From Biowaste to Bioproducts: Phenolic Antioxidants from Oil Palm Waste**

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INTRODUCTION

The oil palm is one of the richest sources of fat-soluble antioxidants such as carotenoids, tocopherols and tocotrienols. While much attention has been focussed on these fat-soluble components, little emphasis has been given to the water-soluble components, most of which are discarded during the extraction of palm oil. In 2008, the Malaysian palm oil industry generated about 42 million tonnes of palm oil mill effluent (POME), a liquid by-product. POME, which is mainly derived from the sterilizer condensate and centrifugal desludging of the raw palm oil from the screw-presses, is currently considered an industrial waste and it requires extensive treatment before discharge. Otherwise, it can pose a potential environmental hazard. Malaysia enforces stringent regulatory environmental standards, the challenge of converting such agricultural waste to high value products has remained elusive until now.

MPOB has developed and patented a breakthrough process (Sambanthamurthi *et al.,* 2008) to recover the concentration of antioxidants from POME. This process provides a solution and financial incentive to reduce pollution by the liquid by-product. The innovation also allows for the recovery of valuable antioxidants with numerous potential health benefits. The extract from the liquid oil palm waste is rich in polyphenols, phenolic acids, water-soluble vitamins and organic acids (*Figure 1*). The free-radical scavenging activities, cytotoxic effects on cancer cells and anti-cancer activity of the bio-products recovered were investigated to assess the potential of the palm phenolic extract for drug purposes.

MATERIALS AND METHODS

The components of the palm phenolic extract was identified by high performance liquid chromatography (HPLC) analysis using a RP C18 column and a PDA detector for detection of peaks absorbing in the range of 220 to 400 nm.

Purification of palm phenolic extract was carried out by using flash chromatography with an Isolute Flash C18 20 g/70 ml column reservoir equilibrated with methanol.

The free radical scavenging activity was measured using a modified method of Cao *et al.* (1998).

Effect of extract on J558 cells (a myeloma cancer cell) *in vitro* was investigated by determining the cell count and viability using the Trypan blue dye exclusion method and MTT assay at different time intervals (0 – 72 hr).

Regression of epithelial tumours was determined by inoculation at the dorsum of BALB/c mice with J558 cells.

RESULTS AND DISCUSSION

Process

The patented process developed for the recovery of polyphenols is completely solvent-free and is based solely on simple separation principles. It involves the use of a three-phase decantor system, and a series of different types of membrane to separating residual oil, ionic contaminants, and the com-

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Figure 1. Production of aqueous bio-products of the palm oil mill.

ponents of high molecular weight. The final filtrate recovered, oil palm phenolics (OPP) is found to be rich in phenolic compounds such as polyphenols and phenolic acids. The yield of the phenolics is 2,4% of the POME on a dry basis.

Flash Chromatography of Extracts

Three-dimensional flash chromatography coupled with biochemical assays confirmed the potent free radical scavenging and reducing activities in several fractions of the extract. The compounds in Fraction 1 have yet to be identified. The major compound in Fraction 2 was determined to be p-hydroxy benzoic acid. Ferulic and p-coumaric acid, rutin hydrate and other unknowns were found in Fraction 3 while Fraction 4 contains unknown methanolic compounds. The 3-D chromatograms for these fractions as shown in *Figure 2.*

Radical Scavenging Activity

The *Essence of Palm* filtrate was found to show good scavenging activity at concentration as low as 300 mg kg^{-1}. *Figure 3* shows that Fraction 3 had relatively higher free radical scavenging activity compared to the other fractions, with activity comparable to that of catechin (found in tea) and gallic acid. All the evaluation tests were conducted with DPPH (1,1-diphenyl-2- picrylhydrazyl), a well-known synthetic stable free radical commonly used in the assaying of antioxidants.

Inhibition of Skin Cancer Cell

Cell culture studies using both human and mouse skin melanoma cancer cells indicated an ability of the *Essence of Palm* extract to inhibit progression of these cancer cells in culture (*Figure 4*). The extract also triggered a programmed cell death process in malignant cells *in vitro* as confirmed in the Tunel assay which is a method for detecting DNA fragmentation by labelling the terminal end of nucleic acids.

