BIOASSAY-GUIDED ISOLATION OF FREE RADICAL SCAVENGING AND ANTIOXIDATIVE FLAVONOID FROM *ZINGIBER OTTENSII* (ZINGIBERACEAE)

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DECLARATION

The materials in this thesis are original except for quotation, excerpts, summaries and references, which have been duly acknowledged.

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ABSTRAK

PEMENCILAN MELALUI BIOCERAKINAN UNTUK PELEKATAN RADIKAL BEBAS AND ANTIOKSIDAN FLAVONOID DARIPADA *ZINGIBER OTTENSII* (*ZINGIBERACEAE*)

Biocerakinan terhadap fraksi mengunakan 2,2-diphenyl-1-picrylhydrazyl telah dijalankan terhadap rizom dan daun *Zingiber ottensii* untuk pemencilan and pengenalpastian kompoun yang menunjukkan aktiviti bioaktif secara pelekatan radikal bebas dan antioksidan yang hadir dalam ekstrak metanol. kompoun aktif yang dipencilkan dikenalpasti menggunakan kaedah spektroskopi sebagai kuersetin-3-*O*-ramnosilglucosida. Kandungan keseluruhan kompoun aktif setara dengan kuersetin juga telah dikira secara kuantitatif dan didapati bahawa kandungan kuersetin dalam rizom lebih banyak berbanding daun. Rizom juga didapati mengadungi 60% gula berdasarkan berat keringnya dan dikenalpasti dengan menggunakan Reagen Bial's sebagai heksos dan pentos. Penemuan ini menyumbang kepada kajian pertama kalinya terhadap kelompok halia kerana ulasan perpustakaan terhadapnya belum wujud. Ujian autoksidasi β-carotene juga telah dijalankan untuk mengkaji kebolehan kompoun yang dipencilkan untuk menghalang oksidasi lipid. Didapati bahawa kompoun ini adalah sama aktif berbanding antioksidan sintetik, BHT, dan lebih aktif berbanding dengan aglaikon kuersetin. Walaupun kuersetin telah diketahui sebagai pelekat radikal dan penghalang oksidasi lipid yang sangat baik, inilah kali pertama kompoun ini dikesan dalam *Z. ottensii*.

ABSTRACT

BIOASSAY-GUIDED ISOLATION OF FREE RADICAL SCAVENGING AND ANTIOXIDATIVE FLAVONOID FROM ZINGIBER OTTENSII (ZINGIBERACEAE)

Bioassay-guided fractionation by 2, 2-diphenyl-1-picrylhydrazyl has been conducted for rhizomes and leaves of Zingiber ottensii to isolate and elucidate the bioactive compound that possess free radical scavenging and antioxidative activities present in the methanol extract. The isolated active compound was identified by means of spectroscopic methods as quercetin-3-O-rhamnosylglucoside. The total content of the active compound in equivalence to quercetin was also quantified and found that the quercetin content was more in rhizomes compared to the leaves. The rhizomes were also found to contain sugars which contributed 60% of the dry weight and was identified by using Bial's Reagent as hexoses and pentoses. This finding can contribute towards the novel study in gingers since there is a void in the present literature on the subject. The autoxidation of β-carotene was also tested in effort to investigate the ability of the isolated compound toward inhibition of lipid peroxidation. It was found that this compound was as active as the synthetic antioxidant, BHT, and was more active than the corresponding quercetin aglycone. Eventhough, quercetin has been known as an excellent radical scavenger and inhibit lipid peroxidation, this is the first time this compound was detected in Z. ottensii.

ABBREVIATIONS

Abs	Absorption
ACCase	Acetyl Co-A carboxyligase
ATBS•$^{+}$	2, 2'-Azinobis-(3-ethylbenzthiazoline-6-sulfonic) acid
BHA	Butylated Hydroxyanisol
BHT	Butylated Hydroxytoluene
CHS	Chalcone synthase
DNA	Deoxyribonucleic acid
DPPH•	2, 2-diphenyl-1-picrylhydrazyl
ED_{50}	Dose of sample required to effect 50% of organism under study
HCl	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
IC_{50}	Concentration of sample required to inhibit 50% of oxidation
LC-MS	Liquid Chromatography- Mass Spectrometry
MeOH	Methanol
PG	Propyl gallate
SD	Standard deviation
TBQH	Tert-butyl hydroquinone
TLC	Thin Layer Chromatography
UV	Ultra Violet

