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CHRYSOPHANOL FROM ALOE SAP: ISOLATION, CHARACTERIZATION AND BIOLOGICAL ACTIVITY STUDY

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ABSTRACT

A compound was isolated by organic solvent extraction and solvent partition method from Aloe sap and purified by chromatographic methods. It was characterized by various spectroscopic methods and was found to be consistent with the structure of chrysophanol (1,8-dihydroxy-3-methylanthraquinon). It was assayed for anti-oxidant activity using ferric thiocyanate-linoleic acid system, DPPH radical scavenging method and reducing power. It was found that it was not an antioxidant.

Keywords: Aloe vera, chrysophanol, structure elucidation, antioxidant activity

1. INTRODUCTION

Aloe vera contains many bioactive compounds such as essential vitamins, minerals, polyphenolic compounds, proteins and enzymes that have medicinal applications\textsuperscript{1,2}. Its medicinal applications include antitumor, antidiabetic and anti-inflammatory\textsuperscript{1,2}. The phenolic constituents such as anthraquinone analogue\textsuperscript{3} of aloe leaf exudates have been recognized to have various physiological actions\textsuperscript{1,2} other than the purgative effects previously reported. We have reported earlier\textsuperscript{4} that the extracts of Aloe vera sap, leaves, and gel exhibited antioxidant activities, the highest being the Aloe vera sap extract. It would be of interest to investigate the components in the extracts of Aloe vera that exhibit antioxidant activity. The objective of this work is to extract, fractionate, purify and characterize the structures of bioactive molecules, anthraquinones in particular, in the extracts and to evaluate their antioxidant activities. This paper focuses only on the extraction, fractionation, purification, structure characterization and antioxidant study of one of the
Antioxidants are compounds, which, while present in low concentrations compared to those of an oxidizable substrate, prevent or delay that substrate from being oxidized. Antioxidants are used as a means of reducing rancidity of fats and oils in food industries. In lipid peroxidation, they function as oxygen quenchers, radical scavengers or quenching radicals such as hydroxyl radicals, and peroxo as well as alkoxy radicals, or as metal ion chelators. In biological systems, antioxidants also play oxidative damages caused by reactive oxygen species in our bodies resulting in the prevention of various diseases such as cancer, cardiovascular diseases, hypertension and diabetes.

2. MATERIALS AND METHODS

2.1 Materials

The *Aloe vera* plants were purchased from Taman Agro Tech, Sepang. All chemicals purchased from chemical companies were of analytical grade and were used without purification.

2.2 Extraction, fractionation and purification of *Aloe vera* sap

*Aloe* fresh leaves were weighed (10 kg) and cut at their base and the yellow sap dripped from the leaves was collected. The exuded yellow sap (70.6 g) was then dissolved in 500 ml methanol and concentrated under pressure to give thick mass (10.2 g). This portion was extracted three times with a mixture of ethyl acetate and water at a ratio of 7:3. The ethyl acetate fractions were combined and concentrated under reduced pressure to give a yellow residue (3.5 g) which was suspended in petroleum ether. The suspension was filtered and the supernatant was treated with 80% aqueous methanol/hexane (1:1). The 80% methanol layer was then extracted with dichloromethane. The dichloromethane layer was collected and the solvent was removed by rotary evaporator. The sap residue (2.4 g) was redissolved in dichloromethane and was introduced onto silica gel column (3.5 x 40 cm) and eluted first with dichloromethane/methanol (98:2) and the polarity of the eluent was increased gradually. About 40 fractions were collected and identified using thin layer chromatography (TLC) and fractions with the same Rf values were pooled. One of the five pooled fractions designated as DCMA (180 mg) was analyzed by TLC and was run on a column again as described earlier. Two pooled fractions were obtained from this column as guided by TLC analysis. One of the pooled fractions designated as DCMX was further purified by preparative TLC. The major intense band was cut from the TLC plate and eluted with dichloromethane/hexane (60:40). This resulted in a single purified compound (SAP A) of weight 12 mg.

