### The Search for New Anti-Oxidative Compounds from Zingiberaceous Species

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### Introduction

In recent years the role of free radicals and reactive oxygen species in human disease process including cancer, atherosclerosis, rheumatoid arthritis, inflammatory, bowel disease, immunosuppression, brain dysfunction, cataracts and malaria has become apparent1. This has led to considerable research on the possible contribution of dietary anti-oxidants to disease prevention, since anti-oxidants are able to remove or prevent the formation of free radicals and reactive oxygen species and prevent oxidative deterioration in vitro. In cosmetics anti-oxidants have heen widely used for prevention of melanin, which causes skin darkening, and in food it is used to reduce rancidity of fats and oils stuff<sup>2</sup>. Although synthetic anti-oxidants, (e.g., BHA and BHT) are effective, concern about possible adverse effects is increasing. As with other synthetic products BHA and BHT have been criticised mainly due to possible toxic effects.

Zingiberaceae consists of 700 species belonging to 45 genera and mainly found in Indomalaysian region. In the Malay society zingiberaceous plants are used widely in traditional medicine and spices. In UPM a collection of more than 30 zingiberaceae species are available in the germ-plasm unit. These specimens were collected from several areas of the peninsular Malaysia.

## **Materials and Methods**

The plant samples obtained from the unit were extracted using various organic solvents. Plants with relevant medicinal claims are primarily of higher interest to our study. The extracts were then subjected to antioxidative assays and the activity were assessed. Plants showing high activity from the assays were selected for detail investigation to isolate and purify the

constituents and finally identified spectroscopically. The activity of each isolate was measured and the bioactivity-structure relationship established.

Twenty-five species of Zingiberacea species available in the germ-plasm unit of UPM have been extracted and tested for their anti-oxidative activity. Most of the species have shown anti-oxidative activity when tested using ferric thiocyanate and thiobarbituric acid methods of assay<sup>3</sup>. A number of these showed stronger activities then the other, which indicate their potential for further detail investigation.

Based on this observation three of the species, Alpinia nutans, Hedychium thyrsiforme, and Alpinia malacensis were subjected for further extraction, isolation and structural elucidation of the active components present in the species.

#### **Results and Discussion**

Several compounds were isolated and identified including 5,6-Dehydrokawain (1), flavokawin-B (2), 1,7-diphenyl-5-hydroxyhepten-3-one (3), (-)-pinocembrin (4) from A. nutans and A. malacensis; and 3,7,4'-trimethoxy-5-hydroxyflavone (5), 3,4'-dimethoxy-5,7-dihydroxyflavone (6), 5,7,4'-trimethoxy-3-hydroxyflavone (7), 3,5,7,4'-tetramethoxyflavone (8) and 7,4'-dimethoxy-3,5-dihydroxyflavone (9) from H. thyrsiforme.

The antioxidant assays carried on the five flavonoids including 3,7,4'trimethoxy-5-hydroxyflavone, 3,4'dimethoxy-5,7-dihydroxyflavone,
5,7,4'-trimethoxy-3-hydroxyflavone,
3,5,7,4'- tetramethoxyflavone and 7,4'dimethoxy-3,5-dihydroxyflavone isolated from the rhizome of *Hedychium*thyrsiforme showed that 5,7,4'trimethoxy-3-hydroxyflavone, 7,4'dimethoxy-3,5-dihydroxyflavone and

3,7-dimethoxy-5,7-dihydroxyflavone had strong antioxidant activities. Only two compounds, 5,7,4'-trimethoxy-3-hydroxyflavone and 7,4'-dimethoxy-3,5-dihydroxyflavone, were found to be as 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals scavenger with the fifty percent inhibition concentration (IC<sub>50</sub>) values of 92 and 119  $\mu$ M, respectively. Antitumor promoting assay indicated that all the flavonoids showed strong inhibition activity towards Epstein-Barr virus (EBV) activation in Raji cells.

In addition to identification of the antioxidative components, it was also discovered that flavokawin-B has strong cytotoxic property specifically against MCR 7 - breast cancer cell line.

#### **Conclusions**

New information on the anti-oxidative property occurring in most of the zingiberaceous species is now available in our country. This information provides support to the claims on their medicinal use in most of the traditional remedies. The information is also useful for the search of new anti-oxidants from natural origin. The results also indicated that there may be some relationship between anti-oxidative with anticancer promoting activity. The study also discovered that flavokawin-B has strong cytotoxic property specifically against MCR 7 - breast cancer cell line.

#### Benefits from the study

New data on the chemical constituents and bio-activities of the compounds isolated to be used by other scientists working in related fields and pharmaceutical industries.

New method of production of bioactive compounds from plant to be benefited by chemical industry. New source of bio-active agents from local plant to be used by pharmaceutical and/or cosmetic industries.

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