Isolation and Identification of Antiradical and Anti-photooxidant Component of *Aloe vera chinensis*

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ABSTRACT

A potent antiradical and antiphotooxidant compound from Aloe vera chinensis was investigated. The results indicated that the methanolic extracts produced stronger antiradical and antiphotooxidant activity also gave higher yields of extract than other organic solvents. Silica gel column separated methanolic extracts into five fractions. Of the five fractions, fraction II possessed significant antiradical activity (70.2 % inhibition on free radical DPPH) and its showed significant antiphotooxidant activity (60.1 % inhibition on hydroperoxide formation). Subsequently, fraction II was separated into eight subfractions by silica gel column. The sub fraction II-b exhibited stronger antiradical and antiphotooxidant activity than others and showed a single spot in thin layer chromatography Silica gel 60 F_{254} plates. On the basis of mass spectrophotometry in combination with reversed phased, high performance chromatography, this compound has been identified as 1,8 dihydroxy-3-hydroxymethylanthraquinone (Aloe emodin).

INTRODUCTION

Natural antioxidants are highly desirable especially from edible plants because the knowledge of antioxidants play an important role not only in food processing, increasing the self life on food product and improving the stability of lipid and lipid-containing foods, but also its prevents radical-induced disease such as cancer and atherosclerosis (Martin et. al., 2000). A mucilagenous juice present in the center of the Aloe vera (Grindley et. al., 1998 and Joshi, 1993) commonly called gel has long history as a home remedy for treatment of variety conditions in traditional medicine (Huttler et. al., 1997). One action of topical application of Aloe is the reversal of skin aging associated with photo-damage. Evidence of the involvement of Reactive Oxygen Species (ROS) in photo damage and age-related skin degeneration suggest that free radical-scavenging phenolics of Aloe gel could account for skin protective effects (Esteban et. al., 2000).

Aloe vera leaves, which are used traditionally for their therapeutic properties, have been studied for their potential antioxidant activity (Hu *et. al.*, 2003 and Hu *et. al.*, 2005). Previous research in our laboratory has focused on the antioxidant defense systems (Dewi *et. al.*, 2005). As part of this effort, we evaluated the antioxidant properties of components of *Aloe vera chinensis*. Many natural antioxidants have been isolated from plants. Phenolic antioxidants have also been found that function as free radical terminators or metal chelators (Shahidi *et. al.*, 2000).

Phenolic constituents of Aloe leaves can be generally classified into two main groups, namely chromones such as Aloesin and anthraquinones

such as Aloin A and B (Okamura *et. al.*, 1997). A potent antioxidant compound was isolated from methanolic extract of *Aloe vera barbadensis* Miller (Lee *et. al.*, 2000). Several studies have been conducted to identify natural phenolic that possesses antioxidant activity, some of these substances are now being extracted from plant sources for commercial production (Schuler, 1990). Despite various highly polar and thermally unstable compounds have been isolated from the organic extract of *Aloe vera* leaves and their obvious antioxidant capacity has been confirm by some in vitro experiments (Lee *et. al.*, 2000). However, only a few articles in the literature have discussed Aloe antioxidants compounds in *Aloe vera chinensis* and their physiological effect in the linoleic acid systems. In this report, we describe the isolation and identification of an antiradical and an antiphotooxidant phenolic compounds in *Aloe vera chinensis*.

MATERIALS AND METHODS

Instrumentation

Analytical separation were performed using a Shimadzu Kyoto, Japan) model SPM 10 PV high performance liquid chromatography (HPLC) system. Mass spectro photometer were acquired using a model 5000 QP. Sample introduction was by means of a direct insertion probe which was heated ballistically until evaporation was complete. Ultraviolet (UV) absorbance measurements were obtained on Shimadzu model UV-Vis 1601 (Kyoto, Japan) spectrophotometer.

Plant material

Aloe vera chinensis leaves were obtained by *Aloe vera* center (Pontianak). Briefly, immediately after harvest, the epidermis of Aloe (green skin) was removed then mucillagenous gel from the parenchymatous cells in the leaf pulp was excised from the leaves and then homogenized. The pulp was removed by passage through a 250 mesh screen then centrifugation at 6000 g. Supernatant was collected and its freeze drying to obtain freeze dried crude *Aloe*.

