Strathclyde Institute of Pharmacy and Biomedical Sciences

PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES OF SOME MEDICINAL PLANTS FROM SOUTH EAST ASIA

KHANITTHA CHAWANANORASEST

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“This thesis is the result of the author’s original research. It has been composed by the author and has not been previously submitted for examination which has led to the award of a degree”.

Signed:

Date:

Dedicated to my parents,

family, husband and son

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Abstract

This thesis presents the isolation and structure elucidation of a range of secondary metabolites from four selected medicinal plants from Southeast Asia; Thailand and Bangladesh, namely, *Cassia tora*, *Piper betel*, *Brugueira gymnorrhiza* and *Avicennia alba*. A Variety of natural products belonging to several classes were isolated and investigated for their biological activity.

The evaluation of *Piper betel* extracts for antimicrobial activity and some second metabolites isolated from *Piper betel* for Antimethicillin-Resistant *Staphylococcus aureus* activity (MRSA) were targeted in this thesis.

A total of eightteen compounds were isolated from the selected plants, including mixtures of two steroids and two sesquiterpenes and two of the compounds were active against MRSA.

Phytochemical investigation of *Cassia tora* leaves resulted in two anthraquinones (physcion and chrysophanol) and a mixture of steroids (*β*-sitosterol and stigmasterol).
Physcion and chrysophanol are reported from the leaves of *Cassia tora* for the first time.
Phytochemical investigation of *Piper betel* leaves led to the isolation of two phenolic compounds (eugenol and 4-allyl pyrocatechol), a mixture of sesquiterpenes (*β*-elemene and trans-calamenene), γ-muurolene and an unidentified cycloartane derivative. Eugenol and 4-allyl pyrocatechol were active against MRSA, *β*-elemene, *trans*-calamenene, γ-muurolene and the unidentified cycloartane derivative are being reported for the first time from the leaves of *Piper betel*.

Phytochemical investigation of *Bruguiera gymnorrhiza* leaves led to six triterpenoids (careaborin and taraxerol, *β*-*Z*-*p*-coumaroyl taraxerol, taraxerone, *β*-lauryl-*β*-amyrin and, 3,4-seco-taraxerol) and one quinone (stenocarpoquinone B). These compounds are being isolated for the first time from the leaves of *Bruguiera gymnorrhiza* and the

Rhizophoraceae family. Phytochemical investigation of *Avicennia alba* stems led to a triterpenoid (betulinic acid) and a steroid (*β*-sitosterol).

Antibacterial activity of isolated compounds was investigated against Antimethicillin-Resistant *Staphylococcus aureus* activity (MRSA). Eugenol and 4-allyl pyrocatechol were active and gave MIC valves, 64 and 128 µg/mL.

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2. Materials and methods

List of Figures

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List of Abbreviations

1D	One Dimensional Nuclear magnetic Resonance Spectroscopy
2D	Two Dimensional Nuclear magnetic Resonance Spectroscopy
3D	Three Dimensional Structure
Acetoned_6	Deuterated acetone
ASE	Accelerated Solvent Extraction
CC	Open Column Chromatography
$CHCl_3$	Chloroform
$CDCl_3$	Deuterated Chloroform
C_5D_5N	Deuterated Pyridine
COSY	Correlation Spectroscopy
DBE	Double bond equivalence
DEPT	Distortionless Enhancement by Polarisation Transfer
DMSO	Dimethyl sulfoxide
DMSOd_6	Deuterated dimethyl sulfoxide
ESI MS	Electrospray Ionisation Mass Spectroscopy
EtOAc	Ethyl acetate
FIC	Fraction Inhibitory Concentration
GF	Gel Filtration
HRCI-MS	High Resolution Chemical Ionisation Mass Spectroscopy
HREI-MS	High Resolution Electron Impact Mass Spectroscopy
HRESI-MS	High Resolution Electrospray Ionisation Mass Spectroscopy
HRFAB-MS	High Resolution Fast Atom Bombardment Mass Spectroscopy
HMBC	Heteronuclear Multiple Bond Coherence
HMQC	Heteronuclear Multiple Quantum Coherence
MeOD	Tetradeuteromethanol
MeOH	Methanol
MIC	Minimum Inhibitory Concentration
NMR	Nuclear Masgnetic Resonance
NOESY	Nuclear Overhauser Enhancement SpectroscopY
ROESY	Rototing Frame Overhauser Experiment Spectroscopy

TLC	Thin layer chromatography
UV	Ultraviolet
VLC	Vacuum Liquid Chromatography

Chapter 1

Introduction

1. Introduction

Phytochemical Study of Medicinal Plants from South East Asia

1.1 Medicinal Plants

Medicinal plants have been used in South East Asia since 5000 B .C. (Joy, P.P. *et al*, 1998). People used the Ayurvedic system as the basic method to remedy illness together with Urani, Siddha, Amchi and Tibetan systems, especially in rural areas where medicinal plants are used for primary health care because they are easy to find, simple to use, and cheaper by comparison with modern medicines. The use of medicinal plants in the Ayurvedic system in Thailand has been transferred from generation to generation and some have kept their medicinal plants as a family secret. There is very little written on the use of medicinal plants in Thailand. The oldest record is the medicinal text of the King of Thailand in 1657-1688. In 1981, the texts recovered, were revised and used as a basic medicinal references nowadays (Farnsworth *et al*, 1992).

Basic principles of Thai medicine are based on the four primary elements of the human body which are earth, water, wind, and fire. The body elements account for 42 elements divided into four groups, 10 of which include 6 elements of wind and 4 of fire while the remaining 32 consist of 20 earth elements and 12 water elements. The wind elements are important for their use with movement and the fire elements have the characteristics of heat. In this system it is believed that diseases are caused by an abnormality of body element systems which may have changed for many reasons. Moreover, Thai medicinal plants comprise of at least two components from plant ingredients and animals such as the Asiatic brush (*Atherurus macrourus*), black softshelled turtle (*Trionyx cartilageneus*), and *Siamese* cobra (Sindhusarn, 2002) which are used to bring the body elements back to normality or equilibrium. The ingredients are related to their major tastes including cold, hot, and astringent. The cold taste is used to bring fire elements back into line; hot and astringent tastes are responsible for the wind and water elements, respectively. Thai traditional plant medicines are divided into two groups which comprise of single and compound ingredient drugs.

1.1.1 Single ingredient medicines

Single drugs are used as remedies for common ailments and they only contain a single ingredient with proven properties, for instance the fruits of *Piper longum* L., the fruit pulp of *Tamarindus indica* L., and the fruit of *Diospyros mollis* Griff are used as a carminative, a laxative, and an intestinal worm remedy, respectively.

1.1.2 Compound ingredient medicines

A variety of medicinal plants are used in some compound traditional medicinal recipes. There are about 25 herbal remedies being used at present and 16 herbs are commonly used as cures for basic ailments. According to UNICEF, there are eight recipes that are recommended for use in rural areas (Punyanubhab *et al*., 1992).

Thai traditional drugs in solid forms include pills, tablets, snuff and suppositories. The liquid forms are used as fluid extracts, alcohol extracts, teas, and expressed juices. Thai people use some of them as snuff, inhalants, and as poultices. (Punyanubham *et al*., 1992).

1.1.3 Thai medicinal plants contribution to modern medicines

Siam cardamoms, gingers, and black pepper are the important Thai medicinal plants as exports of Thailand. Other Thai medicinal plants used for export including Rauwolfia root (*Rauwolfia serpentina)*, Madagascan periwinkle, valerian root, senna pods, and malva nuts. The main countries that import Thai medicinal plants are West Germany, The United States of America, and the United Kingdom. According to the Department of Business Economics, Ministry of Commerce in 1986, there were 7,000 tons of medicinal plants, spices, and related products exported that were important to the income of Thailand. This translated to the value of approximately 400 million Baht. Betel leaves, pepper, ginger, bastard cardamons, rauvolfia root and Siam Cardamoms were exported roughly, 2,500, 2,000, 1,250, 185, 9.7, and 4.6 tons, respectively (Panyanubhab *et al*., 1992). More recent Moreover, there were about 35,000 metric tonnes of medicinal plants and spices exported in 1998 and over 30,000 metric tonnes during 1999 to 2000. The exported were dropped to 25,000 metric tonnes in 2001. The income from export of medicinal plants and spices in

Thailand were over 750, 1,050, and approximately 775 million baht in 1998, 1999-2000, and 2001, respectively (Changtragoon, 2007).

The most popular of Thai medicinal plant, namely *Curcuma longa* Linn., has been used to cure hookworm infestation (Baimai *et al*., 1998). Thai people use *Croton subtyratus* Karz to treat peptic ulcers. At present, this plant is known to contain plannotol which is used commercially by the Sankyo, Co. (Saralamp *et al*., 1996). In recent years, Thai scientists purified curcumin from *Curcuma longa* Linn. It was developed and used as the main ingredient for skin improvements in cosmetic products used on a commercial scale.

