitter taste that stimulates saliva secretion. It is widely used by Bataks (Fakfak, Karoes, Mandailing, Toba and Simalungun) and people of South East and Central Aceh. It has been used to heal several kind of illness such as stomachache and tooth ache. It also has antipyretic properties, enhance appetite and as a free radical scavenging activities (Perry, 1987).

Naturally occurring compounds in andaliman extracts have been reported to exhibit antioxidant properties greater than α-tocoferol and equal to BHT (Wijaya, 1999; Edi Suryanto and Rorong, 2001). Addition of andaliman extract has been shown to provide increased protection from lipid oxidation during cooking. The objective of this research was to determine antiradical activity of andaliman fruits (*Zanthoxylum achanthopodium* DC) extract using DPPH radical decoloration test.

MATERIALS AND METHODS

Material

Andaliman fruits obtained from a local market at North Sumatra were cleaned, freeze dried and ground to 40 mesh. 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co. (St. Lois, MO).

Sample preparation

Andaliman extract was prepared according to method of Su (2000) with minor modification. Five grams of ground andaliman fruits were extracted twice with 100 mL of solvent (hexane, acetone and methanol) and after maceration for 24 hours, the flask was cooled to room temperature. After filtration, residue was extracted once more with an additional 100 mL hexane for 24 hours. The hexane extracts were then pooled and saved. Similarly, the acetone extract was obtained by extracting the residue remaining after twice hexane extraction with 100 mL of acetone each. Finally, the ethanol extract was obtained by extracting the residue remaining with 100 mL of ethanol in a similar way. The solvents in the three extracts were then evaporated using a vacuum evaporator. The resulting three extracts were then weighed and store at –20°C prior to radical scavenging test.

Determination of total phenol content of andaliman extracts

The content of total phenolics in andaliman extracts was measured by Folin-Ciocalteu assay (Conde *et al.*, 1997). The absorbance of extracts was read at 750 nm in a Shimadzu UV 1601 UV-Vis Spectrophotometer. Gallic acid was used as standard and results were expressed as mg per 100 g of extract of gallic acid equivalents.

Determination of free radical scavenging activity (RSA)

The free radical scavenging activity of andaliman extract was measured with DPPH free radical using the method of Tang *et al* (2002) with minor modification. Andaliman extracts (4 mL of 200-1500 ppm ethanol solution) were added to 1 mL, 0.2 mM DPPH in ethanol. After reacting for 30 minutes, the absorbance was read at 517 nm in a Shimadzu UV 1601 UV-Vis Spectrophotometer. Ethanol (4 mL) was mixed with 1 mL DPPH and this served as the control. Radical scavenging activity (%) was calculated as follows:

$$\textit{Radical scavenging activity (\%)} = \left(1 - \frac{\textit{Sampel absorbance at 517 nm}}{\textit{Control absorbance at 517 nm}}\right) x\ 100$$

RESULTS AND DISCUSSION

Phenolic compound was extracted sequentially from andaliman fruit using hexane, acetone and ethanol. Table 1 shows the yield extract and total phenol content in the three solvents. The yield of ethanol extract (EE) gave higher yields from sequential extraction than acetone extract (AE) and hexane extract (HE), whereas higher total phenol content obtained from EE followed AE and HE.

Tabel 1. Extraction yield and total phenol content

Extract	yield of extract[d] (%)	total phenol content (mg/100g)[e]
[a]HE	4.21	3.17±0.09
[b]AE	2.58	9.18±0.02
[c]EE	7.44	13.19±0.11

[a] Hexane extract, [b] Acetone extract, [c] Ethanol extract, [d] Mean value of two measurements.
[e] Mean value of two measurements ± SD (standard deviation).

The extraction was done sequentially with some solvent that possesses different polarity to separate phenolic compound in andaliman fruit. Extraction using hexane could dissolve nonpolar compounds. Whereas acetone could dissolve semi polar compounds and ethanol recovered the more polar compounds. Phenolic compounds such as flavonoid, phenolic acid and other phenolic compound are well known as primary antioxidant from plant was polar (Larson, 1988).

DPPH test is the simplest method to measure the ability of antioxidant to scave free radical. The result of radical scavenging activity (RSA) in DPPH from the three extracts showed all extract posses ability as radical scavenger test (Figure 1, 2 and 3). The result shows that there was positive relationship between the amounts of total phenolic compounds in andaliman fruit with radical scavenging activity. For example, EE showed most phenolic compounds and possessed higher scavenger activity a HE and AE. Many research showed that extract of plant as fruits, leave, and vegetables have positive corellation among total phenol content and antioxidant activity (Velioglu *et al.*, 1998; Duh and Yen, 1997).

