PHYTOCHEMICAL STUDIES ON *HYLOCEREUS POLYRHIZUS* (FRUIT AND STEM) AND *HYLOCEREUS UNDATUS* (FRUIT)

by

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LIST OF ABBREVIATIONS

CC	Column chromatography
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
FID	Flame ionization detector
RI	Retention index
RF	Response factor
R_t	Retention time
TLC	Thin-layer chromatography
UV	Ultra-violet
FT-IR	Fourier-transform infrared
NMR	Nuclear magnetic resonance
DEPT	Distortionless enhancement by polarization transfer
COSY	Correlation spectroscopy
HMQC	Heteronuclear multiple quantum correlation
HMBC	Heteronuclear multiple bond correlation
HSQC	Heteronuclear single quantum correlation
m/z	mass/charge
ppm	part per million
mp	melting point
s	singlet
d	doublet
t	triplet
q	quartet
m	multiplet

dd	doublet of doublets
br	broad
eV	electron volts
amu	atomic mass unit

KAJIAN FITOKIMIA TERHADAP *HYLOCEREUS POLYRHIZUS* (BUAH DAN BATANG) DAN *HYLOCEREUS UNDATUS* (BUAH)

ABSTRAK

Sebatian mudah meruap daripada buah *Hylocereus polyrhizus* dan *Hylocereus undatus* telah diasingkan melalui kaedah penyulingan vakum dan dianalisis dengan GC kapilari dan GC-MS dengan menggunakan dua kolum yang mempunyai kekutuban yang berbeza. Sebanyak 57 dan 58 komponen telah dikenalpasti bagi buah daripada setiap spesies, masing-masing yang mewakili 99.2% dan 99.5% daripada hasil pencilan. Profil sebatian mudah meruap bagi buah daripada kedua-dua spesies tersebut agak serupa, masing-masing didominasi oleh alkohol (*H. polyrhizus*, 36.5%; *H. undatus*, 38.6%) dan asid karboksilik (*H. polyrhizus*, 35.1%; *H. undatus*, 22.3%), dengan 1-heksanol dan skualena sebagai dua komponen utama. Sebatian mudah meruap daripada *H. polyrhizus* menunjukkan aktiviti antibakteria yang lebih baik berbanding sebatian mudah meruap yang dipencilkan daripada *H. undatus* dalam asai antibakteria pencairan mikro terhadap lima jenis bakteria (*Bacillus subtilis* subsp. *spizizenii*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas stutzeri* dan *Staphylococcus aureus*). Ini merupakan laporan pertama bagi sebatian mudah meruap dan aktiviti antibakteria bagi buah daripada kedua-dua spesies *Hylocereus* tersebut. Kajian fitokimia terhadap *H. polyrhizus* telah membawa kepada pengasingan and pengenalpastian enam sebatian tidak mudah meruap. *Myo*-inositol (**C1**) telah diasingkan daripada esktrak metanol buah manakala oktatriakont-1-ena (**C2**), lupeol (**C3**), stigmasterol (**C4**), suatu campuran stigmasterol dan *β*-sitosterol (**C5**) dan kuersetin (**C6**) telah dipencilkan daripada esktrak klorofom batang. Struktur

bagi kesemua sebatian tersebut telah dikenalpasti dengan menggunakan teknik spektroskopi seperti IR, DP-MS, 1D-NMR, 2D-NMR dan UV-Vis. Ini merupakan laporan pertama bagi pengasingan sebatian **C2**, **C3**, **C4**, **C5** dan **C6** daripada batang *H. polyrhizus*.

PHYTOCHEMICAL STUDIES ON *HYLOCEREUS POLYRHIZUS* (FRUIT AND STEM) AND *HYLOCEREUS UNDATUS* (FRUIT)

ABSTRACT

The volatile constituents for the fruits of *Hylocereus polyrhizus* and *Hylocereus undatus* were isolated by vacuum distillation and analysed by capillary GC and GC-MS, using two columns of different polarities. Fifty-seven and 58 components were identified in each of the fruits, representing 99.2% and 99.5% of the isolates, respectively. The volatile profiles of both fruits were quite similar, both of which being dominated by alcohols (*H. polyrhizus*, 36.5%; *H. undatus*, 38.6%) and carboxylic acids (*H. polyrhizus*, 35.1%; *H. undatus*, 22.3%), with 1-hexanol and squalene as the two major components. The volatile constituents of *H. polyrhizus* showed stronger antibacterial activity than those of *H. undatus* when tested against five bacteria (*Bacillus subtilis* subsp. *spizizenii*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas stutzeri* and *Staphylococcus aureus*) using the micro-dilution antibacterial assay. This is the first report for the volatile constituents and antibacterial acitivities of the both *Hylocereus* species. The phytochemical investigation on *H. polyrhizus* had led to the isolation and identification of six compounds. *Myo*-inositol (**C1**) was isolated from the methanolic fruit extract while octatriacont-1-ene (**C2**), lupeol (**C3**), stigmasterol (**C4**), a mixture of stigmasterol and *β*-sitosterol (**C5**) and quercetin (**C6**) were isolated from the chloroform extract of the stems. The structures of these compounds were elucidated by spectroscopic techniques such as IR, DP-MS, 1D-NMR, 2D-NMR and UV-Vis. This is the first report of the isolation of **C2**, **C3**, **C4**, **C5** and **C6** from the stems of *H. polyrhizus*.

CHAPTER 1

INTRODUCTION

1.1. Natural products chemistry

Natural products chemistry is a branch of chemistry which deals with the isolation, identification, structure elucidation, and study of the chemical characteristics of chemical compounds or substances produced by plants, animals and other living organisms that are found in nature. The classes of natural product compounds include terpenoids, polyketides, amino acids, peptides, proteins, carbohydrates, lipids, nucleic acid bases, ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and etcetera. Natural products usually show pharmacological or biological activities which may be used in pharmaceutical drug discovery and drug design or as natural food additives and preservatives (Butler, 2004, Reddy et al., 2003, Singh et al., 2010, Spainhour and Gad, 2010).