1.2 *Cassia tora* L.

Scientific name	*Cassia tora* L.
Family	Leguminosae or Fabaceae
Synonym	*Senna tora*
English name	Foetid cassia, Sickle senna
Vernacular names	Chum- Hed-Thai (Thai), Dau giau, thao quyet minh, muong ngu (Vietnamese), dangwe (Burmese), chueh ming, tsao chueh (Chinese), gelenggang kecil (Malay), ayudham and 10 others names (Sawnskrit), petite casse puante (French) (Wiart, 2002),

Fig1.1 *Cassia tora* image from the website http://www.home.hiroshima-u.ac.jp

1.2.1 Botany

Cassia tora L. is widely distributed in Asian countries and is a well-known herb in India. It is a foetid annual herb reaching about 30-90 cm. in height. The leaves are pinnate and approximately 10 cm long. The leaflets are in 3 pairs which are opposite to each other, obovate, oblong and base oblique.

The flowers are found in pairs in the axils of leaves, have five petals and are pale yellow in colour. The fruit are pods that are obliquely separate. The seeds are long roughly 30-50 cm. rhombohedral. The flowering time of *Cassia tora* L. is after the monsoon (Oudhia *et al*., 2007).

1.2.2 Habitat

C. tora L. is found commonly on the roadsides and wasteland. It tolerates soil with fairly low fertility or moisture level.

1.2.3 Traditional uses

In the Ayurvedic system of medicine, *C. tora* has a great reputation in all kinds of ringworms (Chatterjee *et al*., 1992). The leaves and seeds are a valuable remedy in skin diseases, mainly for ringworm and itch (Kirtikar *et al*., 1975).Various biological and pharmacological activities including antihepatotoxic activity (Wong, 1989), antimutagenic (Choi *et al*., 1998), radical scavenging (Choi *et al*., 1994), antimicrobial (Hatano *et al*., 1999) and antifungal (Kim *et al*., 2004) have been attributed to it.

1.2.4 Medicinal uses

The dried seeds of *C. tora* L. are used as a tonic for eyes in China and Japan. The decoction is applied for conjunctivitis and gonorrhea. The drugs were used for eye treatments, lung diseases, and arthritis remedies many years ago. At present,

C. tora L. is used as a mild laxative. In addition, this plant is used as a laxative and anthelmintic in Indo-China, Malay Peninsula, and the Philippines. The leaves are used to treat a skin rash in Indonesia and also used to smear on the head of restless children in the Malay Peninsula. Moreover, leaf decoctions of *C. tora* L. are used not only to treat skin diseases, but also to relieve vomiting and stomach ache (Perry *et*

al., 1980).*C. tora* L. is used in China as the traditional medicine to remove "heat" from liver, improve visual acuity, and it is used as a laxative. In modern medicines *C. tora* L. is used for hypercholesterolemia and hypertension treatments. A water extract of this plant is used to treat vaginitis by washing the vulva and vagina (Kee, 1999).

1.2.5 Previous Biological Investigation

The seed extract of *Cassia tora* showed displayed strong protease inhibitory activity against *Aspergillus flavus* and *Aspergillus bacillus*. The seed protein fraction 30-60% displayed the most active for trypsin inhibition in caseinolytic assay. This dicated its using as phytoprotecting agent (Tripathi, V.R. *et al*, 2011). The ethanolic extract of *Cassia tora* leaves exhibited strong antioxidant activities in three *in vitro* assays, total antioxidant capacity, DPPH free radical scavenging and ferric ion reducing assays (Ashwini, P. *et al*, 2011). The alcoholic and aqueous extracts from the seeds of *Cassia tora* exhibited significant anthelmintic activity at 100 mg/mL (Deore, S., *et al*, 2009). The methanolic leaf extract induced a marked concentration dependent inhibition on proliferation, reduced DNA content and hypercholesterolemia are not clear (Rejiya, C., S., *et al*, 2009). A cDNA encoding chalone synthase was cloned from the tender leaves by RT-PCR and RACE to analyse chalcone synthase gene of *Cassia tora* (Liao, H., *et al*, 2008). The methanol extract from the raw and roasted seeds exhibited anti-angiotensin converting enzyme activity more than 50% at a concentration of 163.93 µg/mL and an anthraquinone glycoside demonstrated angiotensin-converting enzyme (ACE) inhibitor with IC_{50} value of 30.24±0.20µM (Hyun, S., K., *et al*, 2009). The n-butanol extract from the seeds showed a beneficial effect on postprandial blood glucose levels and insulin secretion in diabetic rats (Nam, J., *et al*, 2008). The water extract of the pulverized seeds (containing emodin, aloe-emodin, chrysophanol, physcion, aurantioobtusin and their glycosides and rubrofusarin or its glycosides) caused no diarrhea when orally administered to mice (Nishikawa, M., *et al*, 2008). The aqueous extract of of *Cassia tora*, *Momordica charan* and *Calendula officinalis* found having effectiveness against bacteria *Staphyllococcus aureus* (Roopashree, T.S., *et al*, 2008). Nine anthraquinones, aurantio-obtusin, chryso-obtusin, obtusin, chryso-obtusin-2-O-β-D-glucoside,

physcion, emodin, chrysophanol, obtusifolin and obtusifolin-2- O-β-D-glucoside were isolated from the ethylacetate extract of the seeds. Emodin and obtusifolin exhibited a significant inhibitory activity on advanced glycation end products (AGEs) with IC_{50} values of 118 and 28.9 μM, respectively whereas aurantio-obtusin, chryso-obtusin-2-O-β-D-glucoside and emodin showed a significant inhibitory activity on rat lens aldose reductase (RLAR) with IC_{50} values of 13.6, 8.8 and 15.9 μm, respectively (Jang, D., S., *et al*, 2007).

The dealcoholised extract of leaves of *C. tora* L. inhibited the growth of *Candida albicans, Aspergillus niger, Saccharomyces cerevisae* and *Tricophyton mentagophytes*. (Mukherjee *et al*., 1996).Anthraquinones isolated from the seeds had strong fungicidal activity against *Botrytis cineria, Erysiphe graminis, Phytophthora infestans, Puccinia recondida, Pyricularia grisen and Rhizoctonia solani*.(Kim *et al*., 2004) The extract of *C.tora* has completely inhibited conidial germination of *Phyllactinia corylea* (Maji *et al*., 2005).

C. tora L. has been found to lower cholesterol in plasma and prevent atherosclerotic plaque formation in arterial walls (Kee, 1992) and its seeds lower the serum levels of lipids (Patil, 2004). It also acts as an antihypertensive, antibacterial, and laxative. *C. tora* L. has gastrointestinal disturbances effects for example nausea, abdominal distension, and loose bowel movements (Kee, 1999). The seeds and the raw seeds of *C. tora* L. are eaten to relieve the bowels of costiveness in Malaysia, Vietnam, and the Philippines. The roasted seeds are used to assuage headache and bowels of costiveness. In addition, it is used to control the excess urination and treat cough symptoms, insomnia, ophthalmic, ocular congestion, and low blood pressure. The seeds macerated with alcohol and vinegar is applied externally for eczema and mycosis remedies. The expressed gum from the seeds is used as an emulsifying, suspending and binding agent for pharmaceutical technology (Joshi, 1964).

1.2.6 Previous phytochemical investigations

Phytochemical investigations on seeds of *C. tora* L. have resulted in the isolation of several anthraquinone and naphthopyrone derivatives (Wang *et al*., 1989; Kim *et al*., 2004). Three naphthalene glycosides were isolated from the seeds (Choi *et al*., 1995). A water-soluble complex polysaccharide was isolated from the defatted seeds (Varshney *et al*., 1973). Thirteen phenolic glycosides have been isolated and characterised from the seeds (Hatano *et al*., 1999), while emodin was isolated from the leaves (Pal *et al*., 1977). (Table 1.1-1.5)

Table1.1 Chemical constituents isolated from *Cassia tora* L.