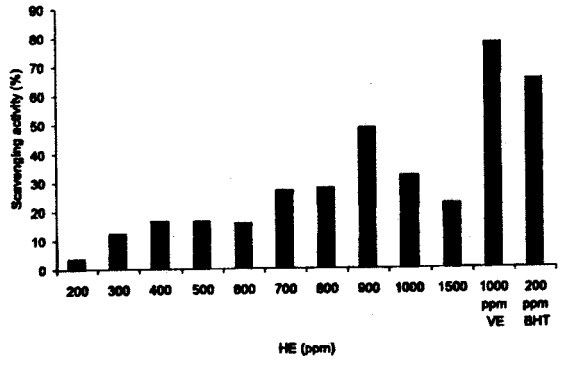

Figure 1. Effects of hexane extract (HE) at different concentrations on scavenging 1,1- diphenyl-2-picrylhydrazyl (DPPH) free radical (2 mM)

Figure 3 shows that all extracts examined were found to possess DPPH-scavenging activity. The effects of andaliman extract on scavenging DPPH free radical at concentration ranging from 200 to 1500 ppm and 1000 ppm α-tocopherol (VE) and 200 ppm BHT were as a positive control in the system. For hexane extract (HE), as the concentrations increased from 200 ppm to 900 ppm, scavenging activity of HE increased, however these different were not significant. When the concentration increased beyond 900 ppm, their scavenging activities decreased at 1000 ppm and 1500 ppm (Figure 1).

Figure 2 show free radical scavenging activity by acetone extract (AE). In this case, concentrations

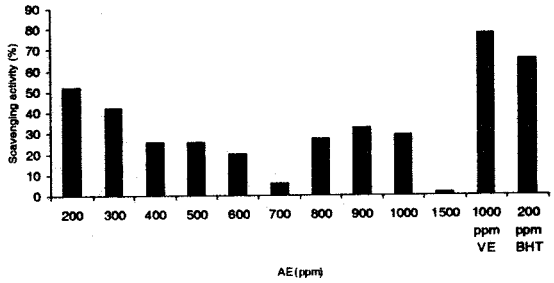

Figure 2. Effects of acetone extract (AE) at different concentrations on scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (2 mM)

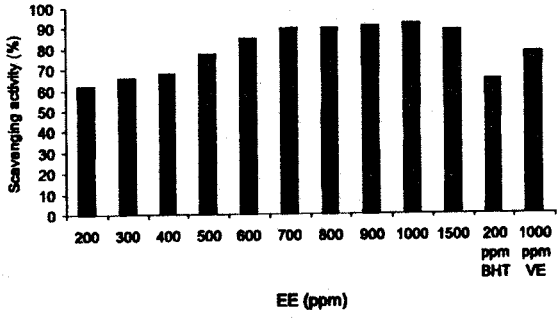

Figure 3. Effects of ethanol extract (EE) at different concentrations on scavenging 1,1- diphenyl-2-picrylhydrazyl (DPPH) free radical (2 mM)

increased from 200-700 ppm resulted in decreased scavenging activities. Conversely, the addition of extracts at concentration of 800 ppm, 900 ppm 1000 ppm showed increasing activity on scavenging DPPH free radical. However, the addition of AE at concentration of 1500 ppm showed lower antiradical activity (1.55 %). Which was not significant different with those of the α-tocoperol and BHT.

Although the differences in the radical scavenging activity (RSA) between HE and AE obtained from the same fruits material were

statistically not significant, some tendency can be observed in favor of AE as compared to HE. It was assumed that most of the essential oils and oleoresin consisting mainly of terpenes and sesquiterpenes were present in the extract. Therefore, it is not likely that the extracted essential oils could decrease antiradical activity of the extract. There, presence of chlorophyll in hexane extract and acetone extract was responsible for the deep-green coloration. Our result showed that hexane extracts and acetone extract were all viscous oils with a darkness green color.

In Figure 3, ethanol extract at concentration 200 ppm to 1000 ppm exhibited excellent antiradical activities from range 61.81 % to 91.99 %. However, at level of addition of 1500 ppm its scavenging activity decreased 88.71 %. At all level of addition of the ethanol extract had scavenging activity higher than the hexane extract and the acetone extract. The addition of ethanol extract at 1000 ppm showed the highest scavenging activity, but it was not statistically difference with level of addition at 600-900 ppm and 1500 ppm. The addition of ethanol extract at 1000 ppm showed higher radical scavenging activity compared to the addition of 200 ppm BHT or 1000 ppm α-tocopherol.

CONCLUSION

The sequential extraction of andaliman fruit using hexane, acetone and ethanol resulted in three extracts with different radical scavenging activity. The ethanolic extract contained highest phenolic compounds and showed the highest radical scavenging activity.

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