2011 2nd International Conference on Biotechnology and Food Science
IPCBEE vol.7 (2011) © (2011) IACSIT Press, Singapore

Inhibition of Lipid Peroxidation by Extract and Fractions of *Dendrobium* Sonia 'Red Bom'

Shafazila T. S., *Pat M. Lee
Materials Technology Program, Faculty of Applied Sciences, Universiti Technologi MARA (UiTM)
Shah Alam, Selangor, Malaysia
*patmlee.1@gmail.com

Lee Kong Hung
Malaysia University of Science and Technology (MUST), Kelana Jaya Square,
Selangor, Malaysia

***Abstract*— Antioxidant activities of extract and solvent-solvent partition fractions from the orchid flower, *Dendrobium* Sonia 'Red Bom' were evaluated. Flower of *Dendrobium* Sonia 'Red Bom' was extracted with acidified methanol. The residue was redissolved in water which was then subjected to solvent–solvent partition with organic solvents. Crude extract (CEDS) and three different fractions namely hexane layer (HXL), ethyl acetate layer (EAL) and water layer (WL) were characterized with UV-Vis spectrophotometer and evaluated for its inhibition of peroxidation of linoleic acid by lipid peroxide-ammonium thiocyanate method. WL and CEDS showed the UV-Vis spectral characteristics of anthocyanins. The percentage inhibition of peroxidation of linoleic acid was found to be higher in WL and CEDS which was comparable to alpha-tocopherol. In this study, a direct relationship between the total anthocyanins content with inhibitory activity towards lipid peroxidation was observed. This indicates that anthocyanins are the main contributors for the observed antioxidant activities for CEDS and WL.**

Keywords-Anthocyanins;antioxidant;Dendrobium;lipid peroxidation;UV-Vis spectroscopy

I. INTRODUCTION

Dendrobium is well known as a largest genera in orchidaceae which include about 1000 species distributed from Himalayas, Asia, Australia, Tasmania and the pacific island [1]. The red-violet color of the *Dendrobium* Sonia *'*Red Bom*'* flower, a hybrid of *Dendrobium* is expected to contain anthocyanins and some other polyphenolic compounds that are believed to have beneficial health effects [2, 3]. This genera is popular and easily hybridized which can be found in various habitat from tropical and to a very cool places [4].

Lipid peroxidation refers to a process that causes polyunsaturated fatty acid to turn rancid and is related to many pathological processes which lead to cancer, degenerative disease, and other diseases [5, 6]. Lipid peroxidation initiators are reactive oxygen species (ROS) such as hydroxyl (OH•) and peroxyl radicals (ROO•) and the superoxide anion radicals (O2•-), which are formed by exogenous chemicals factors and endogenous metabolic processes in the human body or in food systems [3, 7].

Anthocyanin, a natural pigment with intensely red colour have demonstrated to give antioxidant activity and have been used as functional red colour ingredient in food, cosmetic and pharmaceutical industries. Therefore, in order to identify extracts or fractions possessing the ability to inhibit lipid peroxidation, screening plant materials on the basis of their antioxidant potency seems to be essential. In this study, we will characterize the anthocyanin extract and fractions of *Dendrobium* Sonia 'Red Bom" using UV-Vis spectrophotometer and determine their antioxidant properties by their potential in inhibiting the lipid peroxidation.

II. METHODOLOGY

A. Chemicals

Methanol, linoleic acid and Tween 20 were purchased from Systerm® Chemicals, (Malaysia). Trifluoroacetic acid was from Sigma (Germany), n-hexane and ethyl acetate, were purchased from Merck Co. (Darmstadt, Germany). Ethanol was purchased from JT baker (Phillipsburg, USA). α-tocopherol was purchased from Fluka (Germany). Ammonium thiocyanate, potassium chloride and sodium acetate were purchased from BDH Limited Poole (England). Potassium ferricyanide was purchased from COMAK Chemical Limited (London, England).