Extraction of Antiradical and antiphotooxidant compound

Antioxidant compounds were separated from crude Aloe freeze dried using a modification of the method described by Guyod *et al* (1998). The sample (10 g) was extracted with methanol, ethanol or acetone 1000 ml in under magnetic stirring at cool room temperature (4 °C). All extractions were carried out in acidic conditions (addition of acetic acid at 5 ml/L) to avoid oxidation of phenolic compounds. The extracts were filtered through *Whatmann* paper No. 1 and the residue was extracted under same condition. The combined filtrates were concentrated by evaporation under reduced pressure at 40 °C until viscous then immersed in liquid nitrogen and weighed to measured the yield of soluble constituents. The yields, averages of triplicates analyses, are listed in Table 1.

Isolation of Antiradical and antiphotooxidant

The dried extracts (1.5 g) dissolved in 1 ml methanol and fractionated onto a 1.5 x 50 cm chromatographed silica gel neutral column (30 g weight; 70-230 mesh particle size, E. Merck). Ethyl ecetate/methanol (7:3, v/v; 150 ml), ethyl ecetate/ methanol 5:5, v/v; 75 ml) and methanol (50 ml) were used as eluent solvent, respectively. The fractionated material was collected in 5 ml fractions, the absorption of which was monitored at 280 nm. The appropriate fractions were combined, concentrated as above, immersed in liquid nitrogen and weight then stored in 0 °C before analyses. Silica gel chromatography of the material gave 5 fractions. Each fraction were redissolved in methanol to for measuring radical scavenging activity and antiphotooxidant activity.

Purification of Active Antiradical and Antiphotooxidant Component.

Of the 5 fractions, Fractions II showed the highest radical scavenging and antiphotooxidant activity. Fractions II (0.7 g) was dissolved in methanol 0.5 ml and was loaded on the top column. The solution of Ethyl acetate:Methanol:Water (100:10:3, v/v; 100 ml) was used as th eluent solvent.

Silica gel chromatographed column of fraction II yielded eight sub fractions II-a, II-b, II-c, II-d, II-e, II-f, II-g and II-h, respectively. The antiradical and antiphooxidant activity of each sub fraction was determined. Sub fractions II-b, II-c and II-d showed marked antiradical and antiphotooxidant activity and was further investigated. Sub fraction II-b, II-c and II-d were purification by Thin-layer chromatography (TLC) methodology on silica gel plates (4 cm x 6.5 cm, F_{254}, 025 mm, E. Merck)

High-performeance liquid chromato-graphy (HPLC)

HPLC analysis were condition as follows: reverse phase column CLC ODS (M) 4.6 x 250 mm, 80 Å, 5 μM particle size); mobile phase, 79 % methanol: 20 % water: 1 % acetic acid; flow rate, 0.7 ml/min; and diode array detector. The volume injected was 10 µL and Aloe emodin was used as a standard external.

Determination of effect on DPPH radical.

The effects of all extracts (methanol, ethanol or acetone) DPPH radical were estimated using a modification of the method described by Hatano et al. (1988). Extracts were decolorized with cartridges) Sep-Pak C_{18} Waters, Milford, M A) in methanol (3 ml), and added to a methanolic solution (1 ml) of DPPH radical (the final concentration of DPPH 0.2 mM). The mixture was shaken vigorously and left standing at room temperature for 30 min; absorbance of the resulting solution was then masured spectrometrically at 517 nm. The percentage inhibition of free radical formation, 100 – {(absorbance of sample/absorbance of control) x 100}, was calculated to express scavenging activity. All tests and analyses were run in triplicate and averaged.

Antioxidative activity of Aloe vera extracts in the methylene-blue sensitized photooxidation of linoleic acid in methanol.

Methanol, ethanol and acetone were used as extracting solvents to prepare the Aloe vera chinensis extracts as described previously. A mixture of 1.0 ml extract solution (500 ppm), 2.5 ml of 0.3 M linoleic acid in methanol, and 1.5 ml methylene blue in methanol (5 ppm) was, in triplicate, prepared in 30 m capacity serum bottle. The serum bottles were air-tightly sealed with teflon-lined rubber septa and aluminium caps, then randomly placed in light storage box under fluorescent illumination condition as described in detail previously by Jung et al (1995) for 5 h. The light intensity at the sample level was 4000 lux. The oxidation of linoleic acid was determined by measuring the peroxide values according to AOCS Official Method (1990). The percentage inhibition of linoleic acid peroxidation, 100 – {(hydroperoxide value of sample/ hydroperoxide value of control) x 100}, was calculated to express antioxidant activity.