1.2. The family Cactaceae

The family Cactaceae composed of three tribes (*Pereskieae*, *Opuntieae* and *Cereeae*), consisting about 110 genera and more than 1000 species, commonly found in American countries with temperate and tropical climate. This family comprises fleshly perennials, shrubs, trees or vines. The stems are mostly leafless and very spiny, of which the spines vary in size, form and arrangement. The flowers are solitary, rarely clustered and bisexual. The scaly or spiny Cactaceae fruits are juicy

or dry, sometimes edible. The seeds in the fruits are usually numerous (Wu and Raven, 2007, Britton and Rose, 1963).

1.2.1. The genus *Hylocereus*

Hylocereus, which means “forest-cereus” in Greek, is a genus of cacti belonging to the family of Cactaceae and often known as “night-blooming cactus”. *Hylocereus* are climbing cacti, often epiphytic with elongated stems. Their flowers are very large and mostly white in colour. The edible fruits of this genus, generally known as dragon fruits, are spineless with many foliaceous scales on them (Ariffin et al., 2009). *Hylocereus* comprises of 18 species which are native to the West Indies, Mexico, Central America and northern South America. Most of the species are closely related and have similar stems, flowers and fruits (Britton and Rose, 1963). Some of the *Hylocereus* species are commercially grown in many Asian countries (such as Vietnam, Taiwan and Philippines). *Hylocereus polyrhizus* (Weber) Britton & Rose and *Hylocereus undatus* (Haworth) Britton & Rose are among two of the *Hylocereus* species which are commonly grown in Malaysia.

Of late, the fruits of *Hylocereus* cacti have drawn attention of growers worldwide due to their red-purple colour, economic value as fruit products, and antioxidant activities from their betacyanins contents (Wu et al., 2006).

1.2.1.1. *Hylocereus polyrhizus*

Hylocereus polyrhizus (Plate 1.1), commonly known as the “red-fleshed” dragon fruit or “red-fleshed” pitahaya, has three-angled slender vines, sometimes only 3 to 4 cm in thickness. The vines are green or purplish at first but soon become white and green again thereafter. The ribs or wings of the plant are comparatively thin and becoming more turgid with age. Each areole, bearing two to four spines which are stout and brownish, are 2 to 4 mm long and sometimes accompanied by two white hairs. The young flower buds of *H. polyrhizus*, on the other hand, are globular and purple in colour. The flower is 2.5 to 3 dm or longer and strongly fragrant. The outer perianth-segment of the flower is reddish whilst the inner perianth-segment, nearly white. The fruit of this species usually weighs about 300 g and is oblong, about 10 cm long with red peel. The colour of the flesh varies from red to purplish-red with many tiny black seeds (Britton and Rose, 1963, Janick and Paull, 2008, Lim, 2012a).

Plate 1.1 Clockwise from left: The plant, stems, the languish flower and the unripen fruit of *Hylocereus polyrhizus* (Weber) Britton & Rose.

1.2.1.2. *Hylocereus undatus*

Hylocereus undatus is a climbing cactus which is known as “white-fleshed” dragon fruit or “white-fleshed” pitahaya (Plate 1.2). The stem of this plant is long and often clambering over bushes and trees or even creeping up the sides of walls. The rib is mostly three-winged, broad, thin and green in colour. The areole is 3 to 4 cm with each bearing one to three spines. The spines are small and long, each measuring at about 2 to 4 mm. The flower, on the other hand, is large, measuring up to 29 cm or sometimes longer. The outer perianth-segment of the flower is yellowish green whereas the inner perianth-segment is pure white. The fruit is scarlet and oblong with 10 to 12 cm in diameter, weighing 400 to 600 g. Similar to *H. polyrhizus*, the white

fruit pulp of *H. undatus* has many indigestible tiny black seeds (Britton and Rose, 1963, Janick and Paull, 2008, Lim, 2012b).

Plate 1.2 Clockwise from left: The plant with blossoming flowers, stems, the white and large blossom flower and a ripening fruit of *Hylocereus undatus* (Haworth) Britton & Rose.

1.3. Uses of dragon fruits

According to literature, dragon fruits possess several medicinal properties, thus making them useful folkloric medicines. The dragon fruit is an alternative source of natural antioxidant because it is rich in vitamins and phytoalbumins. Antioxidant is an anti-cancer agent which prevents the formation of cancer-causing free radicals. Furthermore, the dragon fruit is rich in fiber which helps in the digestive process in humans. Hence, it helps to prevent colon cancer and diabetes. In Taiwan, dragon

fruits are used as rice substitution by diabetic patients and a source of dietary fiber (Zainoldin and Baba, 2009, Mohd Adzim Khalili et al., 2009, Mohd Adzim Khalili et al., 2010). Mohd Adzim Khalili et al. (2009) revealed that the fruits of *H. polyrhizus* possessed hypocholesterolemic effect on hypercholesterolemia induced rats. The fruit has the potential to reduce dyslipidemia and helps in the prevention of cardiovascular diseases. Besides, Wu *et al.* (2006) also suggested that the dragon fruit peel of *H. polyrhizus*, an inedible waste product of juice manufacturing, should be regarded as a valuable product that may assist in the prevention of chronic diseases due to its antioxidant and anti-melanoma properties. The stems and flowers of the *H. undatus* were used by Mayas as traditional hypoglycemic, diuretic and cicatrizant agents (Gutiérrez et al., 2007). Research has also revealed that the aqueous extracts of leaves, rind, fruit pulp and flowers of *H. undatus* possess wound healing properties on diabetic rats, thus making it a potential traditional medicine for treating injuries on diabetic patients (Perez G et al., 2005).