B. Extraction and Solvent-solvent Partition

The orchid flower, *Dendrobium* Sonia 'Red Bom' (DSF) was purchased from a florist shop in Klang valley, Kuala Lumpur. The flowers (70.4 g) were cut into small pieces and extracted in the dark with 500 ml 0.1% trifluoroacetic acid in methanol for 1 day at low temperature and filtered on a Bunchner funnel. The flower pieces were further submitted to the same process twice for complete extraction of the colour. The acidified methanol extract was collected, filtered, dried under vacuum to yield 3.3 g of dried residue. A 2.3 g of dried acidified methanol extract was suspended in water and partitioned successively with n-hexane and ethyl acetate. The obtained n-hexane layer fraction (HXL), ethyl acetate layer fraction (EAL) and water layer fraction (WL) remaining after solvent-solvent partition were filtered and concentrated under vacuum to give 0.0629 g of HXL, 0.1657 g of EAL and 1.8601 g of WL. The dry residues

were re-dissolved in the same extraction solvent and stored at low temperature for further analysis.

C. *UV–Visible Spectrophotometric Analysis*

All samples (1 mg) were redissolved in 10 ml of respective extraction solvent. The sample solutions were scanned from 220 to 700 nm, using a UV–visible spectrophotometer Lambda 35 (Perkin Elmer).

D. *Lipid Peroxidation- Ammonium Thiocyanate Method (LP-ATM)*

The antioxidant activity of CEDS and all fractions were determined according to the method of [8] with some modifications. A pre-emulsion was prepared by mixing 175 µg Tween 20, 155 µL linoleic acid, and 0.04M potassium phosphate buffer (pH 7.0). A 1 mL of sample (1 mg/mL) in 99.5% ethanol was mixed with 4.1 mL linoleic emulsion, 0.02 M phosphate buffer (pH 7, 8 mL) and distilled water (7.9 mL). The mixed solutions of all samples (21 mL) were incubated in screw cap-tubes under dark conditions at 40˚C at certain time intervals. To 0.1 mL of this mixture was pipeted and added with 9.7 mL of 75% and 0.1 mL of 30% ammonium thiocyanate sequentially. After 3 min, 0.1 mL of 0.02M ferrous chloride in 3.5% hydrochloric acid was added to the reaction mixture. The peroxide level was determined by reading daily of the absorbance at 500 nm in a spectrophotometer (Perkin Elmer, Lamda 35). Antioxidant assay of α-tocopherol were also determined for comparison. All test data was the average of three replicate analyses.

The inhibition of lipid peroxidation in percentage was calculated by the following equation:

$$\% \text{ Inhibition} = [(A_0 - A_1) / A_0] \times 100 \quad (1)$$

Where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of extracts or standard compound.

E. *Statistical analysis*

In all cases analyses were performed in triplicate and data were averaged over the three measurements. The standard deviation (SD) was also calculated. Data were treated for multiple comparisons by analysis of variance (ANOVA), followed by the Duncan's Multiple Range test with significance level $P < 0.05$ to determined significant differences between means. Data in percentages were subjected to arcsine transformation prior to statistical analysis. ANOVA was performed using the statistical software SPSS (SPSS Inc., Chicago, USA).

III. Results and Discussion

A. *UV-vis spectrophotometer Analysis, Total Phenolics Contents and Total Anthocyanins Contents*

The acidified methanol extract was partially purified by solvent-solvent partition. The percentage yields from fractions are in the order of CEDS (4.7 %) > WL (3.84 %) > EAL (0.34 %) > HXL (0.13 %) based on the fresh weight of the DSF petals. The total phenolic content for CEDS, WL, HXL and EAL were found to be 375.48 ± 17.29, 223.36 ± 15.37, 1.14 ± 0.22, 55.80± 7.31, respectively and total anthocyanin contents for CEDS and WL were 84.55 ± 5.10, 76.69 ± 3.82, respectively, with no anthocyanins detected in EAL and HXL [9]. CEDS and WL with higher percentage yields were found to contain higher anthocyanins content and it was noted that WL contained highest ratio of TA with respect to TPC (Table I) [9].

Hexane fraction with the lowest percentage yield obtained as the non-polar solvent used was expected to collect fat soluble compounds for instance lipids or chlorophylls as suggested by [10]. Hexane fraction obtained from solvent-solvent partition was green in colour with λ_{max} of 653 nm and 421 nm in the visible region which possibly correspond to chlorophylls and there is no absorption band in the visible region indicating the anthocyanins band (Fig. 1a). EAL a moderately polar solvent was expected to extract the non-colored polyphenols and flavonoid aglycones [11, 12] with absorption band observed only at 315 nm in the UV region (Fig. 1b) suggesting the presence of some compounds possibly flavonoid or phenolic acids but not anthocyanins. The result agrees with the previous study [9] with no total anthocyanins contents exhibited in the EAL and HXL of the flower as revealed by the results of total phenolics and total anthocyanin contents of EAL and HXL.