Substances	Part Used	Extract	References
Chrysophanol (T1)	Seeds	-	Tabata, 1975
	Seeds	Methanol	Choi, 1997, 1995
	Seeds	Benzene	Poethke, 1968
Chrysophanol diglucoside (T2)	Seeds	Methanol	Poethke, 1968
Chrysophanol triglucoside (T3)	Seeds	Methanol	Poethke, 1968
Emodin (T4)	Seeds	-	Tabata, 1975
	Seeds	Benzene	Poethke, 1968
	Seeds	-	Hatano, 1999
	Seeds	Chloroform	Yong-Mi, 2004
	Seeds	Methanol	Choi, 1995
	Seeds	-	Koshloka, 1978
Physcion (T5)	Seeds	-	Tabata, 1975
	Seeds	Chloroform	Yong –Mi, 2004
	Seeds	Methanol	Choi, 1995
	Seeds	Benzene	Poethke, 1968
Physcion digluside	Seeds	Methanol	Poethke, 1968
Aloe-emodin (T7)	Seeds	-	Hatano, 1999
	Seeds	Chloroform	Yong –Mi, 2004
	Seeds	-	Koshloka, 1978
	Seeds	Benzene	Poethke, 1968
	Leaves	-	Maity, 2001
Aloe-emodin glycoside (T8)	Seeds	Methanol	Poethke, 1968
Chrysophanic acid-9-anthrone (T9)	Seeds	Aqeous	Acharya, 1975
Naphthopyrone glycosides (T10)	Seeds	-	Wong, 1989
	Seeds	Methanol	Choi, 1995
	Seeds	-	Lee, 1997

Table1.2 Chemical constituents isolated from *Cassia tora* L. (continued)

Substances	**Part Used**	**Extract**	**References**
Chryso-obtusin (T11)	Seeds	Methanol	Choi , 1997
	Seeds	-	Choi, 1996
	Seeds	Methanol	Choi, 1995
Cassiaside (T12)	Seeds	Methanol	Choi, 1997
	Seeds	Methanol	Choi,1995
	Seeds	Methanol	Choi,1994
	Seeds	Methanol	Lee, 2006
Rubrofusarin gentiobioside (T13)	Seeds	Methanol	Choi, 1997
	Seeds	Methanol	Choi, 1995
	Seeds	Methanol	Choi,1994
	Seeds	Methanol	Lee, 2006
Rubrofusarin triglucoside (T14)	Seeds	-	Hatano, 1999
Rubrofusarin glucoside (T15)	-	-	Keneda, 1969
Nor rubofuzarin gentiobioside (T16)	Seeds	-	Hatano, 1999
Nor rubofuzarin glucose (T17)	-	Methanol	Choi, 1995
Anthraquinone (T18)	Seeds	Methanol	Choi, 1994
Anthrarufin (T19)	Seeds	-	Lee, 2003
Anthraflavin (T20)	Seeds	-	Lee, 2003
Aurantio obtusin (T21)	Seeds	Methanol	Choi, 1997
	Seeds	Methanol	Choi, 1995
Obtusin (T22)	Seeds	-	Choi, 1996
2-Glycosyl obtusifolin (T23)	Seeds	-	Choi, 1996
Rhein (T24)	Seeds	Chloroform	Yong-Mi, 2004
	Seeds	-	Hatano, 1999
	Seeds	Benzene	Poethke, 1968
Demethylflavasperone gentiobioside (T25)	Seeds	-	Hatano, 1999

Table1.3 Chemical constituents isolated from *Cassia tora* L. (continued)

Substances	Part Used	Extract	References
Torachrysone (T26)	Seeds	-	Hatano, 1999
	-	-	Shibato, 1969
Tarachrysone gentiobioside (T27)	Seeds	-	Hatano, 1999
Toralactone-9-O-β-D-gentiobioside (T28)	Seeds	Butanol	Lee, 2006
Torachrysone tetraglucoside (T29)	Seeds	-	Hatano, 1999
Torachrysone apioglucoside (T30)	Seeds	-	Hatano, 1999
Toralactone (T31)	Seeds	-	Hatano, 1999
Alaternin (T32)	Seeds	Methanol	Choi, 1995
	Seeds	-	Lee, 1997
	Seeds	-	Choi, 1995
Alaternin-2-O-β-D-glucopyranoside (T33)	Seeds	Methanol	Choi, 1995
Adenoside (T34)	Seeds	-	Lee, 1997
Quinizarin (T35)	Seeds	-	Lee, 2003
Sitosterol (T36)	Seeds	Methanol	Choi, 1995
D-galactose (T37)	Seeds	1% acetic acid	Varsey, 1973
D-glucose (T38)	Seeds	1% acetic acid	Varsey, 1973
D-mannose (T39)	Seeds	1% acetic acid	Varsey, 1973
D-Xylose (T40)	Seeds	1% acetic acid	Varsey, 1973

Table1.4 Chemical constituents isolated from *Cassia tora* L. (continued)

Substances	**Part Used**	**Extract**	**References**
8-O-methylchrysophanol (T41)	Seed	-	Jia, Z.-B., 2009
1-O-methylemodin (T42)	Seed	-	Jia, Z.-B., 2009
1, 2-dimethoxy-8-hydroxy-3-methyl-9, 10-anthraquinone (T43)	Seed	-	Jia, Z.-B., 2009
Questin (T44)	Seed	-	Hyun, 2009
2-Hydroxyemodin 1-methylether (T45)	Seed	-	Hyun, 2009
5,7,3'-trihydroxy-4'-methoxyflavone-7-O-α-L-rhamnopyranosyl-(1→3)-O-β-D-xylopyranoside (T46)	Seed	-	Yadava, 2008
(Z, Z)-9, 12-octadecadienoic acid (T47)	Seed	-	Zhang, Y., 2007
Oleic acid (T48)	Seed	-	Zhang, Y., 2007
N-Hexadecanoic acid (T49)	Seed	-	Zhang, Y., 2007
(E)-9-octadecenoic acid (T50)	Seed	-	Zhang, Y., 2007
Octadecanoic acid (T51)	Seed	-	Zhang, Y., 2007

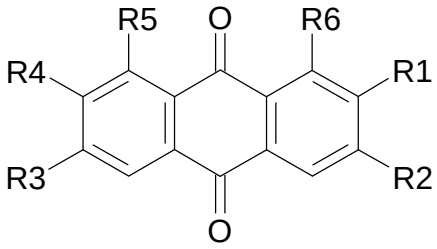
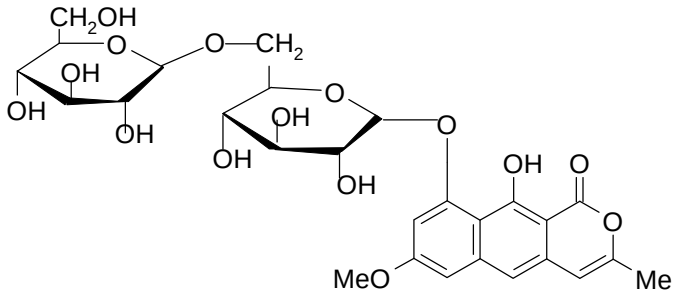


Fig.1.2 Anthraquinone derivatives isolated from *Cassia tora* L.

Table1.5 Anthraquinone derivatives isolated from *Cassia tora* L.

Compounds	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**
T1	H	Me	H	H	OH	OH
T4	H	Me	OH	H	OH	OH
T5	H	Me	OMe	H	OH	OH
T7	H	CH_2OH	H	H	OH	OH
T11	OMe	Me	OMe	OMe	OMe	OMe
T18	H	H	H	H	H	H
T24	H	COOH	H	H	OH	OH



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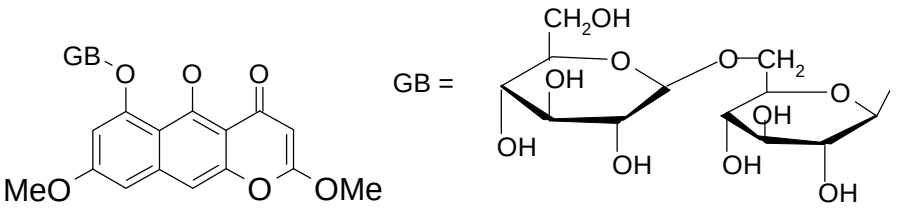

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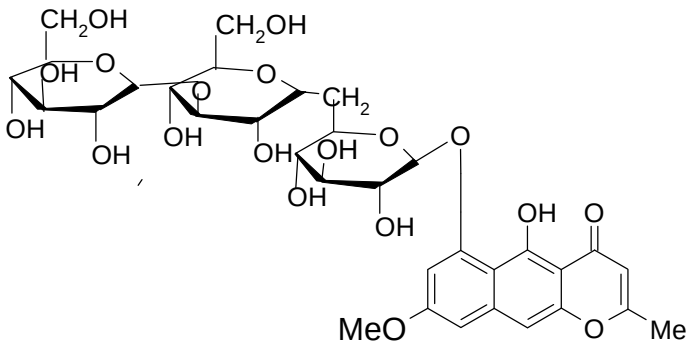
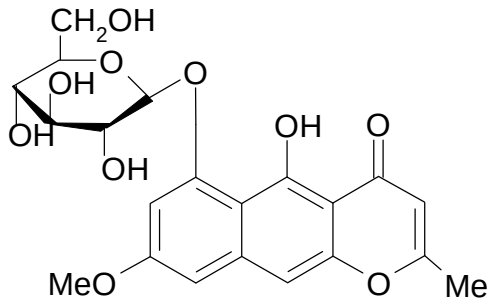
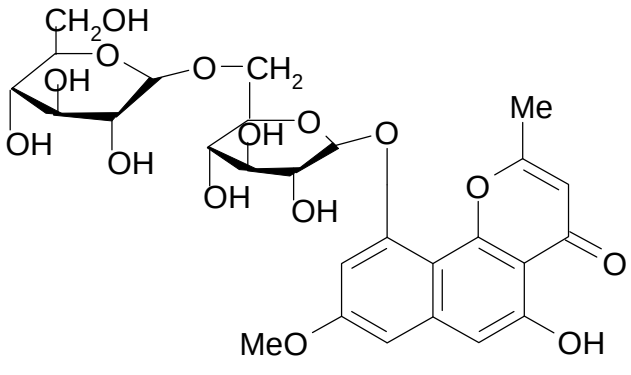


(T14)

Fig. 1.3 Naphthopyrone derivative and glycosides isolated from *Cassia tora* L.