Apart from their medicinal properties, the flesh and pulp of dragon fruits have also drawn attention from scientists worldwide as it can be used as a source of natural colorant in food and cosmetic industries (Harivaindaran et al., 2008, Stintzing et al., 2002). The pulps of *H. polyrhizus* and *H. undatus* fruits resemble that of a kiwi fruit. They are juicy, delicately flavoured and nutritionally rich with many tiny, indigestible seeds. Dragon fruits are often chilled, cut into half and eaten raw as in fruit salad. The flesh can be used to make ice-cream, sorbets, marmalades, jellies, ices and soft drinks by mixing it with milk and sugar. In addition to these, they are used in low-temperature dairy drinks and light drinks with other fruit juices and as wine and pastries flavouring (Janick and Paull, 2008, Radha and Mathews, 2007,

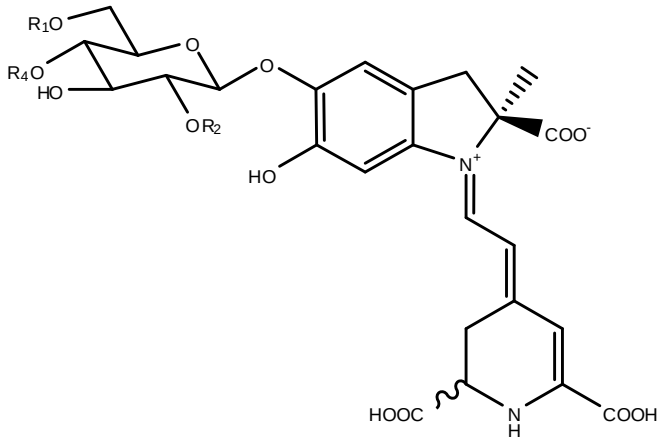
Wybraniec and Mizrahi, 2002). The unopened flower buds, on the other hand, are cooked and eaten as vegetables (Lim, 2012b, Radha and Mathews, 2007). The flowers can also be served together with tea or as vegetables in soup (Gunasena et al., 2004, Lim, 2012b).

1.4. Previous phytochemical studies

1.4.1. *Hylocereus undatus*

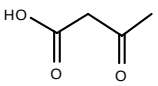
The earliest investigation of *H. undatus* began in 2007 when Wybraniec et al. isolated and identified ten betacyanin pigments from the peel and flesh of *H. undatus* which consisted of betanin (**1**), phyllocactin (**2**), hylocerenin (**3**), 2'-glucosylbetanin (**4**), 2'-*O*-apiosylbetanin (**5**), 4'-malonyl-betanin (**6**), 2'-*O*-apiosylphyllocactin (**7**), 2'-(5"-*O*-*E*-feruloylapiosyl)betanin (**8**), 2'-(5"-*O*-*E*-sinapoylapiosyl)betanin (**9**) and 2'-(5"-*O*-*E*-feruloylapiosyl)phyllocactin (**10**), respectively. Compound **1-10** were found in the peel whilst compounds **1-7** were identified in the flesh. Two betaxanthins, namely *γ*-aminobutyric acid-Bx (**11**) and indicaxanthins (**12**), were also detected in the peel and flesh, respectively. Table 1.1 shows the chemical structures of the betacyanins found in *Hylocereus* cacti.

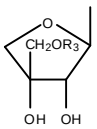
Table 1.1 Chemical structures of betacyanins found in *Hylocereus* cacti

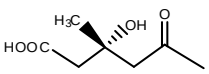


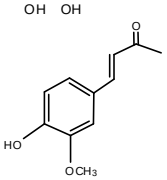
Compound	Substituents			
	R_1	**R_2**	**R_3**	**R_4**
1 and 1'	H	H	-	H
2 and 2'	malonyl	H	-	H
3 and 3'	3-hydroxy-3-methylglutaryl	H	-	H
4	H	glucosyl	-	H
5	H	apiosyl	H	H
6	H	H	-	malonyl
7	malonyl	apiosyl	H	H
8	H	apiosyl	feruroyl	H
9	H	apiosyl	sinapoyl	H
10	malonyl	apiosyl	feruloyl	H

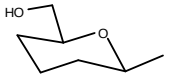
Note:

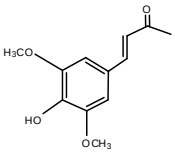
Malonyl: 

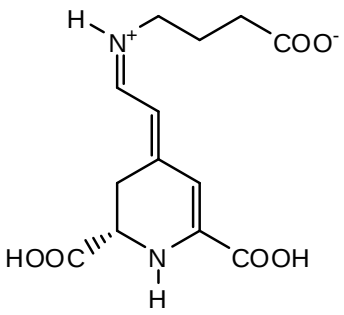
Apiosyl: 

3-Hydroxy-3-methylglutaryl: 

Feruroyl: 

Glucosyl: 

Sinapoyl: 



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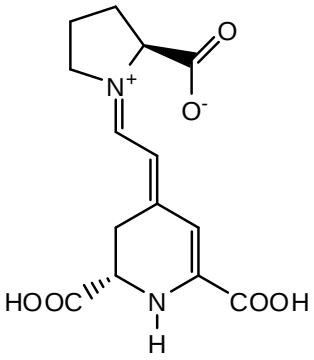
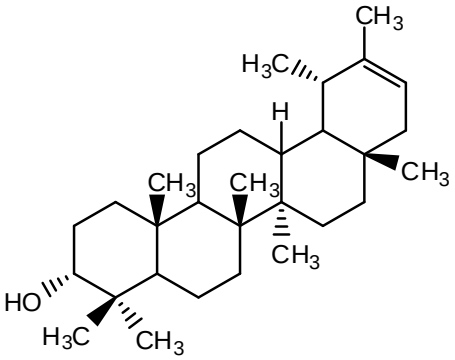


12

Gutiérrez et al. (2007) had isolated and identified two new pentacyclic triterpenes, namely, taraxast-20-ene-3*α*-ol (**13**) and taraxast-12, 20(30)-dien-3α-ol (**14**) from the chloroform extract of the leaves of *H. undatus*.