2.3 Characterization of SAP A

2.3.1 UV-VIS spectrophotometer

The sample was dissolved in 10 ml
methanol and run on UV-VIS spectrophotometer (Perkin Elmer, Lamda 35).

2.3.2 Fourier transform infra red (FTIR) Spectrophotometer

The sample was made into KBr disc and run on FTIR spectrophotometer (Perkin Elmer Spectrum One).

2.3.3 Proton Nuclear magnetic resonance spectroscopy

The sample was dissolved in deuterated chloroform and run on $^1$H NMR Spectrometer (Bruker 300 MHz).

2.3.4 Gas Chromatography-Mass Spectrometry

The sample in chloroform was run on GC-MS (Agilent Technologies 6890N).

2.4 Antioxidant assay

2.4.1 Linoleic acid-ferric thiocyanate (FTC) method

The purified compound was assayed for its antioxidant activity by using linoleic acid-FTC system with slight modification. Typically, a mixture of weighed sample in ethanol, 4.1 ml of 2.51% linoleic acid in ethanol, 8 ml of 0.02 M phosphate buffer (pH 7.0) and 3.9 ml of water was placed in a screw cap vial and incubated in at 40 °C in the dark. The control sample contains the same constituents as stated above except that it does not contain the purified compound. Aliquots of 0.1 ml were taken at different time intervals during incubation. The degree of oxidation was measured according to the thiocyanate method by sequentially adding 9.7 ml 75% ethanol, 0.1 ml 30% ammonium thiocyanate, 0.1 ml sample solution. Precisely 3 min after addition of 0.1 ml of 20 mM ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance of red colour was measured at 500 nm using UV-VIS spectrophotometer (Perkin Elmer, Lamda 35) every 48 hours until one day after absorbance of the control reached maximum.

2.4.2 Radical scavenging activity assay using DPPH radical

The purified compound was assayed for the radical scavenging activities according to the method of Duh et al. Various amounts of the purified compound were mixed with 2.0 ml of methanolic solution of 0.1 mM DPPH. After vortexing, samples were kept at room temperature for 60 minutes, and the absorbance of the mixture was measured at 517 nm.

2.4.3 Determination of reducing capacity

The purified compound was assayed for its reducing activity according to the method of Yang (2000). Typically, various amounts of samples (0 - 15 mg) were mixed with 2.0 M phosphate buffer (pH 6.6) and 1.0% potassium ferricyanide. The mixtures were allowed to incubate at 37 °C for 30 minutes. Five ml of 10% trichloroacetic acid was added, and then the mixture was centrifuged at
650 x g for 10 min. The supernatant was mixed with 5 ml of distilled water and 1 ml of 10% ferric chloride, and then the absorbance of the reaction mixture was measured at 700 nm using UV-VIS spectrophotometer (Perkin Elmer, Lambda 35).

3. RESULTS AND DISCUSSION

3.1 Extraction, fractionation, and purification of compound

To isolate bioactive molecules from the crude extract of Aloe vera sap, the crude extract was first subjected to liquid-liquid fractionation and followed by chromatographic separation as described in the experimental section. TLC profile of the fraction (DCMA) from the first column chromatography was shown in Figure 1. It shows several spots with a major intense spot of Rf value of 0.59. Further purification of this fraction by column chromatography removed some of the components (Figure 2). This fraction after purification by preparative TLC gave 12 mg of the pure compound, SAP A. The percentage yield of SAP A was about 0.1% based on the crude extract.