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CHAPTER 1

INTRODUCTION

1.1 Background

Herbs have been used by mankind as remedies since time immemorial and still continue nowadays as an alternative to modern medicine. Among the herbs used worldwide are members of the Zingiberaceae family. The Zingiberaceae is part of the order Zingiberales which form an isolated group among the monocotyledons. These plants has been an important part of the tropical flora appreciated and used worldwide whether as ornamental, spices or in medicinal preparations (Larsen *et al.*, 1999). Although the morphology of the many gingers have been well-described especially for Peninsular Malaysia and Singapore by the extensive research done by Ridley in 1899 and Holttum in 1950 both (Larsen *et al.* 1999), the chemical content in gingers have not been as extensively documented.

Ethnobotanical literature indicate that *Zingiber ottensii* which is known locally as 'Lempoyang Hitam' by the Malays in Peninsular Malaysia and 'Lempoyang' by the Brunei ethnic in Sabah, had long been used as post-partum medicine and appetizer (Ibrahim *et al.*, 2000) as well as taken raw in their diet. The consumption of fruits, vegetable and flowering parts has been prescribed as a form of cancer prevention in individuals and is attributed to the free radical scavenging properties arising from the presence of substances such as carotenes, fiber and polyphenol in foods which display antimutagenic or anticarcinogenic properties (Bronzette, 1994; Steinmetz & Potter,1996; Satvic,1994). In addition, free radical scavenging properties have been associated with the slowing down of the aging processes.

Flavonoids are phenolic chemical compounds, which constitute a group of C15 aromatic plant pigment, biosynthesized via a confluence of the acetate malonate and shikimate pathways (Markham & Bloor, 1998). The term flavonoid is a collective noun for a plant pigment, mostly derived from benzo-γ-pyrone (Havsteen, 2000). A single plant may contain different flavonoids and therefore, its distribution within a plant family is useful for taxonomical classification.

Beside their contribution to plant colour, flavonoids which occur widely in fruits and green vegetables have broad pharmacological activities. Flavonoids preparation had been long used in medical practice to treat disorders of the peripheral, the circulation to lower blood pressure (Pietta, 2000).

Numerous phytomedicines containing flavonoids are marketed in different countries as anti-inflammatory, antispasmodic, anti-allergic and antiviral remedies (Pietta, 2000). Flavonoids potency as an antioxidant and free radical scavenging activity has also been reported in many papers and articles (Braca *et al.*, 2003; Owen *et al.*, 2003).

Flavonoids such as quercetin, myricetin, kaemferol and (+)-catechin has also been reported to synergy and inhibit the decomposition of the natural antioxidant vitamin E (α-tocopherol) in methyl linoleate system (Pekkarinen *et al.*, 1999).

Work on free radical scavenging and antioxidative flavonoid from *Zingiber ottensii* have not been reported to-date. Therefore, the aim of this study is to contribute to acquiring the chemical content in *Zingiber ottensii* and to explore the potential of this plant as an alternative to the synthetic antioxidant in the interest of the safety of food and additives. The findings of this research can be used as a basis for the development and commercialization of local natural products.

1.2 Research justification

The present study conducted on the *Zingiber ottensii* focuses both its rhizomes and leaves because these parts have long been used as traditional medicine and food. Among the Malay women in Peninsular Malaysia, the rhizomes have been used as post-partum medicine and appetizer (Ibrahim *et al.*, 2000) while the Brunei ethnic in Sabah consume the leaves in raw form (personal observation).

In carrying out this research, claims by the indigenous people can be scientifically validated. The commercial potential of this plant can also be explored. In term of knowledge advancements, this research can contribute to the chemical study on the Zingiberaceae family which is the largest family in the order Zingiberales in Asia but not well documented chemically.