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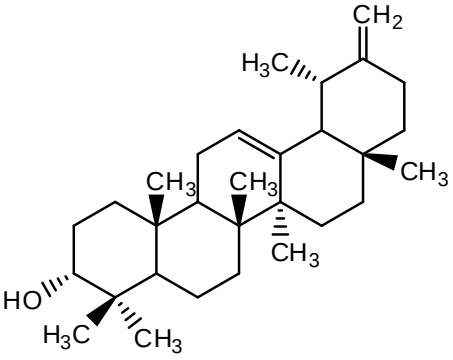
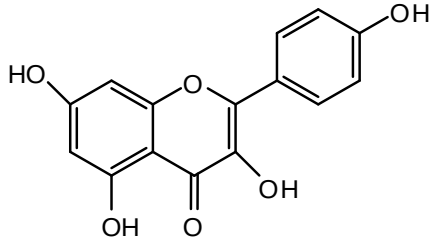


14

Yi et al. (2011) had reported 13 flavonoids from the flowers of *H. undatus* by using HPLC. They are: kaempferol (**15**), quercetin (**16**), isorhamnetin (**17**), kaempferol 3-*O*-*α*-*L*-arabinfuranoside (**18**), kaempferol 3-*O*-*β*-*D*-glucopyranoside (**19**), quercetin 3-*O*-*β*-*D*-glucopyranoside (**20**), isorhamnetin 3-*O*-*β*-*D*-glucopyranoside (**21**), kaempferol 3-*O*-*β*-*D*-galactopyranoside (**22**), quercetin 3-*O*-*β*-*D*-galactopyranoside (**23**), kaempferol 3-*O*-*β*-*D*-rutinoside (**24**), isorhamnetin 3-*O*-*β*-*D*-rutinoside (**25**),

kaempferol 3-*O*-*α*-*L*-rhamopyranosyl-(1→6)-*β*-*D*-galactopyranoside (**26**), and isorhamnetin 3-*O*-*α*-*L*-rhamopyranosyl-(1→6)-*β*-*D*-galactopyranoside (**27**).



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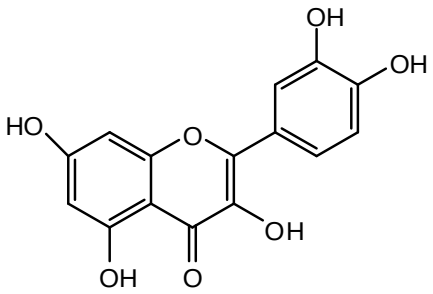

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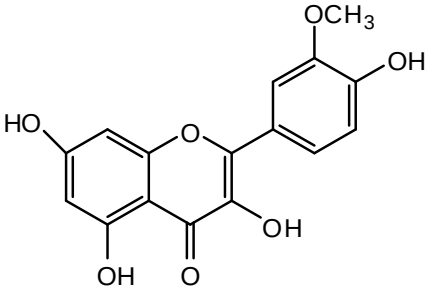

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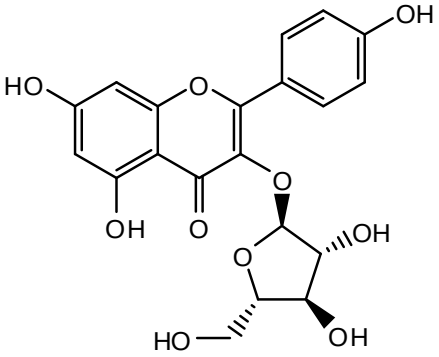

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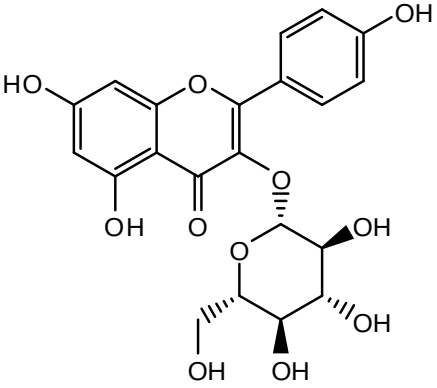

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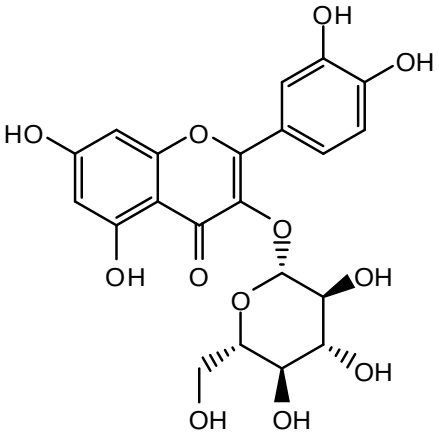

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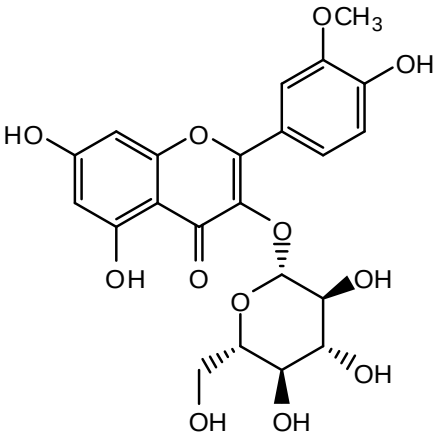
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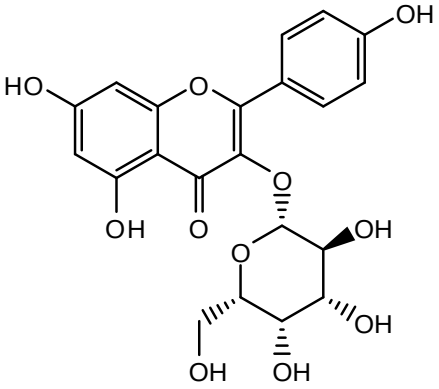
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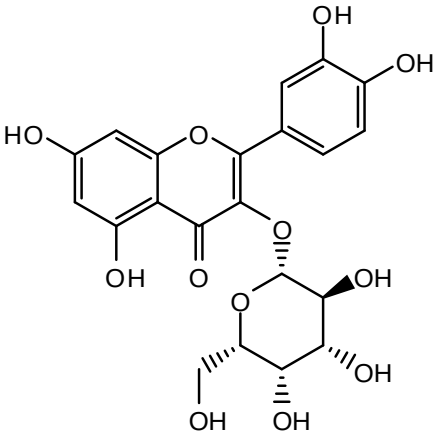
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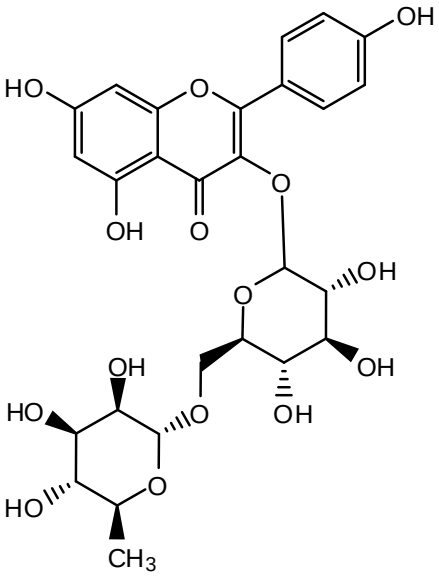
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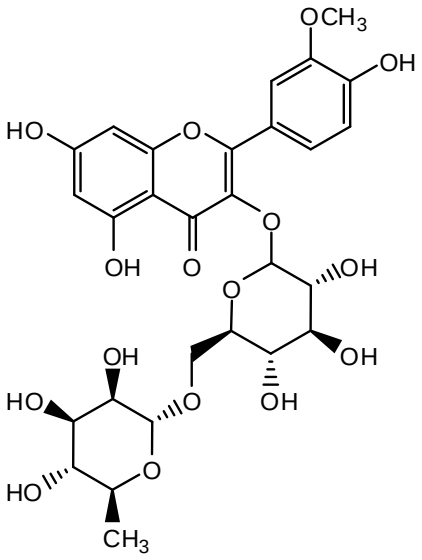