Statistical Analysis

Analyses of variance were performed by ANOVA procedure. Significant difference between the means were determined by LSD test. Statistical analysis were performed using Microsoft excel Software package system.

RESULTS AND DISCUSSION

Of three solvents extracts, methanol extracts exhibited the greatest yielded so do the antiradical and the antiphotooxidant activity (Table 1.). Despite, antiradical and antiphotooxidant have different reaction mechanisms, the methanol extracts have great potential in both activity. This observation is in agreement with some reports (Duh, 1998 and Yen et. al., 1998 and Lee et. al., 2000) that methanol is a widely used and effective solvent for extraction of antiradicals and antiphotooxidants (Jung et. al., 1999). Therefore, we focused on methanolic extracts of Aloe vera chinensis in the following study. To isolate the component responsible for antiradical and antiphotooxidant activity, the methanolic extract of freeze dried crude Aloe vera chinensis was further fractionated using column and thin layer chromatography.

Table 1. Yields, antiradical and antiphotooxidant activity of extracts from Aloe vera chinensis with various solvents

Solvent	Yield*	Antiradical (%)**	Antiphotooxidant (%)***
Metanol	6.20 ± 0.25^{c}****	52.80 ± 1.74^{c}	60.12^{c}
Etanol	5.50 ± 0.10^{b}	49.60 ± 1.15^{b}	57.00^{b}
Aceton	3.80 ± 0.26^{a}	30.10 ± 2.07^{a}	45.90^{a}

* Based on 100 ml of each solvent.

** The antiradical activity; determined by a 0.2 mM DPPH free radical system.

*** The antiphotooxidant activity was measured by the hydroperoxide value.

**** Values are mean ± standard deviation of three replicate analyses. Mean within a column with different superscript letters (a-c) are significant different on LSD test (P ≤0,05)

Of the three solvent extract, methanolic extracts exhibited the highest yield and the strongest antiradical and antiphotooxidant activity. Methanol was, therefore chosen for extraction for antiradical and antiphotooxidant isolation and identification.

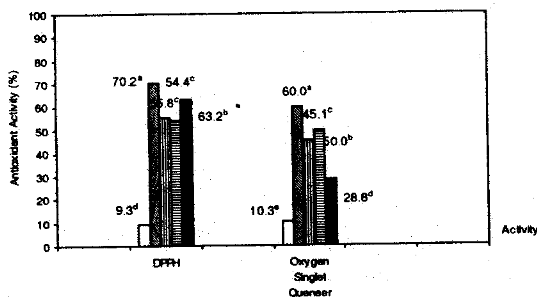

Figure 1. Antiradical (% DPPH) and Antiphotooxidant Activity (% Oxygen Singlet Quenser) of Methanolic Extracts of Aloe vera

Methanolic extract was separated into five major fractions on silica gel column chromatography. Ethyl acetat/methanol, (7:3 v/v, 150 ml), ethyl acetate/methanol (5:5, v/v; 75 ml) and methanol (50 ml), was used as eluent solvent, respectively. The antiradical and antiphotooxidant activity of each fraction are shown in Fig 1. The antiradical and antiphotooxidant activity of fraction II was the strongest of all fractions.

Furthermore, fraction II was cleaned up into eight subfraction on a silica gel column chromatography eluted with ethyl acetat/metanol/ water (100:10:3, v/v, 100 ml). All eight sub fractions showed antiradical and antiphotooxidant activity, although the activity of all eight sub-fractions was less than that of fraction II. In other words, the antiradical and antiphotooxidant activity of fraction II was greater than that the sum of its sub-fractions. This may be due to the fact that the synergism of each sub fraction with one other may contribute to this observation. The antiradical and antiphotooxidant activity of sub-fraction II-b, II-c and II-d showed significant different than one other sub fraction.