1.3 Research objectives

The objectives of this study are:

1. To isolate flavonoids with free radical scavenging and antioxidative activities from rhizomes and leaves of *Z. ottensii* using bioassay-guided isolation.
2. To identify and determine the structure the isolated flavonoids using spectroscopic techniques.
3. To quantify the active flavonoids content in rhizomes and leaves of *Z. ottensii.*

CHAPTER 2

LITERATURE REVIEW

2.1 Background

Gathering food and medicinal herbs are among the earliest activities carried out by mankind. For millions of years, guided by instinct, followed by experience and rational thought, man had used herbs as food and medicine. These knowledge afterwards handed down through generations orally and still continue nowadays especially in isolated localities where medical facilities is difficult to access or as an alternative to modern medicine. In some cases, the 'classic' treatment carried out by the traditional medicine practitioners can be effective and therefore, it deserves to be examined with modern scientific method.

Among the numerous substances identified in medicinal plants, flavonoids represent one of the most interesting groups of biologically active compounds. The most important reported biological property of flavonoids is due to their antioxidant activity by scavenging radicals and inhibiting peroxidation as reported by Hanaski *et al.* (1994). The ability of the plant polyphenols to act as antioxidant in biological systems was recognized in the 1930's (Jovanovic *et al.*, 1998). Recently, there has been considerable interest in the nutraceutical industry and in preventive medicine in the quest for the natural antioxidant from plant materials. Various phytomedical components, such as flavonoids, phenylpropanoids and phenolic acids are known to be responsible for the antioxidant capacity of fruits and vegetables. Consumers are now including phytonutrients in their diet, in the belief that antioxidant compounds may reduce the incidents of cancer, cardiovascular diseases, arthritis and aging in

general, which are correlated with the damaging effects of uncontrolled free radical production.

2.2 Zingiberaceae as food and remedies

Zingiberaceae is one of the largest monocotyledonous families of the order Zingiberales (Ibrahim *et al.*, 2000). The Zingiberaceae comprises more than 1200 species with almost 1000 species found in the Asia tropics (Larsen *et al.*, 1999). More than 150 wild and cultivated zingiberaceous species have been reported for Peninsular Malaysia (Holttum, 1950). The Zingiberales order can be divided into two categories which are the family with five staments and families with one stament and this is summarized in Figure 2.1:

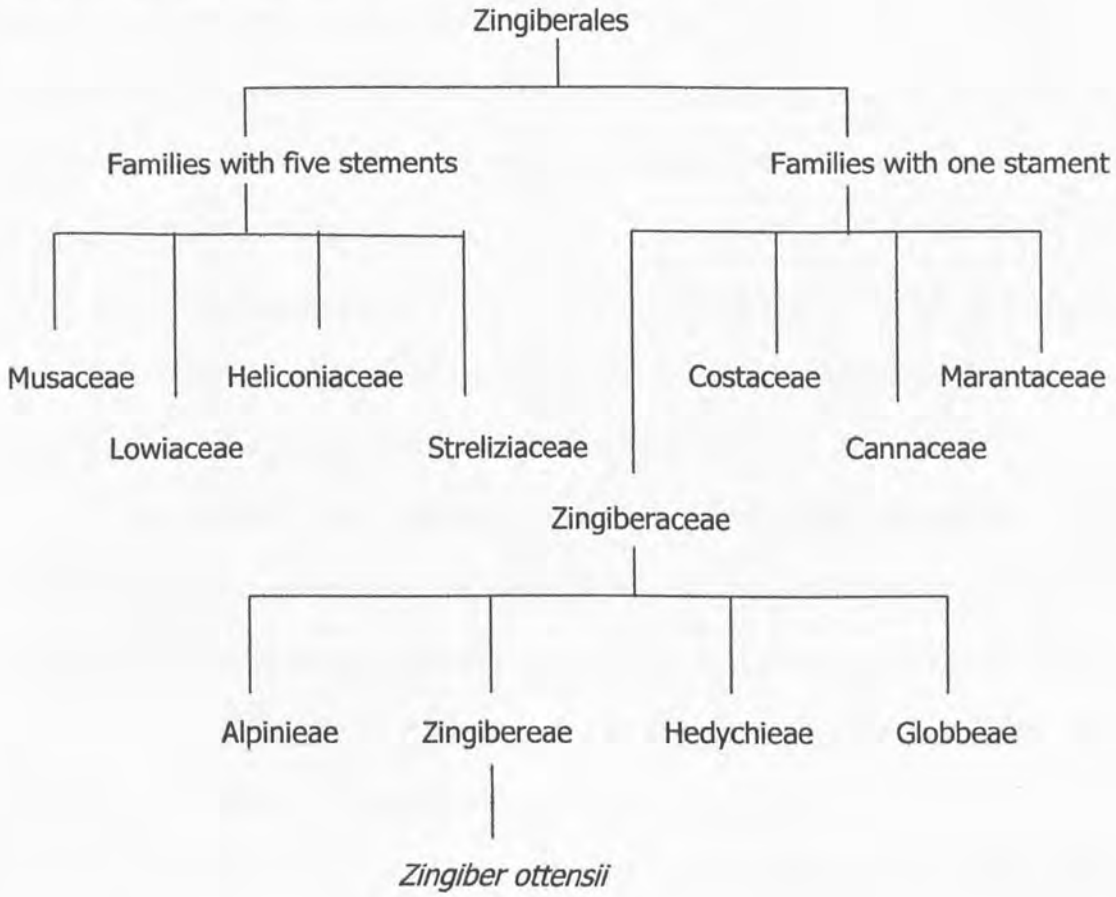


Figure 2.1: The classification of the Zingiberales (After Larsen *et al*, 1999)

The first families with five staments, regarded as an older line of evolution while the last four families with one stament are the more advanced groups in that the non-functional staments have been developed as petaloid staminodes (petal-like-structure). The largest families are the Zingiberaceae and Marantaceae. The first is predominantly found in the Asian tropics while the Marantaceae have their centre of diversity in the American tropics (Larsen *et al.*, 1999). Further, the Zingiberaceae can be divided into four tribes namely Alpinieae, Zingibereae, Hedychieae and Globbeae.

The Costaceae which used to be one of the Zingiberaceae is now distinguished from this family after flavonoid studies done by William & Harborne in 1977 (William & Harborne, 1997). In this study, it was found that the Costaceae having only cyanidin 3- glucoside and no 3-rutiniside together with the absence of myricitin and methylated flavonols of the *Costus* species supported the separation of this genus at the family level as suggested by Tomlinson in 1969 from his anatomical evidence (William & Harborne, 1988).

The Zingiberaceae species are perennial, aromatic herbs which part of the undergrowth flora of the tropical and subtropical forests with orchid like flowers and thrived well in damp, shaded habitats (Ibrahim *et al.*, 2000).

Zingiberaceae rarely found in secondary forest or bushes because only certain species are tolerant to the direct sunlight. Some of the members of the Zingiberaceae family would 'disappear' i.e. become dormant at certain period of time during the drought season and grow again during the rainy season as the rhizomes became active again (Ibrahim, 1989).

The Zingiberaceae family have been used by mankind for many purposes. From the ethobotanical survey of the ginger family in selected Malay villages in Peninsular Malaysia (Ibrahim *et al.*, 2000), this family have reportedly been used as

food, traditional medicine, spice, condiment, dye and flavours. The significance of Zingiberaceous species in traditional cosmetics was also reported (Riswad & Sagat-Roemantyo, 1992) as mentioned by (Ibrahim *et al.*, 2000). Several species from the following genera, *Alpinia, Ammomum, Curcuma, Kaempferia* and *Zingiber* are major ingredients in traditional preparations such as tonics called 'jamu' which are also commercially available (Habsah *et al.*, 2000).

Zingiberaceae species such as *Curcuma zedoaria, Curcuma mangga, Curcuma aeruginosa* and *Zingiber montanum* had been used in post-partum preparations either in pure or mixture form for general health (Larsen *et al.*, 1999). Other than post-partum preparation, Zingiberaceae species also had been used for treatment of rheumatism such as *Alpinia conchigera, Alpinia galanga, Curcuma domestica, Curcuma xanthorrhiza, Kaempferia galanga, Zingiber aromaticum, Zingiber montanum and Zingiber officinale* (Jalil *et al.*, 2000).

Zingiber ottensii (Figure 2.2) was described for the first time by Valeton. In his description, he mentioned that this plant was found in the Malays villages as cultivated plants. The rhizome of this plant is greyish purple within and has a pungent smell. The stems are close together about 1.5m tall bearing many leaves that are slightly hairy underneath. The inflorescence is about 10 cm long with 4 cm long closely imbracating bracts. The colour of the bract is dull reddish at first and bright red when old. The labellum is faint yellow mottled with pink (Holttum, 1950).