UV-Vis absorption spectra showed 3 main absorption bands at 533, 319 and 296 nm for CEDS and WL at 531, 326 and 283 nm (Fig. 1c and 1d) which were consistent with spectral characteristics of anthocyanins at acidic pH [13]. The UV-Vis data were shown in Table II. Furthermore, higher intensity of UV absorption was observed for both spectra of CEDS and WL and these absorption bands may due to water soluble phenolic acids or flavonoid compounds which were also present in the sample.

The analysis of $A_{440\ nm}/A_{vis}$ ratio and $A_{uv\text{-}max}/A_{vis}$ give an indication of the glycosyl pattern and the degree of acylation on the anthocyanidin molecule, respectively. The $A_{440\ nm}/A_{vis}$ ratio values of 26 % and 31 % for CEDS and WL respectively (Table II) indicates that only the 3-position of anthocyanidin is glycosylated [14].

TABLE I. TPC and TA in Respective Fraction and TA/TPC Ratio (%) of CEDS, HXL, EAL and WL. TPC is Expressed as Gallic Acid Equivalents (mg GAE /100 g FW) and Total Anthocyanins (mg cyanidin 3-glucoside /100 g FW)

Sample	TPC	TA	TA/TPC (%)
CEDS*	375.48 ± 17.29 [d]	84.55 ± 5.10 [b]	23
HXL	1.14 ± 0.22 [a]	ND	-
EAL	55.80± 7.31 [b]	ND	-
WL	223.36± 15.37 [c]	76.69 ± 3.82 [a]	34

*Data are the means of three replications ± standard deviation. Values within column followed by the same letter are not significantly different at $P < 0.05$ (Duncan's Multiple Range test).

ND= not detected.

'adapted from [9]

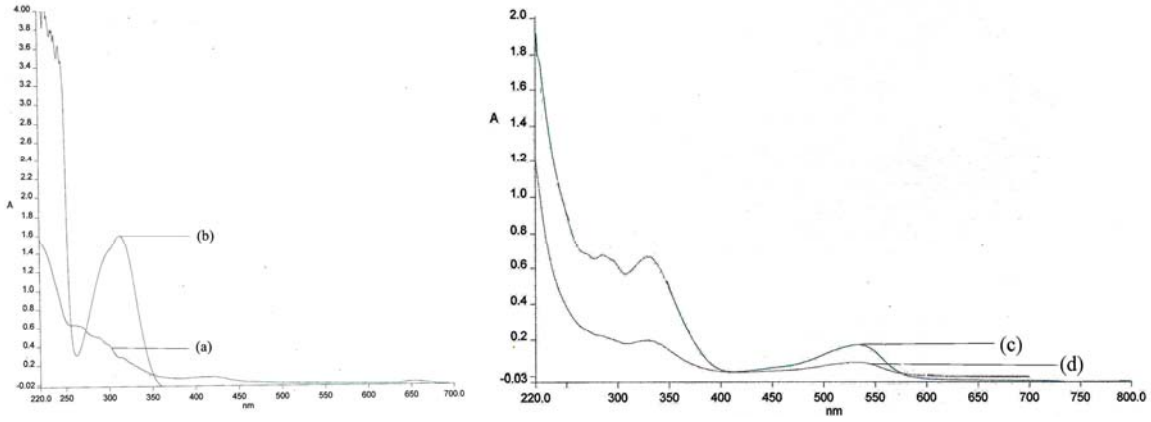

Figure 1. UV-Vis spectra of (a) HXL , (b) EAL, (c) CEDS and (d) WL.

TABLE II. UV-VIS DATA OF CEDS, HXL, EAL AND WL IN RESPECTIVE EXTRACTION SOLVENT

Sample	$\lambda_{vis\ max}$ (nm)	$\lambda_{UV\ max}$ (nm)	A_{uv}/A_{vis} (%)[A]	A_{440nm}/A_{vis} (%)
CEDS	533	319, 296	490	26
HXL	421, 653	268[s], 288[s], 315[s]	-	-
EAL	-	312, 293[s],243	-	-
WL	531	326, 283	271	31

[A]A_{UV} refer to the $\lambda_{UV\ max}$ within the range of 310 to 330, [s] =shoulder

Moreover, a strong UV absorption was detected around 320 nm region with the ratios of $A_{uv\text{-}max}/A_{vis}$ > 271 % indicates that the anthocyanins are highly acylated with two or more aromatic acids. It is also possible that CEDS and WL contain high proportions of free phenolic acids.

