UNIVERSITI PUTRA MALAYSIA

ANALYSIS OF FLAVONOIDS IN ONCIDIUM TAKA FLOWERS AT VARIOUS STAGES OF DEVELOPMENT

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ANALYSIS OF FLAVONOIDS IN *ONCIDIUM* TAKA FLOWERS AT VARIOUS STAGES OF DEVELOPMENT

By

PUTRI NOOR FAIZAH BT MEGAT MOHD TAHIR

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Chairman: **Associate Professor Abdullah Sipat, PhD**

Faculty: **Biotechnology and Biomolecular Sciences**

Colour is an important factor in determining the aesthetic value, and hence the economic value of flowers. Thus there is active research and development in chemistry of colour pigments especially in flowers and in the breeding of cultivars for preferred colours. Understanding the chemistry of colour pigments, especially the changes in their composition and concentration as the flower develops, is essential as a background for any attempt at genetic manipulation of flower colour. It is important to know which pigments are normally present so that specific steps in their synthesis can be targeted.

In this study the concentration of flavonoids in the flower of the orchid *Oncidium* Taka, as it develops from the bud stage to the mature flower, was determined. Four stages of flower development were arbitrarily selected, viz. the bud stage, partially

open flower, fully opened flower and the mature flower. The flavonoids were extracted using methanol and the extract concentrated to dryness. The separation step was by TLC followed by analysis using HPLC and UV Vis spectrophotometer. The standard compounds for flavonoids were kaempferol, luteolin, naringenin and myricetin.

The HPLC analysis result showed that kaempferol, luteolin and myricetin were detected in at the level of 14.54 ± 3.86, 1.80 ± 0.27 and 3.04 ± 1.51 μg/g FW respectively at the bud stage. Throughout the experiment, kaempferol concentration decreased as the flower developed to maturity (0.12 ± 0.03 μg/g FW). The same trend was also observed for myricetin (0.24 ± 0.11 μg/g FW) at the third stage but increased at the fourth stage. Luteolin on the other hand remained consistent at approximately 1.35 ± 0.44 μg/g FW throughout the development of the flower from the bud to the mature stage. Naringenin could not be detected using HPLC at all stages of flower development.

The qualitative analysis using TLC and UV Vis spectrophotometer also indicated the presence of kaempferol, luteolin and myricetin in *Oncidium* Taka petals extract. Naringenin was only detected using TLC technique with solvent A (chloroform:acetic acid:water (90:45:6 v/v/v)) and solvent C (chloroform:methanol (100:20 v/v)). All the TLC plates were fumed with iodine vapour.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

ANALISIS FLAVONOID DI DALAM *ONCIDIUM* TAKA PADA SETIAP PERINGKAT PERKEMBANGANNYA

Oleh

PUTRI NOOR FAIZAH BT MEGAT MOHD TAHIR

Mac 2006

Pengerusi: Profesor Madya Abdullah Sipat, PhD

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Warna bunga adalah faktor penting yang menentukan nilai estetik dan ekonomi dalam industri bunga keratan. Dengan itu, penyelidikan dan perkembangan aktif dalam bidang kimia pigmen warna dan pemilihan serta pembiakan kultivar untuk warna yang dikehendaki aktif dijalankan. Pengetahuan mengenai kimia pigmen warna terutamanya dari segi perubahan komposisi pigmen warna dan juga kepekatannya di setiap peringkat perkembangan bunga adalah amat penting sebagai asas untuk percubaan memanipulasi genetik warna bunga. Adalah penting juga untuk mengetahui pigmen yang wujud dengan itu langkah yang spesifik untuk sintesis warna berkenaan boleh disasarkan.

Dalam kajian ini, penentuan kepekatan flavonoid di dalam bunga orkid *Oncidium* Taka di setiap peringkat peringkat perkembangannya dari peringkat kudup bunga

hingga ke peringkat matang dikaji. Empat peringkat perkembangan bunga dipilih, iaitu kudup bunga, bunga separuh buka, bunga kembang dan bunga matang. Flavonoid diekstrak dengan menggunakan metanol dan dikeringkan. Langkah pemisahan telah dijalankan dengan menggunakan teknik kromatografi lapisan tipis (TLC) dan sampel dianalisis dengan kromatografi cecair berprestasi tinggi (HPLC) serta UV Vis spektrofotometer. Terdapat empat sebatian flavonoid piawai tulen iaitu kaempferol, luteolin, myricetin dan naringenin.

Keputusan analisis HPLC menunjukkan kaempferol, luteolin dan myricetin telah dapat dikesan pada 14.54 ± 3.86, 1.80 ± 0.27 dan 3.04 ± 1.51μg/g BB, masing-masing di peringkat kudup bunga. Sepanjang ujikaji, kepekatan kaempferol menurun apabila bunga berkembang menjadi matang (0.12 ± 0.03 μg/g BB). Begitu juga dengan myricetin (0.24 ± 0.11 μg/g BB pada peringkat tiga tetapi meningkat pada peringkat empat). Sebaliknya, luteolin menunjukkan kadar kepekatan yang konsisten lebih kurang pada 1.35 ± 0.44 μg/g BB sepanjang perkembangan bunga di peringkat satu hingga empat. Naringenin tidak dapat dikesan dengan menggunakan HPLC pada semua peringkat perkembangan bunga.

Analisis kualitatif dengan menggunakan TLC dan UV Vis spektrofotometer menunjukkan kehadiran kaempferol, luteolin dan myricetin di dalam bunga *Oncidium* Taka. Naringenin hanya dapat dikesan dengan melalui teknik TLC dengan menggunakan pelarut A (kloroform: asid asetik:air (90:45:6 v/v/v)) dan pelarut C

(kloroform: methanol (100:20 v/v)). Semua kepingan TLC didedahkan pada wap iodine.