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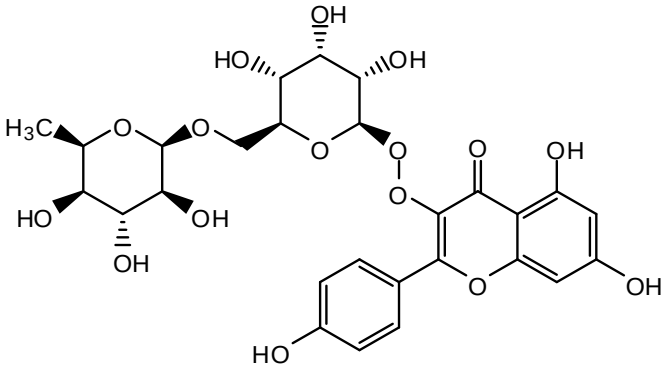

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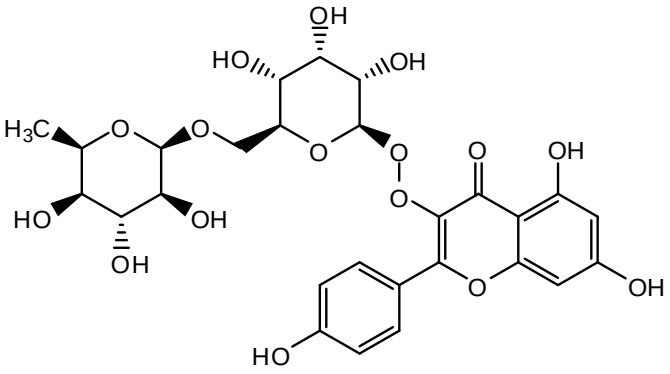
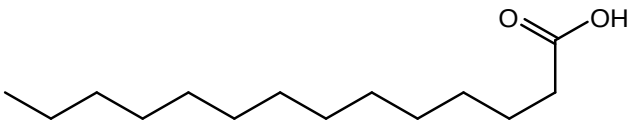


27

On the other hand, Ariffin et al. (2009) had investigated the seed oil extract of *H. undatus* and it was found to contain myristic acid (**28**), 0.30%; palmitic acid (**29**), 17.1%; stearic acid (**30**), 4.37%; palmitoleic acid (**31**), 0.61%; oleic acid (**32**), 23.8%; *cis*-vaccenic acid (**33**), 2.81%; linoleic acid (**34**), 50.1%; and linolenic acid (**35**), 0.98%. Lim et al. (2010) reported that the seed oil of *H. undatus* contained high amount of oil (28.37%) of which linoleic acid, oleic acid and palmitic acid were found to be the three major fatty acids. The tocopherol content in the seed oil was 36.70 mg/100 g. Seven phenolic acids: gallic (**36**), protocatechuic (**37**), *p*-hydroxybenzoic acid (**38**), vanillic acid (**39**), caffeic acid (**40**), syringic acid (**41**) and *p*-coumaric acid (**42**) and four phytosterol compounds: cholesterol (**43**), campesterol (**44**), stigmasterol (**45**) and *β*-sitosterol (**46**) were also identified.



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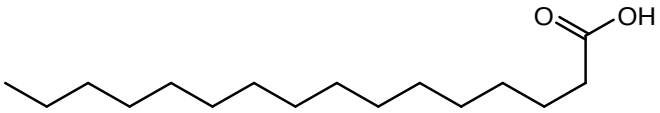

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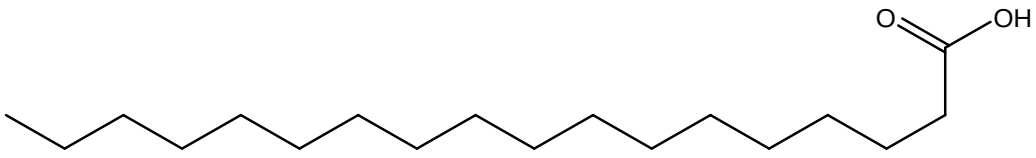

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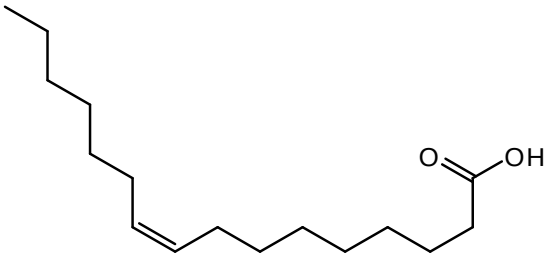

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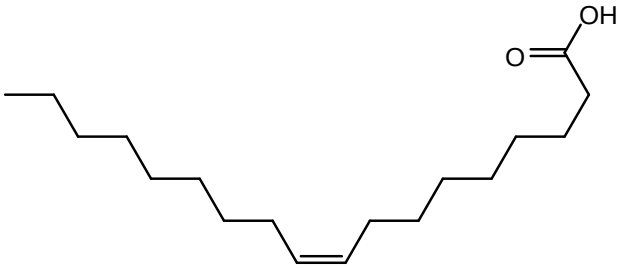