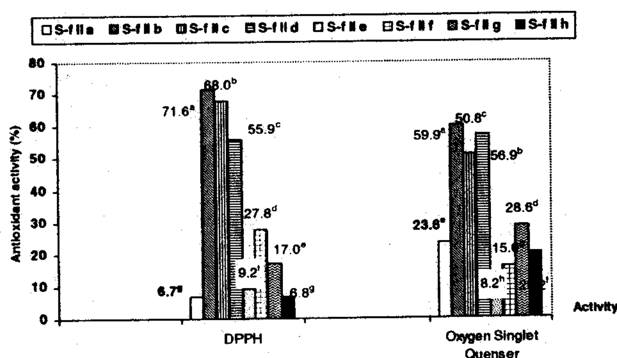

Figure 2. Antiradical Activity (% DPPH) and Antiphotooxidant Activity (% Oxygen Singlet Quenser) of Eight Subfractions

Furthermore, Sub fraction II-b, II-c and II-d were loader on a thin layer chromatography, were developed on silica gel 60 F_{254} using ethyl acetat/ methanol/water (100:10:3, v/v); ethyl acetat/ methanol/water (100:9:4, v/v) and butanol/etil asetat/ water(4:3:1, v/v). After drying, each plate was examined by UV light The sub- fraction II-b showed a yellow single spots and was Rf 0.90, 0.94 and 0.26. Furthermore, sub fraction II-b were investigated by mass spectrometry and tentative identification of this compound was made by comparison with the HPLC. Therefore, the chemical structure of subfraction II-b was determined by instrument analysis as follows. Subfraction II-b, orange amorphous solid, had molecular weight of m/z 257 by mass spctrophotometer.. Yen et.al. (1998) reported that the presence of carbonyl group and hydroxyl group

in the molecule of emodin, which appears to be necessary contributed to the antioxidant activity. Figure 3, emodin of Aloe vera chinensis extracts contains two carbonyl group and three hydroxyl group possesses antiradical and antiphotooxidant activity. The UV spectrum, with absorption band at 429, 288, 255 and 225 nm and showed only one peak which observed at 8.67 min on the HPLC chromatogram. The UV spectrum of subfraction II-b, which obtained by a PDA on our HPLC instrument, showed similar pattern with Aloe emodin standard. On the basis of these data, the structure of subfraction II-b was identified as 1,8 dihydroxy-3-hydroxymethylanthraquinone (Fig. 4a and 4b).

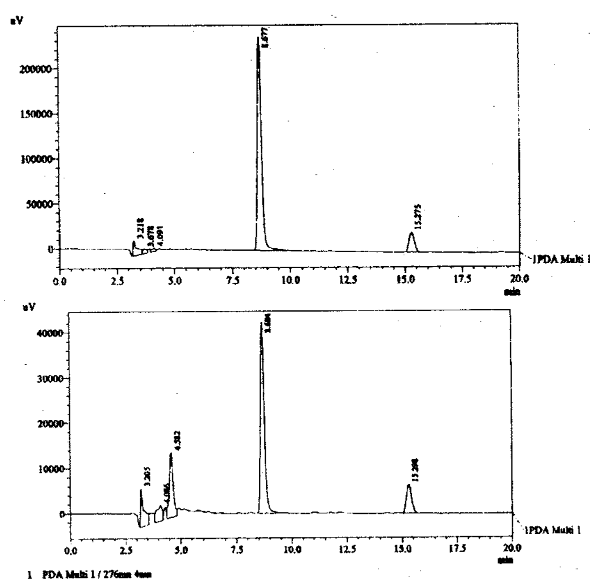

Figure 3. Moss Spectroof include of subfraction II-b (1,8 dihydroxy-3-hydroxymethylan-thraquinone)

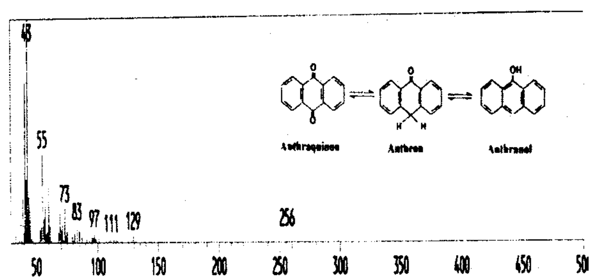

Figure 4. HPLC analysis of subfraction II-b (a) and Aloe emodin standard (b)

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In conclusion, a 1,8 dihydroxy-3-hydroxymethylan-thraquinone (Aloe emodin) was an antiradical and an antiphotooxidant compound from Aloe vera chinensis that it was responsible to protect of oxidation. Future research should include investigation of quensing mechanism and kinetics of Aloe emodin in singlet oxygen oxidation of linoleic acid.