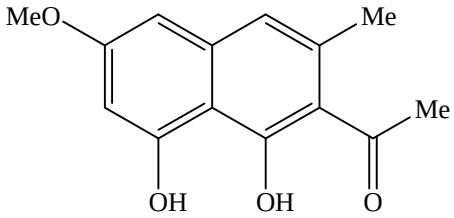


(T15)

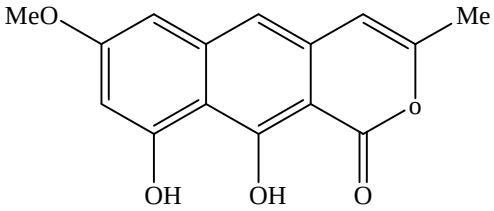


(T25)

Fig.1.4 Naphthopyrone glycosides isolated from *Cassia tora* L.



(T26)



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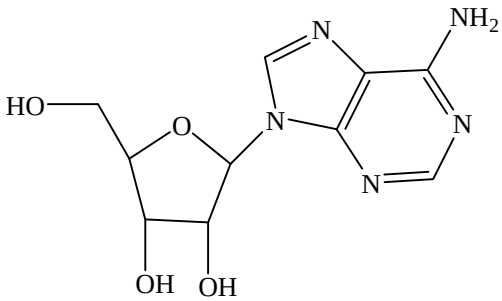
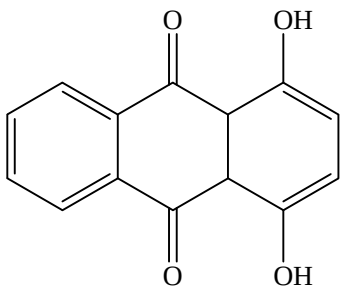


(T34)



(T35)

Fig.1.5 Miscellaneous compounds isolated from *Cassia tora* L.

1.3 The Leguminosae family

The Leguminosae or Fabaceae is a very large family of herbs, shrubs and trees with a great variety of habitat, including aquatics, xerophytes and climbers. Many species are of enormous importance to human communities.

1.3.1 Distribution

There are approximately 700 genera and 17,000 species that have a cosmopolitan distribution in tropical, subtropical and temperate zones. Only Papilionaeae that is one of the three subgroups is extensive in temperate regions (Zomlefer, 1994).

1.3.2 Characteristics of the family

The Leguminosae are trees, shrubs, woody vines, and annual or perennial herbs. The leaves are usually alternate and compound such as bipinnate, simply pinnate, or palmate, rarely simple. Flowers are zygomorphic and the adaxial petal overlap to lateral petals. Stamens are 10 and the gynaeceum comprises of 1 carpel which is formed a single-celled ovary (Wiart, 2002). Fruits are characteristically as pods which are dehiscent or indehiscent (Allen *et al*., 1981).

1.3.3 Significance

A number of plants in this family are useful products, for example *Pisum sativum* L. (peas), *Arachis hypogaea* L. (ground nuts), *Glycine max* (L.) Merr. (soya beans), and *Indigofera tinctoria* L. (indigo). Thailand, *Pueraria mirifica* has rejuvenating properties, hormone-like activity according to their phytoestrogenic compounds such as deoxymiroestrol, miroestrol, daidzein, genistein, kwakhurin, puerarine and isoflavonoids. This plant is used in commercial products in Thailand for breast enlargement and body firming (Chukeatirote, E. and Saisavoey, T., 2009). In Burma, people used the roots of *Abrus precatorius* L. to alleviate cough and adulterate liquorice. *Alysicarpus vaginalis* (L.) DC. is used to alleviate cough, treat mumps in Malaysia and it is used to promote digestion in Taiwan. Moreover, the bark of *Erythrina subumbrans* (Hassk.) Merr. is used to stop vomiting and alleviate coughs in Malaysia. The roots of this plant are used to as diuretic in Vietnam. In India, the roots of *Pongamia pinnata* (L.) Merr. are used to sooth inflammation and counteract

putrefaction (Wiart, 2002). In the Philippines, the leaves of *Indigofera tinctoria* L. contain a numbers of peptides which exhibit antimicrobial and antifungal properties *in vitro* (Daho *et al*., 1999).

1.3.4 The subfamilies

According to Bentham, the Leguminosae is treated as a vast family, clearly natural, and divided into three subfamilies, Papilionaceae, Caesalpinieae, and Mimoseae following by their characters. These subfamilies are sometimes used separately as a single family, the Leguminosae (Fabaceae)-Caesalpinioideae, Mimosoideae and Faboideae (Harborone *et al*., 1971).

1.3.5 Characteristics of the subfamilies

1.3.5.1 Mimoseae: There are trees, shrubs, woody vines and a few perennial herbs. The leaves are pinnate or bipinnate. Flowers are regular and crowded into globose heads or cylindric spikes. Fruits are pods which are straight, curved or spirally twisted and they are usually dehiscent.

1.3.5.2 Caesalpinieae: There are trees, shrubs, rarely scandent and herbs. The leaves are pinnate or bipinnate. Flowers are irregular and they are usually in showy racemes. Fruits are pods which are indehiscent and some with winged sutures.

1.3.5.3 Papilioideae: There are trees, shrubs, and herbs. The leaves are palmate or pinnate, but they are not bipinnate. Flowers are very irregular. Fruits are pods with straight, curved, and winged or moniliform. The fruits are usually 2-valved and dehiscent.

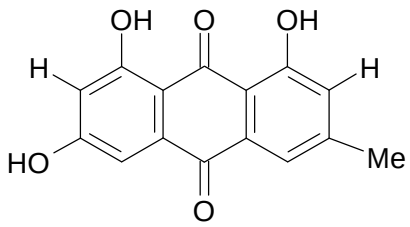
1.3.6 Economic uses

The subfamily Mimoseae has a variety of species yielding high value products, for example the Australian black wattle (*Acacia decurrens*) and golden wattle (*Acacia pycnantha*) which yield wattle bark used in tanning. In addition, the white-flowering and spinose *Acacia senegal* gives a slightly water-soluble sticky substance known as Gum Arabic used in textile, sweet, liqueur and pharmaceutical industries. The oil of *Acacia farresiana* is used as a ingredient for perfumes (Wit *et al*., 1963). *Cassia* species in the subfamily Caesalpinieae are useful in various ways; for instance the dried leaves of *Cassia acutifolia* and *Cassia angustifolia* are used as the source of

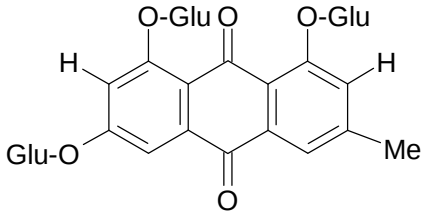
the purgative senna. Several spices in these subfamilies are the sources of dyes, resins, and timbers. Moreover, the pods of tamarind (*Tamarindus indica*) are used for fruits and medicines in India. The seeds and pods of several species of Papilionaceae are the sources of human and animal foods which are rich in proteins and minerals for example *Pisum sativum* L. (Nisar, M. *et al*, 2008; Zomlefer, 1994).