Figure 2.2: *Zingiber ottensii* inflorescence

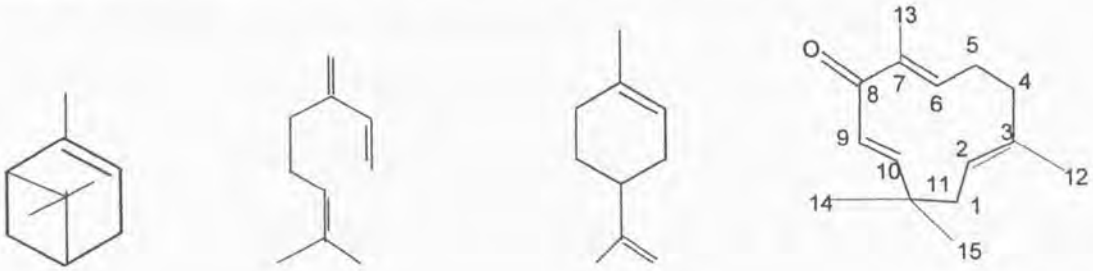
Zingiber ottensii is very closely related to *Zingiber zerumbet,* morphologically. The differences are: the bract of *Z. zerumbet* is green in colour at first and turn to bright red when old and the labellum is creamy in colour and not mottled. In addition, cross sections of rhizomes of *Z. zerumbet* is yellow whereas it is greyish purple in *Z. ottensii.* Aside from closely related vegetative characteristics, *Z. ottensii* and *Z. zerumbet* are also genetically closely related and only differ very slightly in the peroxidase isoenzymes of the rhizomes (Larsen *et al.*, 1999).

2.2.1 Previous research on *Z. ottensii* and *Z. zerumbet* chemical constituents

Members of Zingiberaceae are usually aromatic in all or most parts or at least one of the parts. Many species are known to be rich in terpenoids, other compounds such as alkaloid and phenolics are not well documented (Larsen *et al.*, 1999).

Research carried out on *Z. ottensii* by Lee & Hasnah, (2000) lead to the identification of monoterpene substances such as α-tujune, α-pinene, mirsene,

α-phalendrene, camphene, α-terpine, sabinene, β-pinene, limonene,δ-3-carene, γ-terpenolene, 1,8-cineol, linalool, terpine-4-ol, α-terpineol and sesquiterpine such as α-gurjunene, α-copaene, zerumbone, β-caryophelene, α-humulene, β-bisabolene, β-sesquiphelandrene, β-elemene, humulene epoxide II and β-eudesmol.



α-pinene Mirsene Limonene Zerumbone

Figure 2.3: Terpenoids identified in *Z. ottensii* (After Lee & Hasnah, 2000)

The isolation from the chloroform extract (Lee & Hasnah, 2000) gave three pure compounds namely humulene, humulene epoxide II and zerumbone.

The bioassay studies on the chloroform crude extract (Lee & Hasnah, 2000) showed inhibiting activities on *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* with 0.1 mg/mL concentration. While on the toxicity study by using *Artemia salina*, the chloroform crude extract performed ED_{50} at 551.48 ppm and the pure compounds humulene epoxide II and zerumbone were 183.28ppm and 128.72 ppm respectively.

The screening of the antimicrobial and antioxidant activities carried out in the crude extract by Habsah *et al.* (2000), based on the minimum inhibitory dose and linoleic acid assay, reported that the dichloromethane extract shows moderate activities for both assays and less active for methanol extract. Other than this research, there is no other research done for this plant either locally or internationally.

Z. zerumbet the closely related species of the *Z. ottensii* is more studied especially Zerumbone, the main component in the essential oil because of the ability of this compound to exhibit a variety of interesting reactions e.g. regio and stereoselective conjugate addition, transannular ring contration and cyclization, and several regiospecific reaction which cleave the 11-membered ring. The zerumbone also become a versatile stating material for conversion to the other useful compound such as precursor of the potent anticancer agent paclitaxel (Kitayama *et al.*, 2002).