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I certify that an Examination Committee has met on 3rd March 2006 to conduct the final examination of Putri Noor Faizah Bt Megat Mohd Tahir on her Master of Science thesis entitled "Analysis of Flavonoids in *Oncidium* Taka Flowers at Various Stages of Development " in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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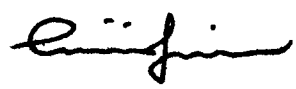

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

PUTRI NOOR FAIZAH BT MEGAT MOHD TAHIR

Date: 18/9/06

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nm	nanometer
A	absorbance
TLC	thin layer chromatography
HPLC	high performance liquid chromatography
NMR	nuclear magnetic resonance
IR	infrared
FW	fresh weight
BB	berat basah
UV Vis	ultraviolet visible
UV	ultraviolet
DFR	dihydroflavanol-4-reductase
CHS	chalcone synthase
CHI	chalcone isomerase
F3H	flavanone 3-hydroxylase
FNS	flavone synthase
FLS	flavonol synthase
ANS	anthocyanidin synthase
F3'5'H	flavonoid 3',5' - hydroxylase
BAW	t- butanol: acetic acid: water
HOAc	acetic acid

TFA	trifluoroacetic acid
MeOH	methanol
PE	petroleum ether
R_f	retention factor

CHAPTER I

INTRODUCTION

The Malaysian floriculture industry has been identified as a dynamic industry and both the Second Industrial Master Plan (IMP2) and the Third National Agriculture Policy recognize orchid as a major floriculture product. Some of the sympodial major types of orchid flowers grown commercially are as *Dendrobium*, *Aranda* and *Oncidium*. The *Oncidium* is a major commercial orchid, having a production value of about RM 80 million per year, mainly for export (Manickam, 2000).

Malaysia has developed fewer orchid varieties for cut flowers than its competitors from Singapore and Thailand. In response to fulfilled market requirements, such as wide colour range blue and solid yellow or to change colour in established varieties for instance yellow *Oncidium* to white which is in higher demand for Japan's market, it is important to do a research on flower colour. Flower colour is an important feature that adds to the aesthetic appeal of ornamental plant species. The increasing knowledge of the flavonoids biosynthesis has made this biosynthetic pathway target for metabolic engineering. Because no species generate the full spectrum of colours, novelty of flower colour is one of the major topics in ornamental plant breeding. Moreover, flower colour is often based on flavonoids compounds, such as flavones, flavonols, carotenoids and anthocyanins. Therefore, the development of strategies to

create missing hues or to manipulate the flower colour in a selected plant species is of special interest.

Flower colour is greatly influenced by a combination of factors such as the type of pigments present in the flower, translocation of such pigments from the site of production, pH and co-pigment. The major pigments responsible for flower colour are the flavonoids, carotenoids and chlorophylls. It is essential to know the existing pigments in the plant before new colour variations can be developed. In this project, only flavonoids are focused upon. Flavonoid such as cyanidin, peonidin, quercetin and myricetin were detected in *Dendrobium* orchid species and hybrids (Kuehnle *et al.,* 1997. Williams *et al.,* 2002 also reported that the flavonoid cyanidin 3- (6-malonylglucoside)–7,3'–(6- sinapylglucoside) and flavonols glycosides (3-rutinoside-7-glucosides of kaempferol and quercetin) were identified in *Dendrobium* cv. Pampadour. Not much research has been done on flavonoids in *Oncidium* species or hybrids. As a group, flavonoids have a strong absorption in the ultraviolet particularly in the region 265-340 nm (Mishio *et al.,* 2006). These pigments are responsible for the orange, scarlet, crimson, mauve and blue colours as well as contributing much to yellow, ivory and cream flower colours. Flavonoids are actually water-soluble pigment (Harborne, 1984).

The objective of this project is to get basic knowledge on the concentration of flavonoids present at various stages of flower development in orchids *Oncidium* Taka.

It is hoped that results obtained will provide a better understanding of the levels of the flavonoid present as the flower develops to maturity. Indirectly this gives an indication of the activity of the key enzyme involved in flavonoid biosynthesis and also the expression of their respective genes.

OBJECTIVES

The objectives of this project are:

1. To determine the presence and concentration of flavonoid from the bud stage to fully opened *Oncidium* Taka flowers.
2. To compare different extraction methods on the yields of flavonoid.
3. To analyse on flavonoids extracted from different stages of flower development by TLC, UV spectrophotometer and HPLC.

CHAPTER II

LITERATURE REVIEW

Orchids and Flower Industry

Orchids form the largest family of flowering plants with more than 800 genera and over 25,000 species that are commercially grown globally (Arditti, 1992). The majority of the exotic, beautiful and aromatic orchid species are native to Malaysia. Peninsular Malaysia, together with Sabah and Sarawak, is home to 3,000 species of the most attractive and beautiful orchids found today. These species occur in their greatest diversity at an altitude of between 1,000 and 6,000 metres above sea level (Arditti, 1992). The flowers of orchids appear to be very complex but they all have the same basic structure. Orchids have three sepals and three petals. Orchids are perennial and herbaceous. Because of the size of this family, the characteristics vary a great deal. The sizes of orchids range from very small to quite large in more tropical climates. The flowers can be solitary, in panicles, racemes, spikes heads or umbels. The flowers are irregular and can be fragrant or odorless. The leaves can be basal, but some plants are void of any leaves. The leaves are most often alternate (Revette, 2000).

Orchids are often bisexual. Orchids have a distinct reproductive feature. Instead of having separate male and female organs, the stamen and pistils are combined into a