1.3.7 The Genus *Cassia*

Cassia is the fourth largest genus of the Leguminosae family and the largest in subfamily Caesalpinieae as well as the 25 largest genera of dicotyledonous plants. *Cassia* is divided into 3 subgenera namely, *Fistula*, *Senna*, and *Lasiorhegma* (Allen *et al*., 1981). Besides, *Cassia* is the large genus with 400 species. A number of species are ornamentals, for example *Cassia alata* from tropical America and *Cassia echenultiana angustifolia* from Southern Asia. A variety of species are important medicinal plants such as *Cassia* and *Cassia acutifolia*. In addition, *Cassia* wood is used to make posts, beams, and as fuel. There is about 20 percent of tannin yielding from *Cassia auriculata* (Wit *et al*., 1969). People in tropical countries used the decoction of plants in this genus to treat ringworm, eye diseases, rheumatism and other ailments. Moreover, the main laxative property in this genus is emodin, **$C_{15}H_{10}O_5$**, and its related glycosides (Allen *et al*., 1981) as shown below.



Emodin



Emodin glycoside

1.4 *Piper betel* Linn.

Scientific name *Piper betel* Linn.

Family Piperaceae

Synonym Chavica betel

English names Betel, Betel pepper, Betelvine, Betel vine

Vernacular names Plu (Thai), Vetrilai (Tamil), Vettila (Malayalam), Tanbul (Arabic), Ju jiang (Chinese), Pan (Hindu), Bakik serasa (Malay), Betel, Poivrier betel (French), Betelpfeffer (German) (Barlow *et al.*, 2008)

Fig 1.6 *Piper betel* images, taken by Colonel Worachet Chawananorasest

1.4.1 Botany

It is a climber shrub with swollen nodes. The leaves are heart-shaped 15 cm. long (Robertson *et al*., 1989) and 3-11 cm. wide, cordate or obliquely rounded at the base with 5-7 nerves. The flowers are dioecious or monoecious. The berry is green and 1-1.5 cm. in diameter (Li *et al*., 1976). The dioecious spikes have 0.25-0.50 foot long on pedunde which one opposite to the upper leaves. There are two stamens and four to five stigmas. The fruits, globse sessible, are the size of a small pea. The root is found around lower nodes (Baker *et al*., 1877).

1.4.2 Habitat

It is indigenous throughout the Indian Malay region and cultivated in Madagascar, Bourbon, and West Indies. (Grieve *et al*., 1974) This plant is also widespread in tropical Asia, cultivated in Mauritius and the Seychelles (Baker *et al*., 1877). It is widely grown in Thailand.

1.4.3 Traditional uses

The leaf, mixed with areca nut and lime, is used as a stimulant masticatory (Rimando *et al*., 1989) and to preserve teeth (Watt *et al*., 1962). The leaves are used as a mouth freshener, for their wound healing property, and to stimulate digestive and pancreatic enzymes (Bhattacharya *et al*., 2007). The leaf is used as a carminative, aphrodisiac, tonic, laxative and to improve the appetite (Ghosh *et al*., 2005).The fresh crushed leaves are used externally to cure cuts and wounds and as a poultice for boils in the Philippines (Rimando *et al*., 1989). The fresh leaf is used as an antiflatulent in Thailand (Chaveerach *et al*., 2006).The leaves and the sap are used to cleanse wounds (Perry *et al*., 1980).The roots are used for treating rheumatism (Thanh *et al*., 1996/1997). The plant has been used to remedy “gravel” and as a diuretic (Watt *et al*., 1962). The leaf extracts are antibacterial which benefits to treat purulent parodontosis. A poultice of the leaves and a decoction has been used to remedy wounds, burns, impetigo furunculosis, eczema and lymphangitis. The decoction of this plant was found to be an effective long-lasting oral contraceptive (Thanh *et al*., 1996/1997). *Piper betel* is still in use from its fresh leaves for the healing property and chewing in the combination of areca nut and lime wrapped in betel leaf, called

"betel quid" as a stimulant masticatory in Thailand, especially in the rural areas. It is one of the most important and popular medicinal plants in Thailand. It has been consumed by 200-600 million betel quid chewers around the world (Chang, M., C., *et al*, 2007).

1.4.4 Previous Biological Investigation

The extracts of *Piper betel*, *Catharanthus roseus*, *Dendropthoe petandra* and *Curcuma mangga* Val. exhibited T47D cell proliferation and showed DPPH scavenging activity antioxidant activity (Widowati, W., *et al*, 2011). The petroleum ether extract displayed highest antioxidant activity followed respectively by ethyl acetate methanol and aqueous extracts. None of the extracts showed the best results for their antihaemolytic activity (Chakraborty, D and Shah, B., 2011). Allylpyrocatecol and chavibetol have shown protection against photosensitization-mediated lipid peroxidation of rat liver mitochondria, allylpyrocatechol was found to be more potent than chavibetol (Mula, S., *et al*, 2008). The extracts from *Piper betel* leaf extract exhibited antioxidant activity (Arambewela, L., *et al*, 2006). Allylpyrocatechol, chavibetol and the ethanol extract showed antioxidant activity; allylpyrocatechol exhibited the best results in all the in *vitro* experiments (Rathee, J., *et al*, 2006). The ethanol extract of *Piper betel* leaf showed anti-oxidant activity against erythrocytes from patients with HbE-beta thalassemia (Srimani, P., *et al*, 2009). The water, methanol ethyl acetate and petroleum ether of *Piper betel* leaves were evaluated for their antioxidant (Fathilah, A.R., 2011). The leaves showed antioxidant properties (Thanh *et al*., 1996/1997).

The essential oil with fifty two volatile constituents was screened for antibacterial activity against one Gram positive, two Gram negative bacteria and yeast (Kumar, R., *et al*, 2009). The leaves extracted with ethanol had a potent antibacterial action against *Staphylococcus aureus* and *Streptococcus sp.* (Savaspun *et al*., 2003). The essential oil showed antimicrobial activity against a wide range of micro-organisms. Eugenol, chavibetol acetate, 4-allylphenyl acetate and 4-allylphenol were its main components. The ethanol extract showed a healing property effect against indomethacin-induced stomach ulceration in rats (Bhattacharya, S., *et al*, 2007). The

water, methanol ethyl acetate and petroleum ether of *Piper betel* leaves were tested against four different bacteria (*Streptococcus pyogenes, Staphylococcus aureus, Proteus vulgaris and Escherichia coli*) and antihaemolytic activities. The aqueous extracts of *Piper betel* and *Psidium guajava* exhibited antimicrobial activities with their MIC values in the range of 2.61 to 4.69 mg/mL (Fathilah, A.R., 2011). The aqueous extract exhibited maximum inhibition against *Staphylococcus aureus,* ethyl acetate extract found its inhibition against *Escherichia coli* ether extract was active against *Proteus vulgaris*. Safrole, isolated from *Piper betel* inflorescences showed bactericidal activity and released superoxide anions by polymorphonuclear leukocytes. Hydroxychavicol had an effect on phagocytic activity, the intracellular production of reactive oxygen species and the activity of the lysosomal enzyme myeloperoxidase (Chang, L.-Y., *et al*, 2009). Hydroxychavicol isolated from the aqueous extract of *Piper betel* leaf was active against oral cavity pathogens (MICs in the range of 62.5 to 500 µg/mL), it showed potent antioxidant and anti-inflammatory activity (Sharma, S., *et al*, 2009). This plant exhibited showed antimicrobial activity (Rimando *et al*., 1986). The essential oil had a bactericidal effect which can be used to treat the nose affections of the mucous membrane of and throat (Perry *et al*., 1980). The aqueous and methanol extracts of *Piper betel* leaf and other two plants were evaluated against antimicrobial activity against ten Gram positive, twelve Gram negative bacteria. Only the methanol extract of *Piper betel* exhibited the most active against *Steptococcus species*. None of active compounds have been isolated as yet (Nair, R., *et al*, 2008). The *Piper betel* extract showed moderate acivity against *Bacillus cereus* and no active principles were investigated. (Vaghasiya, Y., *et al*, 2007). The leaves showed antibacterial properties (Thanh *et al*., 1996/1997)

The ethanol extract of *Piper betel* leaf showed anti-inflammatory activity possibly mediated by allylpyrocatechol (Sarkar, D., *et al*, 2008). The leaf extract showed gastrocytoprotective properties. Its leaf extract has been investigated for anti-inflammatory activity (Bhattachaya *et al*., 2007). Allylpyrocatechol showed significant lowering of pro-inflammatory (Th1) cytokine levels in arthritic paw tissue at doses levels of 2 and 4 mg/Kg p.o. and enhanced the production of anti-inflammatory (Th2) cytokines IL-4 and IL-5 by cytometric bead array immunoassay

(Pandey, A., *et al*, 2010). Hydroxycharvical showed ulcer healing activity compared to omeprazole (Yadove, S., *et al*, 2008). The *Piper betel* extract showed pro-inflammatory potency. The active principles were not investigated. (Vaghasiya, Y., *et al*, 2007).