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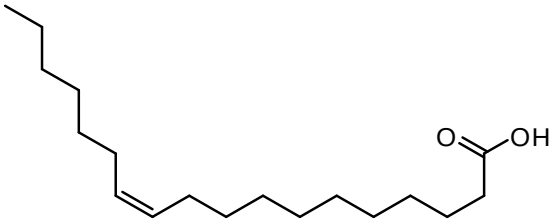

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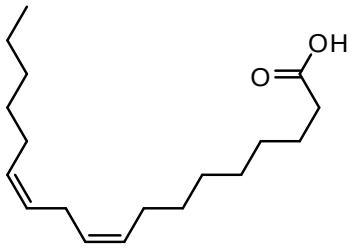

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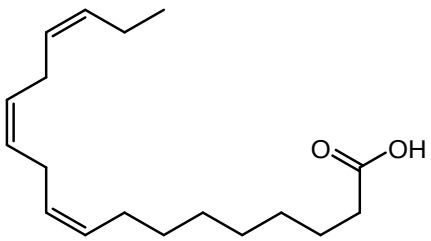

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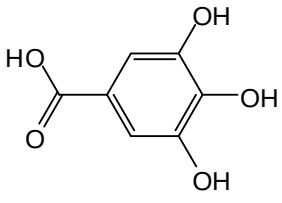

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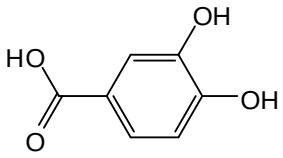

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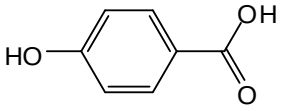

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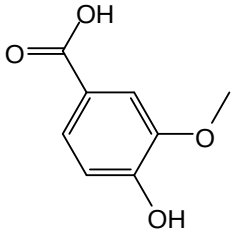

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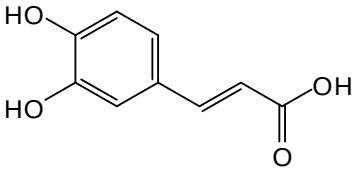

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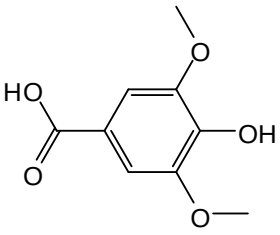

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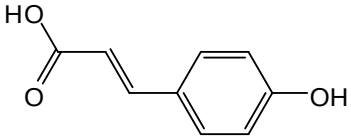

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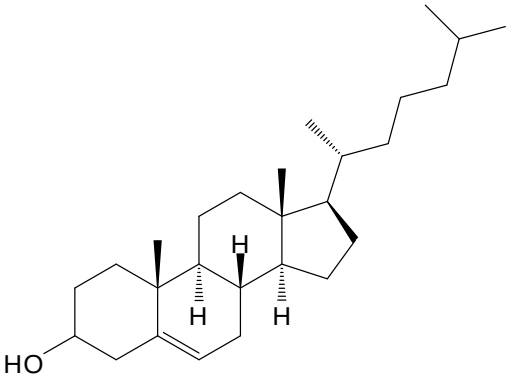

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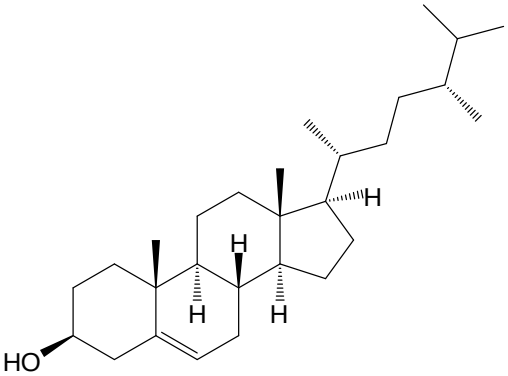

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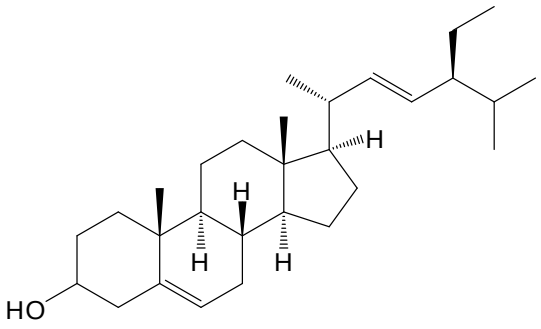

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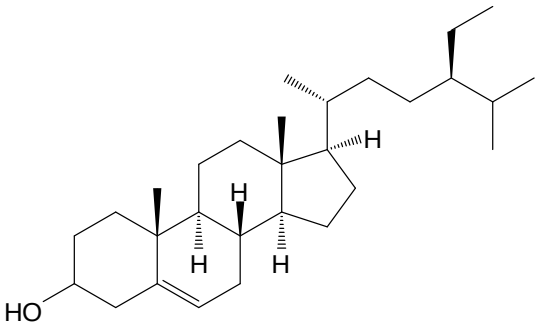

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1.4.2. *Hylocereus polyrhizus*

The earliest phytochemical investigation on *H. polyrhizus* began when Wybraniec et al. (2001) reported betanin (**1**), phyllocactin (**2**) and their 15*R*-isoforms, namely isobetanin (**1'**) and isophyllocactin (**2'**), from the fruit pulp. Hylocerenin (**3**) and its isomer (**3'**) were also identified as new betacyanins in his study.

In another study, Stintzing et al. (2002) had separated ten betacyanins from the aqueous extract of the fruit pulp of *H. polyrhizus* by HPLC. Eight of these betacyanins were identified from their mass spectra using positive ion electrospray mass spectrometry; five of which were bougainvillein-r-I (**4**), betanin (**1**), isobetanin (**1'**), phyllocactin (**2**) and isophyllocactin (**2'**). The remaining betacyanins were tentatively identified as (6'-*O*-3-hydroxy-3-methyl-glutaryl)-betanin, its C15 stereoisomers and (6'-*O*-3-hydroxy-3-butyryl)-betanin.