Suppression of Tumour Growth in Mice

The extract showed potent anticancer activity in BALB/c mice injected with skin melanoma (*Figure 5*) as evidenced by the significantly lower tumour numbers occurring in mice supplemented with the extract as a drink (B) as compared to a control group provided with drinking water (A). It is also noted that the presence or absence of the extract did not affect the mice general health and body weight.

CONCLUSION

The oil palm is a very rich source of antioxidants with potential nutraceutical applications. The innovative process developed by MPOB has shown that phenolic antioxidants extracted from liquid by-products of palm oil milling have a huge potential in nutraceutical applications. This new technology simultaneously yields premium products while solving the problem of pollution from POME. Considering the large volume of this biowaste produced annually from the palm oil mills in the country, there is a huge potential to utilize it as a renewable source of raw material for this high value addition application. With the increasing global demand for phenolic-rich functional food supplement, this discovery appears especially attractive for entrepreneurs.

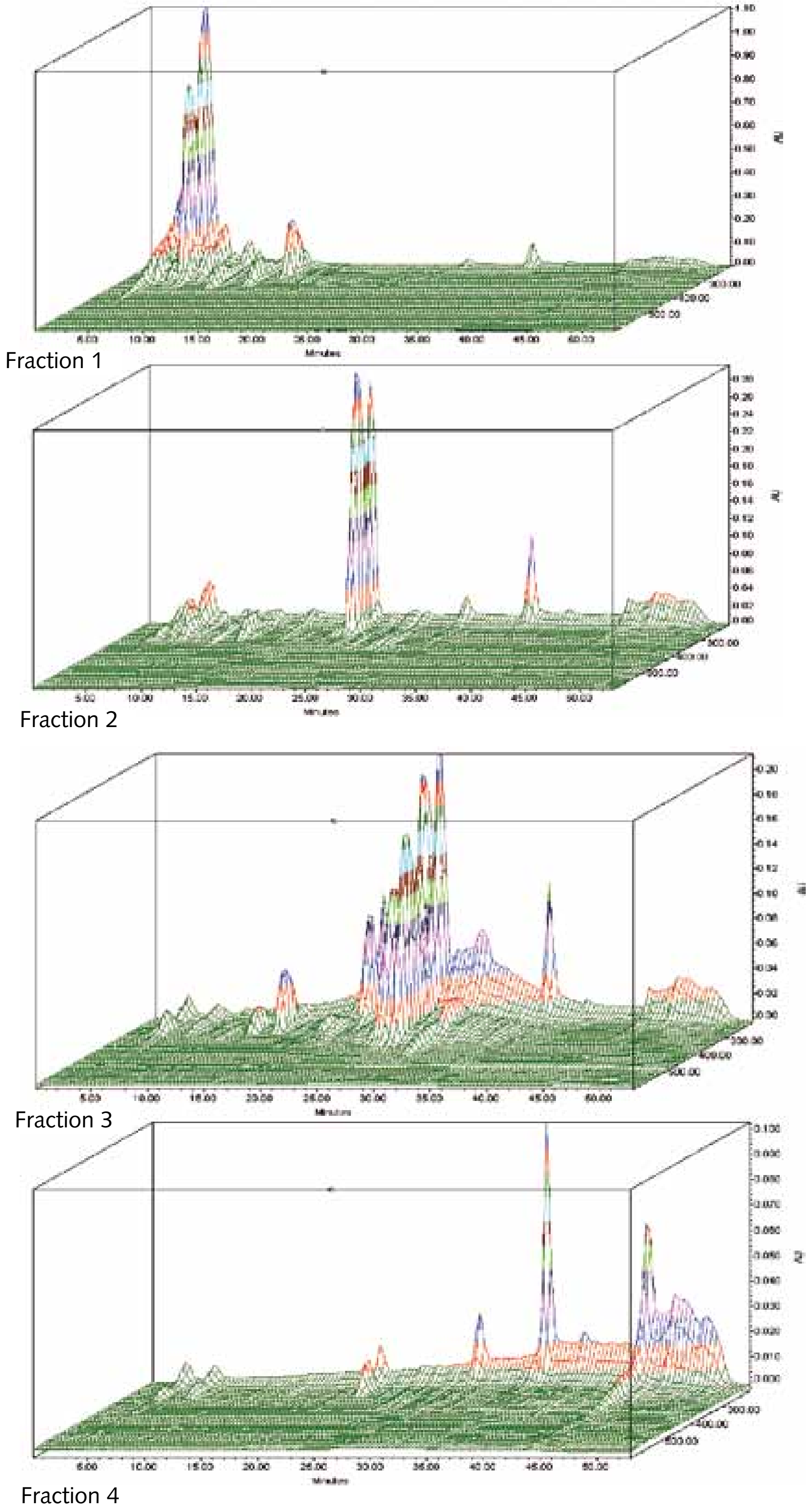

Figure 2. The 3D chromatograms of fractions 1, 2, 3 and 4 from flash chromatography.

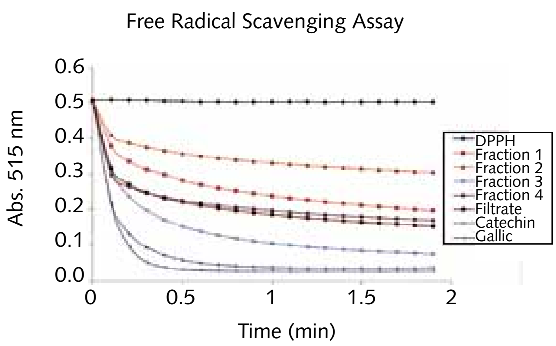

Figure 3. Free radical scavenging activity of various fractions.

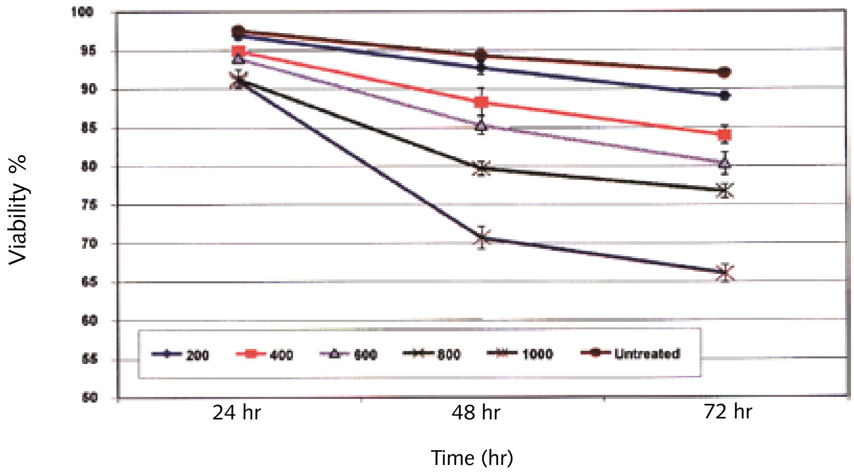

Figure 4. J558 tumour cell viability (%) after 24, 48 and 72 hr following treatment with palm extract at various concentrations.

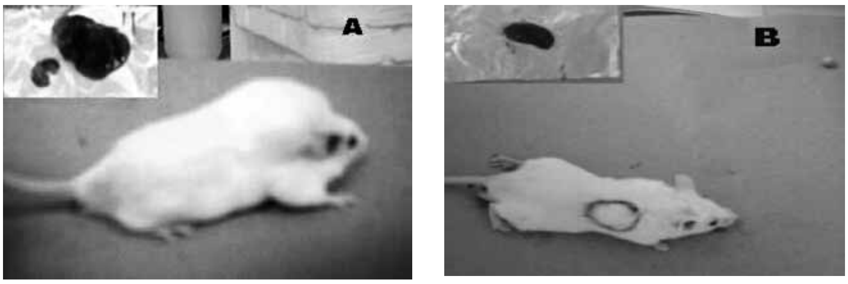

Figure 5. Tumour shape in mice that develop tumours from different groups before excising. Excised tumours shown at the upper left side of each picture.

REFERENCES

CAO, G; BOOTH, S L; SADOWSKI, J A and PRIOR, R L (1998). Increases in human plasma antioxidant capacity after consumption of controlled diets high in fruit and vegetables. *Am. J. Clin. Nutr. 68:* 1081-1087.

SAMBANTHAMURTHI, R; TAN, Y A and SUNDRAM, K (2008). Treatment of vegetation liquors derived from oil-bearing fruit. United States patent 7387802.