3.2 Structure elucidation

SAP A was an orange yellowish amorphous solid compound with melting point of 193 – 195 °C. UV-Vis spectrum (Figure 3 (a)) of this compound in absolute ethanol shows five peaks with λ\text{max} at 203, 225, 255, 287 and 430 nm. When a few drops of 0.3M NaOH solution were added into the sample solution, the orange-yellowish coloured solution immediately changed to red colour and shifted the λ\text{max} from 430 nm to 516 nm indicating the presence of hydroxyl group at the peri-position. Mass spectrometry of this compound showed an intense peak m/z at 254 (Figure 3(b)) corresponding to molecular weight of C_{15}H_{10}O_{4}. The IR spectrum (Figure 3 (c)) shows a broad absorption band at 3435 cm\(^{-1}\) which confirms the presence of hydroxyl group in this compound. The absorption band at 1628 cm\(^{-1}\) is due to the absorption of chelated carbonyl group, while the absorption band at 1670 cm\(^{-1}\) arises from the absorption of free or unchelated carbonyl group. The \(^1\)H-NMR spectrum (Figure 3 (d)) shows two singlet peaks at the downfield region at 12.13 ppm and 12.03 ppm. The occurrence of these two peaks also indicated the presence of two chelated hydroxyl group in this compound. Signals at region between 7.19 – 7.83 ppm are due to the five aromatic ring protons from rings A and C (Refer to Figure 4). Signals at 7.29 ppm (doublet, J = 9 Hz) and 7.83 (doublet, J= 7.8 Hz) are assigned to C-5 and C-7 respectively while a triplet signal at 7.67 ppm is assigned to C-6. Two singlet peaks at 7.70 ppm and 7.19 ppm are assigned to C-2 and C-4 respectively. At the upfield region, one sharp intense singlet peak at 2.47 ppm indicated the presence of CH\(_3\) group at C-3. The spectroscopic analysis as discussed above is consistent with the structure of the compound chrysophanol (1,8-dihydroxy-3-methylanthraquinone) (Figure 4). It is noteworthy that the \(^1\)H-NMR data obtained in this work is
agreeable to that reported by Liu et al.\(^8\) (2004) on the compound chrysophanol isolated from a Chinese herb.

### 3.3 Anti-oxidant activity

The results of antioxidant activity of chrysophanol and Vitamin E are summarized in Figures 5-7. It is clear that chrysophanol at the concentration range of 100-500 \(\mu g\) does not inhibit lipid peroxidation and does not reduce iron (III) ion in solution. It shows low radical scavenging activity, about 5.3% as compared to Vitamin E. The absence of antioxidant activity of chrysophanol has also been observed by Yen et al.\(^9\). They suggested that chrysophanol acts as a pro-oxidant rather than as an antioxidant. However, it is likely that the hydrophobic nature of the \(-\text{CH}_3\) substituent at the C-3 position of the anthraquinone hinders the interaction with oxygen molecules or iron (III) ions. Further work will be required to understand its biological action. It was demonstrated that the crude *Aloe vera* sap extract exhibited antioxidant activity. The observed antioxidant activity of the crude extract is likely to be attributed to other bioactive molecules\(^10\).

![Figure 1: TLC profile of DCMA fraction](SHAPE v MERGEFORMAT)

![Figure 2: TLC profile of DCMX fraction](SHAPE v MERGEFORMAT)
Figure 3: Spectroscopic data of the compound isolated from Aloe sap. (a) UV-Vis spectrum (b) GC-MS chromatogram, (c) FTIR spectrum (d) NMR spectrum

Figure 4: Structure of chrysophanol with $^1$H-NMR signal
**Figure 5:** Antioxidant study of chrysophanol by linoleic acid-ferric thiocyanate method

**Figure 6:** Radical scavenging effect of chrysophanol on DPPH

**Figure 7:** Determination of reducing power of chrysophanol
4. CONCLUSION

Spectroscopic data suggested that the compound isolated from the sap extract in this work was chrysophanol, 1,8-dihydroxy-3-methylanthraquinone. Chrysophanol did not inhibit lipid peroxidation and has no reducing activity. It also showed weak radical scavenging activity. It is concluded that the observed antioxidant activity of the sap extract was not attributed to chrysophanol but is likely to be attributed to other bioactive molecules.10

ACKNOWLEDGEMENTS

This work was supported by IRPA grant (Project no. 09-02-01-0014-EA013), Ministry of Science, Technology and Innovation, Malaysia and a short term research grant from the Institute of Research, Development and Commercialization, Universiti Teknologi MARA. The authors would like to thank the University for the support and for providing the facilities for this research.

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