B. Lipid Peroxidation-Ammonium Thiocyanate (LP-AT) assay

Lipid Peroxidation-Ammonium Thiocyanate (LP-AT) assay in the present study monitored the amount of lipid peroxide formed every 24 h, over a period of 4 days, by monitoring the colored complex of lipid peroxide which absorbs light at 520 nm. It was found that on the third day, the absorbance of the control solution reached a maximum indicating the maximum formation of lipid peroxides. Fig. 3 showed the absorbance value of CEDS and all fractions as compared to α-tocopherol as a positive control. Low absorbance value at 500 nm indicated high level of inhibition of lipid peroxidation and therefore high antioxidant activity. The percentages inhibition of linoleic acid peroxidation for all samples were between 37 to 54 %. It was found that CEDS and WL have comparable values of lipid peroxide inhibition activity (50.90 ± 0.67 % and 54.84 ± 0.76 %, respectively) which is not significantly different from alpha-tocopherol (55.01 ± 2.26 %) (Table III). The results showed strong linear correlation between antioxidant activity and TA (r^2=0.97) and the HXL and EAL which were non-anthocyanins fractions showed lower activities. The activity showed weaker linear correlations with TPC (r^2=0.84). It is noted that the lipid peroxidation inhibitory activity of CEDS was comparable to the chestnut flower extract [15] with the value of 61.0 % at 1.5 mg/mL of concentration but found to be lower from dill flower extract (76.12 % at 0.4 mg/mL) [10].

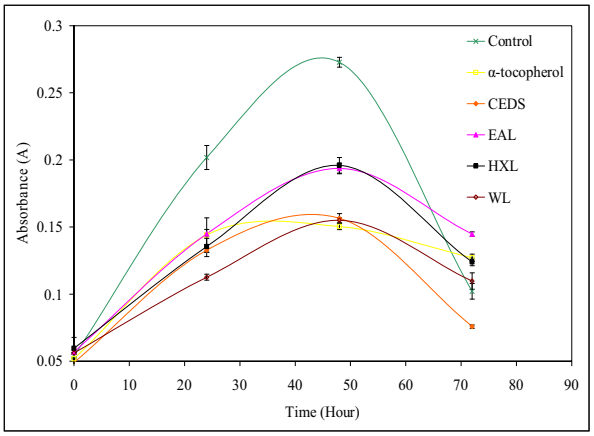

Figure 2. Variation of absorbance values of lipid peroxide colored complex with time for extract and fractions from DSF and alpha-tocopherol.

TABLE III. INHIBITION OF LIPID PEROXIDATION OF EXTRACT AND SOLVENT-SOLVENT PARTITION FRACTIONS FROM DSF AND ALPHA-TOCOPHEROL.

Sample	LP-AM (%)*
CEDS	50.90 ± 0.67^{b}
HXL	37.50 ± 2.22^{a}
EAL	37.76 ± 4.75^{a}
WL	54.84 ± 0.76^{b}
alpha-tocopherol	55.01 ± 2.26^{b}

*Values expressed at concentration 1 mg/mL.

Data are the means of three replications ± standard deviation. Values within column followed by the same letter are not significantly different at P < 0.05 (Duncan's Multiple Range test).

IV. CONCLUSION

In this study, among the crude extract and solvent-solvent partition fractions obtained from *Dendrobium* Sonia 'Red Bom' flower, the crude extract and water layer fraction contain anthocyanins as shown by UV-Vis spectral analysis. Inhibitory activity of peroxidation of linoleic acid as determined in this study showed strong correlation with the total anthocyanins contents in CEDS and WL. This indicates that anthocyanins are the main contributors for the antioxidant activity observed for *Dendrobium* Sonia 'Red Bom' flower. This warrants the isolation, purification and characterization of anthocyanins from the water layer of the extract of this flower. This work is currently in progress in our laboratory.