Matthes *et al.* (1980), also reported the potential of the zerumbone which was isolated from the pentene extract, as cytotoxic compound together with zerumbone epoxide, while, diferuloylmethane, feruloyl-p-coumaroylmethane and di-p-coumaroylmethane isolated from ether extract also show highly cytotoxic activity and 3" 4"-O-diacetylafzelin with moderate activity. These tests were carried out using hepatoma tissue culture, a neoplastic rat liver cell strain culture *in vitro*.

The flavonoids study of *Z. zerumbet* was carried out by Masuda *et al.* (1991). From this research, it was reported that three new acelated flavonols glycoside and a known flavonol glycoside were isolated from the rhizomes acetone extract. The compounds were determined as Kaempferol 3-O-(2-O-acetyl-α-L-rhamnopyranoside), Kaempferol 3-O-(3-O-acetyl-α-L-rhamnopyranoside), Kaempferol 3-O-(4-O-acetyl-α-L-rhamnopyranoside), and Kaempferol 3-O-α-L-rhamnopyranoside). No further bioassay was done on this study.

REFERENCES

Amic, D., Davidovic-Amic, D., Belso, D & Trinajstic, N. 2003. Structure-radical scavenging activity relationship of flavonoids. *CROATICA CHEMICA ACTA* **76**: 55-61

Bondet, V., Brand-Williams, W & Berset, C.1997. Kinetics and mechanisms of antioxidant activity using the DPPH• free radical method. *Lebensm-Wiss. U-Technol.* **30**: 609-615

Bors, W., Heller, W & Michel, C. 1998. *The chemistry of flavonoids* in *"Flavonoids in health and disease"* Rice-Evans, C & Parker, L (eds). New York: Marcel Dekker, Inc pp 111-136

Braca, A., Sortino, C., Politi, M., Morelli, I & Mandez, J. 2002. Antioxidant activity of flavonoids from *Licania licaniaeflora. J. Ethnopharm.* **79**: 379-381

Braca, A., Fico, G., Morelli, I., De Simon, F., Tome, F & De Tommasi, N. 2003. Antioxidant and free radical scavenging activity of flavonol glycoside from different *Aconitum* species. *J. Ethnopharm.* **86**: 63-67

Bronzette, G. 1994. Antimutagens in food. *Trends in Food Sci. Tech.* **5**: 390-395

Choe, M., Jackson, C & Yu, B. P. 1995. Lipid peroxidation contributes to age-related membrane rigidity. *Free Radical Biology & medicine.* **18**: 977-984

Cornard, J.P., Merlin, J.C., Boudet, A.C & Vrielynck, L. 1996. structure study of quercetin by vibrational and electronic spectroscopies combined with semiemperical calculations. *Biospect* **3:**183-193

Fernandez, M.T., Mira, M.L., Flarencio, M.H & Jennings, K.R. 2002. Iron and copper chelation by flavonoids: an electrospray mass spectrometry study. *Journal of Inorganic Biochemistry.* **92**: 105-111

Forkmann, G & Heller, W. 1999. *Biosynthesis of flavonoids* in "*Comprehensive Natural product Chemistry Vol 1.* Sangkawa, U (ed). Oxford: Alsevier pp 714, 716

Gomathi, k., Gopinath, D., Rafiuddin ahmed, M., Jayakumar, R. 3003. Quercetin incorporated collagen matrices for dermal wound healing processes in rat. *Biomaterials* **24:** *2767-2772*

Habsah, M., Amran, M., Mackeen, M.M., Lajis, N.H., Kikuzaki, H., Nakatani, N., Rahman, A.A., Ghafar & Ali, A.M. 2000. Screening of *Zingiberaceae* extracts for antimicrobial and antioxidant activities. *J. Ethnopharm.* **72**: 403-410

Havsteen, B. H. 2000. The biochemistry and medical significance of the flavonoids. *Pharmacology & Therapeutics.* **96**: 67-202

Hanaski, Y., Ogawar, S., Fukui, S. 1994. The correlation between active oxygen scavenging and antioxidative effect of flavonoids. *Free Radical Biology and Medicine* **16:** 845-450

Holttum, R.E. 1950. The *Zingiberaceae* of Malay Peninsular. *The garden' Bulletin Singapore.* **13**: 56-57