According to Ariffin et al. (2009), the seed oil extract of *H. polyrhizus* has a similar profile as that of *H. undatus*. The fatty acid composition of *H. polyrhizus* seed oil was reported to be made up of myristic acid (**28**), 0.20%; palmitic acid (**29**), 17.9%;

stearic acid (**30**), 5.49%; palmitoleic acid (**31**), 0.91%; oleic acid (**32**), 21.6%; *cis*-vaccenic acid (**33**), 3.14%; linoleic acid (**34**), 49.6% and linolenic acid (**35**), 1.21%. However, the seed oil of *H. polyrhizus* was found to have a lower oil content (18.33%) but higher in tocopherol content (43.5 mg/100g) as compared to that of *H. undatus*. The phenolic acids and phytosterol compounds identified in the seed oil of *H. polyrhizus* are identical to those found in *H. undatus* (Lim et al., 2010).

1.5 Problem statements

The fruits of *H. undatus and H. polyrhizus* have drawn attention of farmers and scientists worldwide due to their colour pigment and economic value as fruit products. According to literature, the fruits of this species also possess several medicinal properties, thus making them useful folkloric medicines. The dragon fruits are also an alternative source of natural antioxidants because they are rich in vitamins, phytoalbumins and betacyanins. Previously reported phytochemical investigations were found to focus on the non-volatile compositions of the flowers, fruits, seeds and leaves of the two species. However, a literature search reviewed no information about the volatile compositions and the anti-bacterial activities of the volatile extracts of the fruits of *H. undatus and H. polyrhizus*, and the non-volatiles of stems of *H. polyrhizus*.

1.6. Research objectives

The objectives of the present study are:

1. To isolate the volatile constituents from the fruits of *H. polyrhizus* and *H. undatus,* using vacuum distillation.
2. To identify the volatile constituents in the fruits of *H. polyrhizus* and *H. undatus*, by using capillary GC and GC-MS.
3. To evaluate the antibacterial activities of the volatile extracts from the fruits of *H. polyrhizus* and *H. undatus.*
4. To isolate the non-volatile compounds from the stems of *H. polyrhizus*.
5. To characterize the isolated compounds using various spectroscopic techniques such as FT-IR, 1D and 2D NMR, EI-MS and UV.

CHAPTER 2

MATERIALS AND METHODS

2.1. Chemicals and reagents

All chemicals and reagents were used without further purification unless stated otherwise.

1. *Acetone, AR grade (Qrec, Malaysia)
2. Aluminium chloride, $AlCl_3.6H_2O$ (Merck, Germany)
3. Anhydrous sodium acetate (R&M Chemicals, UK)
4. Boric acid (Sigma-Aldirch, USA)
5. Boron trifluoride-methanol, 10% (w/w) (Supelco, USA)
6. *Chloroform, AR grade (Qrec, Malaysia)
7. Chloroform-*d*, with 0.03% (v/v) TMS, 99.8 atom % D stabilized with silver foil (Sigma-Aldrich, USA)
8. Deuterium oxide, 99.9 atom % D (Merck, Germany)
9. *Dichloromethane (Qrec, Malaysia)
10. Diethyl Ether, AR grade (Qrec, Malaysia)
11. Dotriacontane 99% (Sigma-Adrich, USA)
12. *Ethyl Acetate, AR grade (Qrec, Malaysia)
13. Ethyl 6-methylsalicylate (TCI, Japan)
14. *Ethanol 99.7%, AR grade (Qrec, Malaysia)
15. Heptadecane 99% (Sigma-Aldrich, USA)
16. Hydrochloric acid 37% (Fisher Chemicals, UK)
17. *Methanol, AR grade (Qrec, Malaysia)
18. Methanol, HPLC grade (Merck, Germany)

19. Methyl-d_3 alcohol-*d* 99.8 atom % D (Merck, Germany)
20. Mixture of geranyllinalool isomers (TCI, Japan)
21. Nerol 97% (Sigma-Aldrich, USA)
22. Sephadex LH-20 (Sigma-Aldrich, USA)
23. Silica gel 60 for column chromatography, 0.040-0.063 mm and 230-400 mesh ASTM (Merck, Germany)
24. Silica gel 60 F254, pre-coated glass plates 20 cm × 20 cm × 0.5 mm (Merck, Germany)
25. Sulphuric acid 95-98% (Merck, Germany)
26. TLC aluminium sheets, silica gel 60 F254, 20 cm × 20 cm × 0.5 mm (Merck, Germany)

*Solvents were distilled prior to use

2.2. Collection of plant materials

The fruits of *H. polyrhizus* were obtained from a local market whilst those of *H. undatus* were purchased from Tesco hypermarket in Penang. The fruits of both species were originated from Vietnam. The stems of *H. polyrhizus* were collected from the Tropical Fruit Farm at Teluk Bahang, Penang. The plant species were identified by the staff of the Tropical Fruit Farm, Teluk Bahang, and the School of Biological Sciences, Universiti Sains Malaysia. Voucher specimens of these two species have been deposited at the herbarium of Universiti Sains Malaysia (*H. polyrhizus*, #11265; *H. undatus*, #11266, respectively).

2.3 Isolation and analysis of volatile constituents

2.3.1. Isolation of volatile constituents

All fruits were washed with distilled water and their peels were removed. The pulp (flesh and seeds) (3.4 kg) of each of the *Hylocereus* fruits was blended for 1 min then immediately vacuum-distilled (28-30ºC, 0.3 mmHg) for 5 h, yielding approximately 160 mL of distillate for each species. The distillates were collected in two separate liquid nitrogen-cooled traps. Each distillate was extracted five times (5 × 25 mL) with freshly distilled dichloromethane at room temperature. After drying with anhydrous sodium sulphate, the extract was concentrated using a Kuderna-Danish concentrator at a bath temperature of 50ºC, and reduced to a final volume of 0.1 mL under a gentle stream of N_2 gas at room temperature prior to GC and GC-MS analyses. The resultant essence possessed a characteristic aroma of the fruit. The procedures were repeated with a mixture of heptadecane (2.4 mg), dotriacontane (2.3 mg), and nerol (2.0 mg) was added as an internal standard to assess the recovery of the procedure and for the calculation of the absolute amounts of the major identified constituents. The resultant puree was blended for another 30 s, after which it was immediately vacuum-distilled. The isolation of the volatile components of each of the *Hylocereus* fruits was carried out in duplicates. In all cases, the relative standard deviations for each of the major gas chromatographic peak were less than 10%.