ACKNOWLEDGMENTS

The authors thank the Ministry of Science, Technology and Innovation (MOSTI), Malaysia for the eScience Fund (project nos. 02-01-01-SF0214 and 03-01-01-SF0092) and the Faculty of Applied Sciences, Universiti Teknologi MARA for the facilities and Research Management Institute (RMI) for the support.

REFERENCES

[1] H. Kamemoto, T.D. Amore, and A.R. Kuehnle, "Breeding dendrobium orchids in Hawaii," Canada: University of Hawaii Press, 1999.

[2] A.R. Kuehnle, "Orchid. *Dendrobium*," in Flower Breeding and Genetics, N.O. Anderson, Editor, Springer: Carlifonia, USA, 2007.

[3] I. Gülçin, Ö. I. Küfrevioglu, M. Oktay, and M. E. Büyükokuroglu, "Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (Urtica dioica L.)," J. of Ethnopharmacology, vol. 90, pp. 205-215, 2004.

[4] T.W. Purviance and J.F. Salventi, "*Dendrobium*," in The best orchids for indoors, C.M. Fitch, Editor. Brooklyn Botanic Garden, Inc.: Brooklyn, NY, 2004.

[5] D. Cvetkovic, and D. Markovic, 'Beta-carotene suppression of benzophenone-sensitized lipid peroxidation in hexane through additional chain-breaking activities," Radiat. Phys. Chem., vol. 80, pp. 76-84, 2010.

[6] J.T. Salonen, "The role of lipid peroxidation and natural antioxidants in cardiovascular diseases, in Natural antioxidants and food quality in atherosclerosis and cancer prevention," J.T. Kumpulainen, and J.T. Salonen, The Royal Society of Chemistry: Cambridge, UK, 1996.

[7] I. Gülçin, D. Berashvili, and A. Gepdiremen, "Antiradical and antioxidant activity of total anthocyanins from Perilla pankinensis decne," J. of Ethnopharmacology, vol. 101, 287-293, 2005.

[8] İ. Gülçin, I. Güngör Sat, S. Beydemir, M. Elmastas and Ö. Irfan Küfrevioglu, Comparison of antioxidant activity of clove (Eugenia caryophylata Thunb) buds and lavender (Lavandula stoechas L.). Food Chem., vol. 87, pp. 393-400, 2004.

[9] T.S. Shafazila, P.M. Lee, and L.K. Hung, "*Radical Scavenging* Activities of Extract and Solvent-Solvent Partition Fractions from *Dendrobium* Sonia 'Red Bom' Flower," Proc. Conference on Science & Social Research, CSSR 2010, pp. 768-771, Dec 2010.

[10] Y.-S. Shyu, J.-T. Lin, Y.-T. Chang, C.-J. Chiang, and D.-J. Yang, "Evaluation of antioxidant ability of ethanolic extract from dill (Anethum graveolens L.) flower," Food Chem., vol. 115, pp. 515-521, 2009.

[11] K.N. Prasad, E. Yang, C. Yi, M. Zhao and Jiang, Y., "Effects of high pressure extraction on the extraction yield, total phenolic content and antioxidant activity of longan fruit pericarp," Innovat. Food Sci. Emerg. Tech., vol. 10, pp. 155–159, 2009.

[12] M. Schwarz, S. Hillebrand, S. Habben, A. Degenhardt and P. Winterhalter, "Application of high-speed countercurrent chromatography to the large-scale isolation of anthocyanins," Biochemical Engineering Journal, vol. 14, pp. 179–189, 2003.

[13] E. Erhard Pfundel; Giovanni Agati and Cerovic, Z.G., "Optical properties of plant surfaces, in Biology of the plant cuticle," Riederer, M. and Müller, C., Wiley-Blackwell: Victoria, Australia, 2006.

[14] A. Solomon, S. Golubowicz, Z. Yablowicz, S. Grossman, M. Bergman, H. E. Gottlieb, A. Altman, Z Kerem, and M.A. Flaishman, "Antioxidant Activities and Anthocyanin Content of Fresh Fruits of Common Fig (Ficus carica L.), " J. Agric. Food Chem. , vol. 54, pp. 7717-7723, 2006.

[15] J.C.M. Barreira, I.C.F.R. Ferreira, M.B.P.P. Oliveira, and J.A. Pereira, "Antioxidant activities of the extracts from chestnut flower, leaf, skins and fruit," Food Chem., vol. 107, pp. 1106–1113, 2008.