Hollman, P. C. H & Katan, M. B. 1999. Dietary flavonoids: Intake, health effects and bioavailability. *Food. Chem. Toxicol.* **37**: 937-942

Hollman, P.C.H., Trijp, J. M. P., Buysman, M.N.C.P., Gaag, M. S. V. d., Mengelers, M.J.B., de Veries, J. H. M., Katan, M. B. 1997. Relativity bioavailability of the antioxidant flavonoid quercetin from various food in man. *FEBS letters* **418:** *152-156*

Hou, L., Zhou, B., Yang, L & Liu, Z.L. 2004. Inhibition of human low density lipoprotein oxidation by flavonol and their glycosides. *Chemistry and Physical of Lipid.* **129**: 209-219

Hristea, E.N., Hillebrand, M., Caproiu, M.T., Caldararu, H., Constantinescu, T & Balaban, T. 2002. Scavenging the hydroxyl radical by 2,2-diphenyl-1-picrylhydrazyl. *ARKIVOC* **II**: 123-132

Hu, H.L., Forsey, R.J., Bladers, T.J., Barratt, Mike E.J., Parmar, P & Powell, J.R. 2000. Antioxidants may contribute in the fight against ageing: an in vitro model. *Mechanisms of Ageing and Development.* **121**: 217-230

Ibrahim, H. 1989. Preliminary survey of gingers at Tabin forest. *Sabah Museum Monograph.* **3**: 57-62

Ibrahim, H., Ong, H.C & Hassan, R. 2000. Ethnobiological survey of the ginger family in selected Malay villages in Peninsular Malaysia. *Malaysia Journal of Science* **19**: 93-99

Ikan, R.1991. *Natural products a laboratory guide.* (2nd edition). London: Acedemic Press

Ismail, A., Marjan, Z.M & Chin, W. F. 2004. Total antioxidant and phenolic content in selected vegetables. *Food Chemistry* **87**: 581-586

Jalil, J., Jantan, I., Husain, K., Jamal, J.A., & Salleh, A.M. 2000. Tumbuhan ubatan untuk merawat reumatisme. Rahmani, M., Mahmood, M., Sukri, M. A., Gwendoline Ee C.L., Taufiq,. Yap, Y.H & Israf Ali, D.A(eds.). *Interdisciplinary Approaches in Natural Product Research* : Proceeding of the 16th National Seminar on Natural Product 24-25 October, 2000. 28-32.

Jovanovic, S.V., Steen, S., Simic, M.G & Hara, Y. 1998. *Antioxidant properties of flavonoids: reduction potentions and electron transfer reaction of flavonoids radicals* In *"Flavonoids in health and disease"* Rice-Evans, C & Parker, L (eds). New York: Marcel Dekker, Inc pp 137-162.

Kitayama, T., Nagao, R., Masuda, T., Hill, R.K., Morita, M., Takatani, M., Sawada, S & Okamoto, T. 2002. The chemistry IV asymmetric synthesis of zerumbol. *Journal of Molecular Catalysis B: Enzymatic.* **17**: 75-79

Larsen, K., Ibrahim, H., Shaw, S.H., & Saw, L.G. 1999. *Gingers of Peninsular Malaysia and Singapore.* Kota Kinabalu: Natural History Publication (Borneo)

Larson, R.A. 1997. *Naturally occurring antioxidants.* New York: Lewis Publisher

Lombard, K., Peffley, E., Geoffriau, E., Thompson, L., Herring, A. 2005. Quercetin in onion (*Allium cepa* L.) after heat-treatment simulating home preparation. *Journal of food composition and analysis* **18:** *571-581*

Lee, L. M & Sirat, H.M. 2000. Kandungan kimia dan bioaktiviti daripada dua spesies *Zingiber.* Rahmani, M., Mahmood, M., Sukri, M. A., Gwendoline Ee C.L., Taufiq,. Yap, Y.H & Israf Ali, D.A(eds.). *Interdisciplinary Approaches in Natural Product Research* : Proceeding of the 16th National Seminar on Natural Product 24-25 October, 2000. 28-32.

Markham, K. R & Bloor, S.J.1998. *Analysis and identification of flavonoids in practice.* In *"Flavonoids in health and disease"* Rice-Evans, C & Parker, L (eds). New York: Marcel Dekker, Inc pp 1-33.