The various extracts of eight medicinal plants inclding *Piper betel* showed antifungal activity against plant pathogen (Prince, L. and Prabakaran, P., 2011). The aqueous leaf extract of *Piper betel* exhibited antifungal activity against 124 strains of selected fungi with MIC in the range of 15.62 to 500 µg/mL for yeasts, 125 to 500 µg/mL for *Aspergillus species* and 7.81 to 62.5 µg/mL for dermatophytes (Ali, I., *et al*, 2010). The aqueous extract from the leaves had antifungal activity against *Phaeoisariopsis personata* and *Puccinia Arachidis* in *vitro* (Krishna *et al*., 2005). The leaf extract showed gastrocytoprotective properties. Its leaf extract has been investigated for antifungal activity (Bhattachaya *et al*., 2007). The methanolic and aqueous ectracts of *Piper betel* exhibited strong activity against *Candida albicans* and *Malassezia pachydermatis* (found in skin of animals). The leaves extracted with ethanol had a potent antifungal activity against *Trichophyton mentagrophytes* (Savaspun *et al*., 2003). The essential oil and the leaf extracts also had antifungal activity against *Aspergillus niger*, *Aspergillus oryzae*, *Curvularia lunata*, and *Fusarium oxysporum* (Duke, 1929). The chloroform extract from fresh frozen leaves has investigated for antifungal activity against *Cladosporium cucumerinum* (Evans *et al*., 1984). The ethanol extract of this plant inhibited fungal pathogen of plant (Mohamed *et al*., 1996). This plant showed fungicidal activity (Rimando *et al*., 1986). The dried powder of the leaves showed antifungal activity against the fungal pathogen causing Chalkbrood disease in honey bee larvae (Chantawanakul *et al*., abstract). The leaves showed antifungal activity against a fungal pathogen of rice (Tewari *et al*., 1984). The extract also acted as a protective agent in the early phase of wound healing by increasing total protein content and wound contraction rate in rats (Keat, E., C., *et al*, 2010). The aqueous and methanol extracts of *Piper betel* leaf and other two plants were evaluated against *Candida tropicalis*. Only the methanol extract of *Piper betel* exhibited the most active against *Candida tropicalis*, (Nair, R., *et al*, 2008).

The ethanol and essential oil extract of *Piper betel* leaf possess antihistaminic activity on isolated guinea pig tracheal chain preparation (Hajare, R., *et al*, 2011). The extract of *Piper betel* leaves showed potent xanthine oxidase inhibition, hydroxychavicol was isolated as an active principle (Murata, K., *et al*, 2009). The ethanol extract fro *Piper betel* accelerated intestinal transit in mice up to 90% at 800mg/Kg (Dhaked, P., S., *et al*, 2010). The hexane and chloroform fractions exhibited activity against human lymphatic filarid *Brugia malayi* and triggered an immune response in mice (Singh, M., *et al*, 2009). The methanolic extract from two landrances of *Piper betel* showed its pro-apoptotic effect on *Leishmania donovani*, possibly attributable to its high content in eugenol (Mira, P., *et al*, 2009). The expectorant effect found from the leaves was used for coughs, asthma and bronchitis (Chaveerach *et al*., 2006).

In addition, this plant showed hypotensive, cardiac and respiratory depressant effects, smooth and skeletal muscles relaxant action and nematocidal activity (Rimando *et al*., 1986). Hydroxychavicol isolated from *Piper betel* leaf inhibited platelet aggregation and it could be the agent to prevent and treatment of atherosclerosis, other cardiovascular through its anti-inflammatory and antiplatelet effects on hemostatic functions (Chang, M., C., *et al*, 2007). Its essential oil showed hypotensive, cardiac and respiratory effects (Thanh *et al*., 1996/1997). The alcoholic extract of the leaf-stalk has significant antifertility effects in both male and female rats (Ghosh *et al*., 2005). The methanol extract containing phenols, flavonoids, tannins and polysaccharides was active to various immune disorders including autoimmune disorders (Kanjwani, D., G., *et al*, 2008). The extracts of the leaf was investigated for inhibitory activities against HIV-1 intergrase with 76.32±0.68 % (chloroform extract), 49.86±4.02 % (methanol extract) and 36.43±4.07 % (water extract) of inhibitions (Tewtrakul *et al*., 2006). The ethanol extract of *Piper betel* leaf exhibited cytotoxicity activity in the brine shrimp (*Astemia salina* Linnaeus) assay. Chavibetol and allylpyrocatechol were the active compounds with LC_{50} values 2.55 and 16.36 µg/mL at 24 h., respectively (Koocharoenpisal *et al*., 1997). Hydroxychavicol showed biotransformation and cytotoxic effects in isolated rat hepatocytes (Nakagawa, Y., *et al*, 2009). The methanol extract of *Piper betel* leaf

investigated for antimalarial activity against *Plasmodium berhei* infections. The extract demonstrated significant schizonticidal activity in three antimalarial evaluation models and showed toxicologically safe (Al-Adhroey, A.H., *et al*, 2011).

1.4.5 Previous phytochemical Investigations

Simple phenolic derivatives (Table 1.6-1.9), neolignans (Table 1.10), phytosterols (Table 1.11), alkaloids (Table 1.12), miscellaneous (Table 1.13) were found from *Piper betel* L.

Table1.6 Simple phenolic derivatives isolated from *Piper betel* Linn.

Compounds	Part used	Solvent	References
Chavibetol (PB1)	Leaves/essential oil	-	Grieve, 1974
(Iso-eugenol)	Leaves /essential oil	-	Watt, 1962
	Leaves	Ethanol	Bhattacharya, 2007
	Leaves/Essential oil	-	Rimand, 1986
	Leaves	-	Duke, 1929
	Leaves	Ethanol	Bhattacharya, 2005
	Fresh frozen leaves	Chloroform	Evans, 1984
	Leaves	Ethanol	Koocharoenpisal,1997
		Water/EtOAc	Vadhanasin, 2007
	Flowers/Essentail oil	-	Hwang, 1992
Chavibetol acetate (PB2)			
	Leaves/Essential oil	-	Rimand, 1986
	Fresh frozen leaves	Chloroform	Evans, 1984
Chavicol (PB3)	Leaves/essential oil	-	Grieve, 1974
	Essential oil	-	Perry, 1980
	Leaves	-	Duke, 1929
	Leaves	-	Duke, 1929
	Fresh frozen leaves	Chloroform	Evans, 1984
Methyl chavibetol (PB4)			
	Leaves/Essential oil	-	Rimand, 1986
Allylpyrocatechol (Hydroxychavicol) (PB5)			
	Leaves/essential oil	-	Watt, 1962
	Leaves/Essential oil	-	Rimand, 1986

Table1.7 Simple phenolic derivatives isolated from *Piper betel* Linn. (continued)

Compounds	Part used	Solvent	References
Allylpyrocatechol (PB5)	Leaves	-	Ramji, 2002
	Water/EtOAc		Vadhanasin, 2007
	Fresh frozen leaves	$CHCl_3$	Evans, 1984
			Bhattacharya, 2007
	Inflorescences	-	Lee-Chan, 1996
	Leaves	-	Chans, 2007
	Inflorescences	-	Tang, 2004
	Leaves & Inflorescences	-	Jeng, 2004
	Flower/Essentail oil	-	Hwang, 1992
4-Allylpyrocatechol(PB5)	Leaves Ethanol	-	Bhattacharya, 2007
Allylpyrocatechol diacetate (PB6)			
	Leaves/Essential oil	-	Rimand, 1986
Allylpyrocatechol monoacetate (PB7)			
	Leaves/Essential oil	-	Rimand, 1986
Allylcatechol(PB8)	Leaves	-	Ghosh, 2005
	Leaves	-	Duke, 1929
4-Allyl resorcinol (PB9)	Roots	Alcohol	Ghosh, 2005

Table1.8 Simple phenolic derivatives isolated from *Piper betel* Linn. (continued)

Compounds	Part used	Solvent	References
Eugenol (PB10)	Essential oil	-	Thanh, 1996/1997
	Inflorescence	-	Chen, 1995
	Flower/Essentail oil	-	Hwang, 1992
Eugenol methyl ether (PB11)	Leaves	-	Ghosh, 2005
	Leaves	-	Duke, 1929
Eugenol methyl ester (PB12)	Flower/Essentail oil	-	Hwang, 1992
Safrole (PB13)	Leaves/Essential oil	-	Rimand, 1986
		-	Wong, 2007
	Inflorescence	-	Chen, 1995
	Flower/Essentail oil	-	Hwang, 1992
Estragol (PB14)	Leaves	-	Duke, 1929
Eugenol diacetate (PB15)	Leaves	-	Duke, 1929
Eugenol acetate (PB16)	Leaves	-	Kumar, R., 2009

Table1.9 Simple phenolic derivatives isolated from *Piper betel* Linn. (continued)

Compounds	Part used	Solvent	References
Gallic acid (PB17)	Leaves & Roots	-	Lavania, 2006
Chlorogenic acid (PB18)	Leaves & Roots	-	Lavania, 2006
Caffeic acid (PB19)	Leaves & Roots	-	Lavania, 2006
Ferulic acid (PB20)	Leaves & Roots	-	Lavania, 2006
Terpene (PB21)	Essential oil	-	Perry, 1980
Menthone (PB22)	Essential oil	-	Perry, 1980
P-Cymene (PB23)	Leaves	-	Ghosh, 2005
Myrcene (PB24)	Flowers/Essentail oil	-	Hwang, 1992
Adiene (PB25)	Leaves	-	Duke, 1929
Carvacrol (PB26)	Leaves	-	Duke, 1929
Cineole (PB27)	Leaves	-	Duke, 1929
Cadinene (PB28)	Leaves/essential oil	-	Grieve, 1974
	Leaves	-	Ghosh, 2005
	Essential oil	-	Perry, 1980
Carotenoids (PB29)	Leaves/essential oil	-	Watt, 1962
Vitamin A (PB30)	Leaves/essential oil	-	Watt, 1962
Caryophyllene (PB31)	Leaves	-	Ghosh, 2005
	Essential oil	-	Perry, 1980

Table 1.10 Neolignans isolated from *Piper betel* Linn.