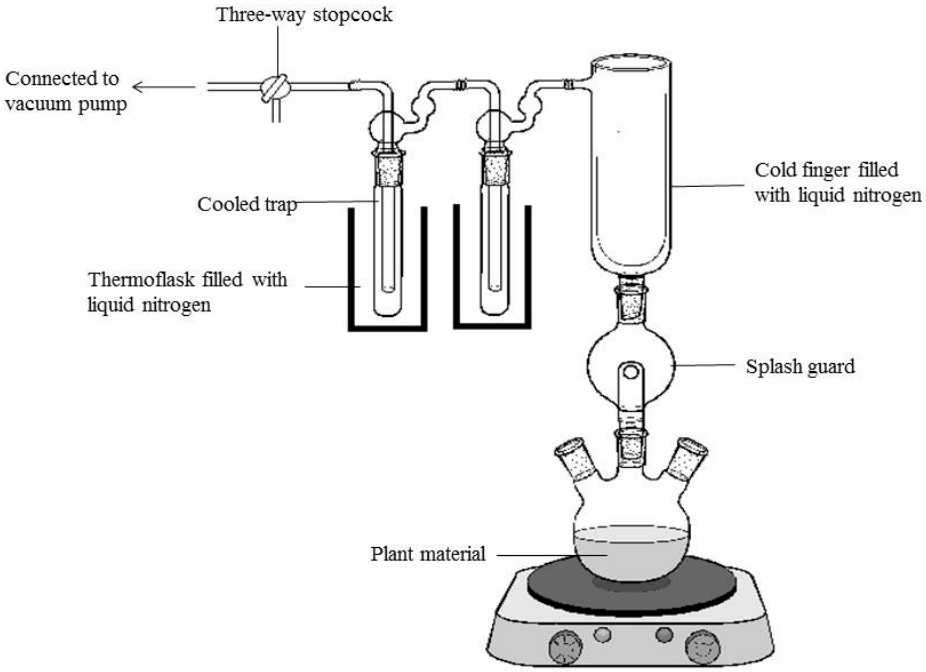

Figure 2.1 Vacuum distillation apparatus

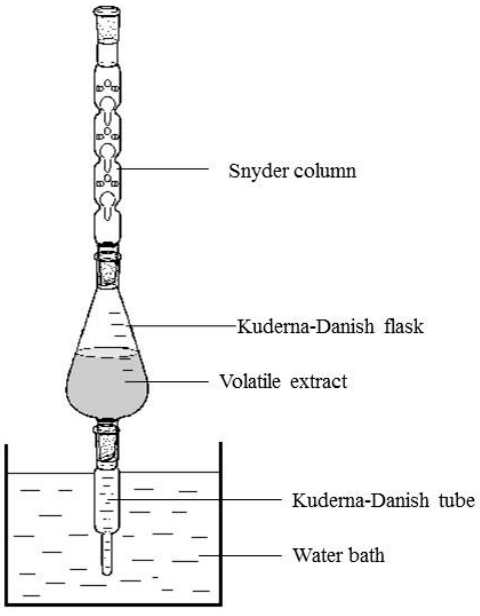

Figure 2.2 Kuderna-Danish concentrator

2.3.2. Chromatographic analysis of volatile constituents

The volatile constituents isolated from the fruits of *H. polyrhizus* and *H. undatus* were analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS).

2.3.2.1. Gas chromatography

GC analyses were carried out using a Thermo Finnigan instrument (San Jose, CA, USA) equipped with a flame ionization detector (FID). Two fused-silica capillary columns were employed: SPB-1 [bonded poly(dimethylsiloxane), 30 m × 0.25 m i.d., film thickness, 0.25 µm; Supelco Inc., USA] and Supelcowax 10 [bonded poly(ethylene glycol), 30 m × 0.25 mm i.d., film thickness, 0.25 µm; Supelco Inc., USA]. The operating conditions of the two columns were: initial oven temperature was at 40ºC for 5 min, then increased to 220ºC at 5ºC min^{-1} and held for 15 min; injector port and detector temperature: 250ºC; carrier gas, 10 mL min^{-1} He; injection volume, 2µL; split ratio, 30:1. Peak areas were determined with a Hitachi D2500 Chromato-Integrator (Tokyo, Japan).

A mixture containing a homologous series of *n*-alkanes ranging from C_5 to C_{32} was injected into the column immediately after each GC analysis of volatiles under identical operating conditions. The hydrocarbons were used as standards in the calculation of the retention indices (RI).

For a temperature-programmed GC, the retention index (RI) of a component in the volatiles was calculated using the following equation (Van Den Dool and Dec. Kratz, 1963):

$$RI = 100i\left[\frac{t-t_{(n)}}{t_{(n+i)}-t_{(n)}}\right] + 100n \quad (1)$$

Under the condition that $t_{(n)} < t < t_{(n+i)}$.

t = retention time of the component of interest

$t_{(n)}$ = retention time of the alkane with *n* carbon atoms which is eluted just before the component of interest

$t_{(n+i)}$ = retention time of the alkane with (*n*+*i*) carbon atoms which is eluted just after the component of interest

i = difference in the number of carbon atoms between two alkanes.

n = the number of carbon atoms in the alkanes.

The RIs obtained based on the calculation using two neighbouring alkanes was found to give best precision. When *i* = 1, equation (1) is simplified to:

$$RI = 100\left[\frac{t-t_{(n)}}{t_{(n+1)}-t_{(n)}}\right] + 100n \quad (2)$$

Hence, equation (2) was used to calculate the RIs of the volatile constituents in the present work.

2.3.2.2. Gas chromatography-mass spectrometry

GC-MS analysis was performed using a Perkin Elmer Clarus 600T (Waltham, MA, USA) equipped with the NIST, MAIN and Wiley Library software. The same capillary columns and GC operating conditions were employed as described in section 2.3.2.1. The temperature of the injection port was set at 250ºC. Significant