Masuda, T., Jitoe, A & Nakatani, N.1991. Acelated flavonol glycoside from *Z. zerumbet. Journal of Phytochemistry* **30** : 2391-2392

Matthes, H.W.D., Luu, B & Ourrison, G. 1980. Cytotoxic components of *Z. zerumbet, Curcuma zedoria* and *C. domestica. Journal of Phytochemistry.* **19**: 2643-2650

Miliauskas, G., Venskutonis, P.R & Van Beek, T. A. 2004. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chemistry.* **85**: 231-237

Moure, A., Cruz, J.M., Franco, D., Dominiguez, J.M., Sineiro, J., Dominiguez, H., Nunez, M.J & Parajo, J.C. 2001. Natural antioxidants from residual sources. *Journal of Food Chemistry* **72**: 145-171

Owen, R.W., Haubner, R., Hull, W.E., Erban, G., Spiegelhalder, B., Bartsch, H & Harbet, B. 2003. Isolation and structure elucidation of the major individual polyphenol in carob fiber. *Food. Chem. Toxic.* **41**: 1727- 1738

Paganga, G & Rice-Evans, C.A .1997. The identification of flavonoids as glycosides in human plasma. *FEBS Letters* **401**: 78-82

Pavia, D.L., Lampman, G.M & Kriz, G.S. 2001. *Introduction to spectroscopy. A guide for students of organic chemistry.* (3rd edition). USA. Brooks/cole. pp 404

Peitta, P.G. 1998. *Flavonoids in medicinal plants* In *"Flavonoids in health and disease"* Rice-Evans, C & Parker, L (eds). New York: Marcel Dekker, Inc pp 61-110.

Pietta,P.G. 2000. Flavonoids as antioxidants. *J. Nat. Prod.* **63**: 1035-1042

Phan, T.T., Lim, I.J., Sun, L., Chan, S.Y., Bay, B.H., Tan, E.K., Lee, S.T. 2003. Quercetin inhibits fibronectin production by keloid-derive fibroblasts. Implication for the treatment of excessive scars. *Journal of Dermatological Science* **192:** *192-194*

Rawel, H.M., Rohn, S., Kroll, J. 2003. Influence of sugar moiety (rhamosylglucoside) at 3-*O* position on the reactivity of quercetin with whey proteins. *International journal of Biological Macromolecules* **32:** *109-120*

Rice-Evan, C. A, Miller, N.J. & Peganga, G. 1996. Structure-antioxidant activity relationship of flavonoids and phenolic acids. *Free Radical Biology and Medicine.* **7**: 933-956

Rotelli, A. E., Guardia, T., Juarez, A.O., De la Rocha, N.E & Pelzer, L. E. 2003. Comparative study of flavonoids in experimental model of inflammation. *Pharmacological Research.* In www.elsevier.com/locate/yphrs. Printed 22nd March 2004

Satvic, B.1994. Antimutagents and anticarcinogens in food. *Food. Chem. Toxic.* **32**: 79-90.

Steinmetz, K & Potter, J. D.1996. Vegetable, fruits and cancer prevention: A review. *J. Am. Diet. Assoc.* **96**: 1027-1039

Tsao, R & Deng, Z. 2004. Separation procedure for naturally occurring antioxidant phytochemicals. *Journal of chromatography B* **812:** 85-99

Vallenjo, F., Tomas-Barberan, F.A., Ferreres, F. 2004. Characterisation of flavonols in broccoli (*Brassica oleracea* L. var. *italica*) by liquid chromatography-UV diod-array detection-electrospry ionisation mass spectrometry. *Journal of chromatography A* in www.elsevier.com/locate/chroma. Printed 22nd March 2005.

William, C.A & Harborne, J.B. 1988. *The flavonoids advance in research since 1980.* London: Chapman and Hall

William, C.A & Harborne, J. B.1977. The leaf flavonoids of the *Zingiberales. Biochemical Systematic and Ecology.* **5**: 221-229

Wu, W., Yan, C., Li,L., Liu, Z & Liu, S. 2004. Study on the flavones using liquid chromatography-electrospray ionization tandem mass spectrometry. *Journal of chromatography A* **1047:** *213-220*