Compounds	Part used	Solvent	References
Piperbetol (PB32)	-	-	Zeng, 1997
Methylpiperbetol (PB33)	-	-	Zeng, 1997
Piperol A (PB34)	-	-	Zeng, 1997
Piperol B (PB35)	-	-	Zeng, 1997

Table 1.11 Phytosterols isolated from *Piper betel* Linn.

Compounds	Part used	Solvent	References
Campesterol (PB36)	Leaves	Ethanol	Koocharoenpisal, 1997
Stigmasterol (PB37)	Leaves	Ethanol	Koocharoenpisal, 1997
β-sitosterol (PB38)	Leaves	Ethanol	Koocharoenpisal, 1997
	Leaves	Petrol.	Parmar, 1998
	Stems	Pet. ether+CH_2Cl_2	Parmar, 1998
	Roots	Pet. ether+CH_2Cl_2	Parmar, 1998
β-sitosterol palmitate (PB39)	Roots	Pet. ether+CH_2Cl_2	Parmar, 1998
Stigmast-4-en- 3, 6-dione (PB40)			
	Roots	Pet. ether	Ghosh, 2005

Table 1.12 Alkaloids isolated from *Piper betel* Linn.

Compounds	Part used	Solvent	References
Piperine (PB41)	Stem	Pet. ether+CH_2Cl_2	Parmar, 1998
Piperlonguminine (PB42)	Stem	Pet. ether+CH_2Cl_2	Parmar, 1998
Cepharadione A (PB43)	Leaves	Pet. ether extract	Parmar, 1998

Table 1.13 Miscellaneous compounds isolated from *Piper betel* Linn.

Compounds	Part used	Solvent	References
Pyrocatechin (PB44)	Leaves	-	Duke, 1929
Protocatechuic (PB45)	Leaves & Roots	-	Lavania, 2006
γ-lactam (PB46)	Leaves	-	Ghosh, 2005
Aristolactam A-II (PB47)	Roots	Alcohol	Ghosh, 2005
Ellagic acid (PB48)	Leaves & Roots	-	Lavania, 2006
Dotriacontanoic acid (PB49)	Leaves	Pet. ether	Parmar, 1998
Tritriacontane (PB50)	Leaves	Pet. ether	Parmar, 1998
Stearic acid (PB51)	Leaves	Pet. ether	Parmar, 1998
	Leaves	-	Duke, 1929
Ceramide (PB52)	Stems	-	Huang, 2010
Germacrene-D (PB53)	Leaves	-	Kumar, 2009
Sabinene (PB54)	Leaves	-	Kumar, 2009

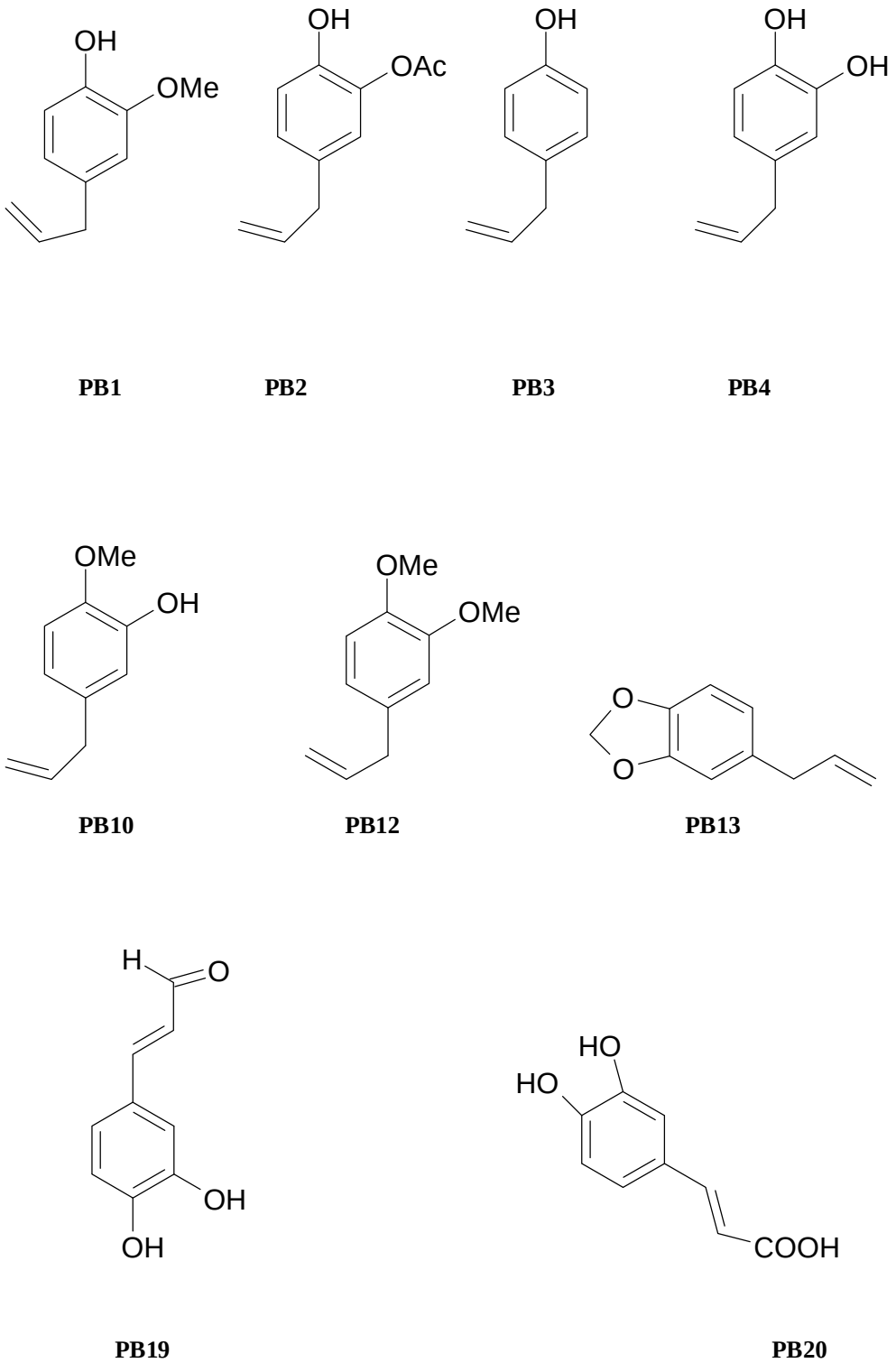
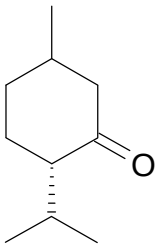
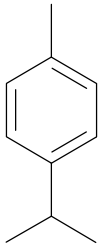


Figure 1.7 Structure of allyl benzenes isolated from *Piper betel* L.



PB22



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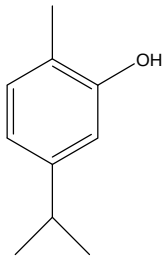

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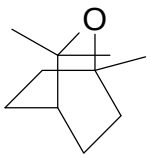
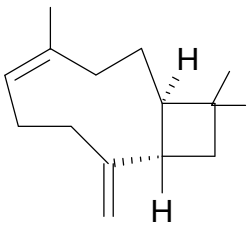
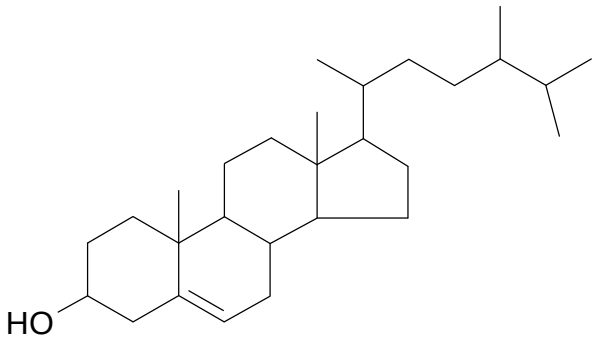


PB27



PB31

Figure 1.8 Structure of terpenoids isolated from *Piper betel* L.