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REFERENCE

AOCS. 1990. Official. Methods and Recommended of the American Oil Chemists *Society. 4 th Ed. Champaign, Il. American Oil Chemist Society.*

Dewi, Y. S. K., Tranggono, S. Raharjo and Pudji Hastuti. 2005. *Extract Aloe vera Sebagai Penangkap Radikal* . Agritech (In Press).

Duh, P.D. 1998. *Antioxidants Activity of Burdock (Arctium lappa Linne'): Its Scavenging Effect on Free-Radical and Active Oxygen.* J. Am. Oil. Chem. Soc. 75:455-461.

Esteban, A., J. M. Zapata, L. Casano, M. Martin dan B. Sabater. 2000. *Peroxidase Activity in Aloe barbadensis Commercial Gel: Probable Role In Skin Protection.* Planta Medica. 66: 724-727.

Grindlay, D. and T. Reynolds. 1986. *The Aloe vera Phenomenon: A Review on The Properties and Modeern Uses of The Leaf Parenchyme Gel.* J. Ethnopharmacol. 16: 117-151.

Guyod, S., N. Marnet, D. Laraba., P. Sannoner, J. F. Drilleau.. 1998. *Reversed Phase HPLC Following Thiolysis for Quantitative Estimation and Characterization of the Four Main Classes of Phenolic Compounds in Different Tissue Zones of a Frech Cider Apple Variety (Malus domestica Var. Kermerrien).* J. Agric. Food Chem. 46:1698-1705.

Hatano, T. H. Kagawa, T. Yasuhara. and T. Okuda. 1988. *Two New Flavonoids and Other Constituents in Licorice Root: Their Relative Astringency and Radical Scavenging Effects*, Chem, Phar, Bull. 36: 2090-2097.

Hu, Y., J. Xu and Q. H. Hu. 2003. *Evaluation of Antioxidant Potential of Aloe vera (Aloe barbadensis) Mill Extracts*. Journal of Agricultural and Food Chemistry. 26: 7788-7791.

Hu, Q, Y. Hu and J. Xu. 2005. *Free Radical- Scavenging Activity of Aloe vera (Aloe barbadensis Miller) Extracts by Supercritical Carbon Dioxide Extraction*. Food Chemistry 91:85-90.

Huttler, J.A., M. Salman. W. B. Stavinova., N. satsangi. R.F. Williams. R.T. Streeper dan S.T. Weintraub. 1996. *Antiinflammmatory C-Glucosyl Chromone from Aloe barbadensis*. J. Nat. Prod. 59: 541-543.

Joshi, S. P.. 1998. *Chemical Constituents and Biological Activity of Aloe barbadensis*- a Review. J. Med. Aromat. Plant. Sci. 20:768-773.

Jung, M. Y. , J. P. kim and S. Y. Kim. 1999. Methanolic Extracts of Coptis Japonica Makino Reduces Photosensitized Oxidation of Oils. Food. Chem. 67: 261-268.

Lee, K. Y., S. T. Weintraub and B. P. Yu. 2000. *Isolation and Identification of Phenolic Antioxidant from Aloe barbadensis.* Free Rad. Bio & Med. 28:261-265.

Martin, T.S., H. Kikuzaki, M. Hisamoto and N. Nakatani. 2000. *Constituents of Amomum tsao-ko and Their Radical Scavenging and Antioxidant Activities*. J. Am. Oil. Chem. Soc. 77:667-673.

Shahidi, F. 1997. *Natural Antioksidants: An Overview*. In *Natural Antioksidants. Chemistry, Health Effects, and Applications*. AOCS Press. Champaign. Illionis. p.1-11.

Schuler, P. 1990. *Natural Antioxidants Exploited Commercially. In Hudson, B. J., ed. Food Antioxidants*, Amsterdam: Elsevier, p. 99.

Yen, G Y., H. W. Chen and P. D. Duh. 1998. *Extraction and Identification of an Antioxidative Component from Jue Ming Zi (Cassia tora L.)*. J. Agric. Food Chem. Vol. 46. 3.

Yen, G.C., P. D. Duh dan D.Y. Chuang. 2000. *Antioxidant Activity of Antraquinones and Anthrone. Food. Chem.* 70:437-441