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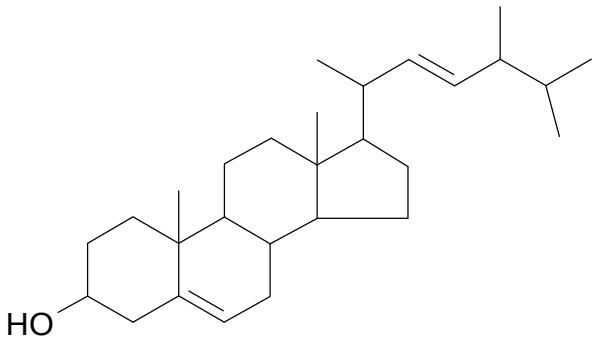
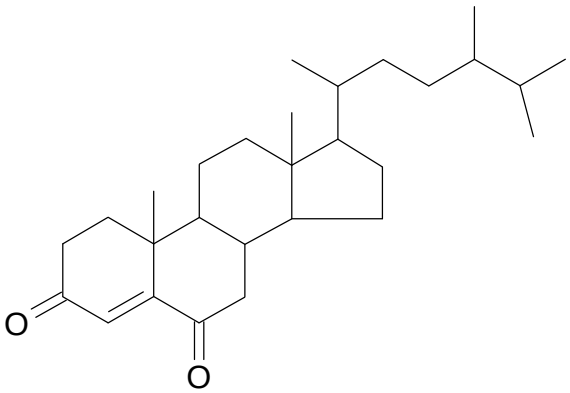
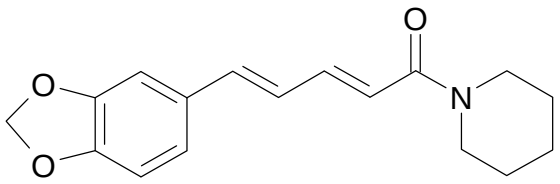


PB37

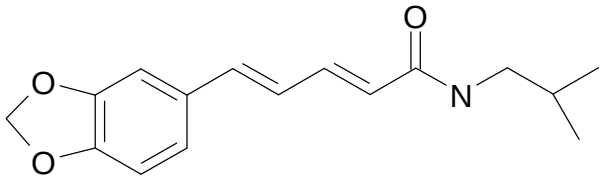


PB43

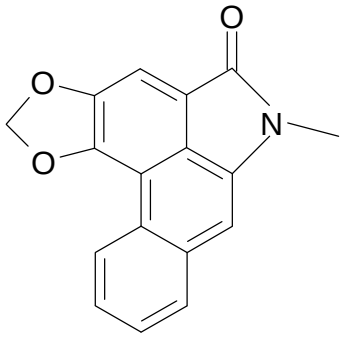
Figure 1.9 Structure of phytosterols isolated from *Piper betel* L.



PB41



PB42



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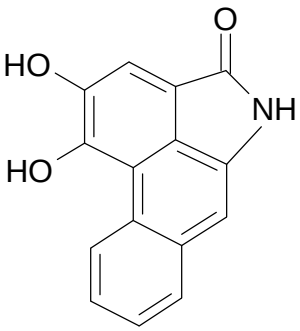
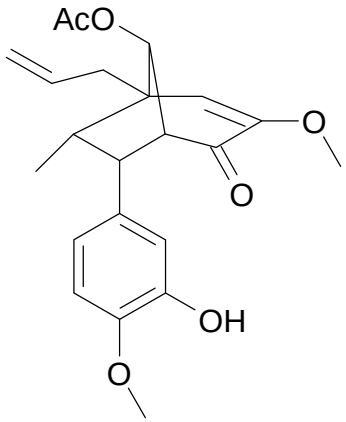
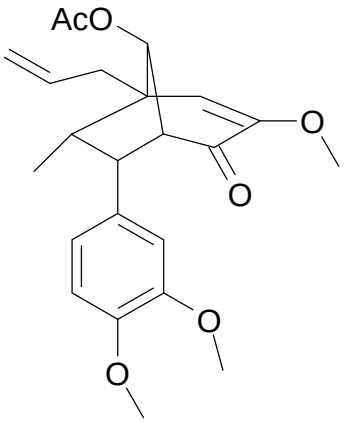


PB47

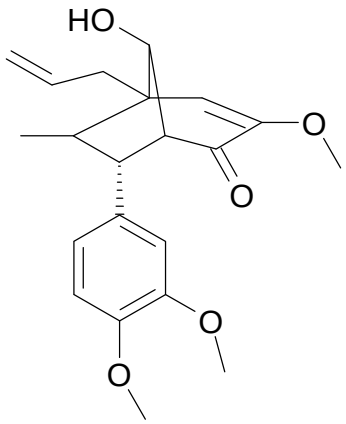
Figure 1.10 Structure of alkaloids isolated from *Piper betel* L.



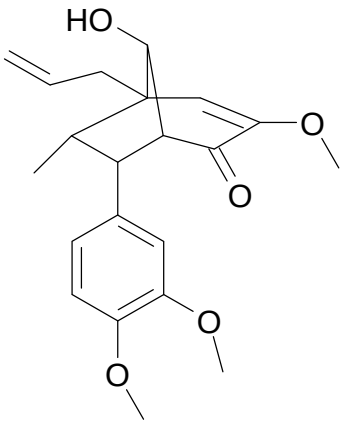
PB32



PB33

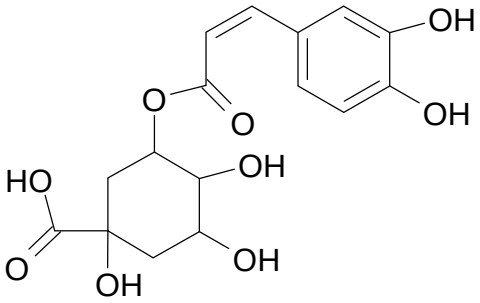


PB34

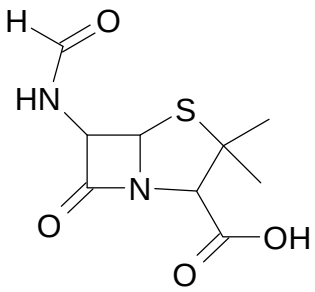


PB35

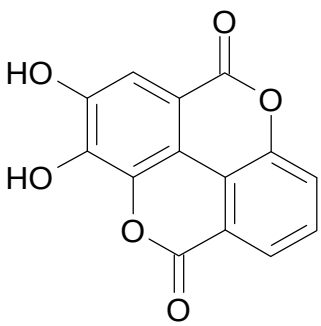
Figure 1.11 Structure of neolignans isolated from *Piper betel* L.



PB18

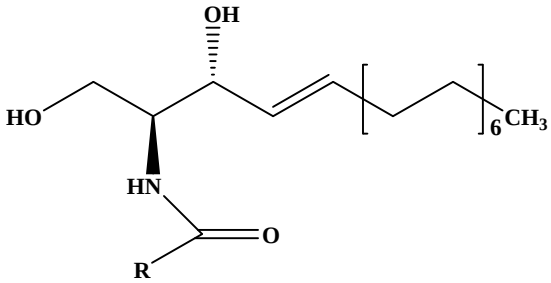


PB46

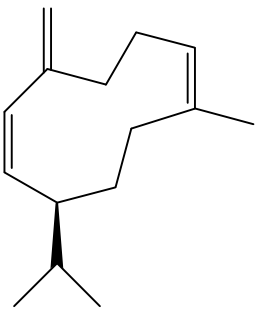


PB48

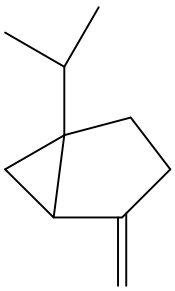
Figure 1.12 Structure of miscellaneous isolated from *Piper betel* L.



PB52



PB53



PB54

Figure1.13 Structure of miscellaneous isolated from *Piper betel* L.

1.5 *Avicennia alba*

Scientific name *Avicennia alba* Blume

Family Avicenniaceae

Synonyms *Avicennia spicata* Kuntze

English name Avicennia

Vernacular names Samae-Khao (Thai), Vellai kandai (Tamil), Gunda, vilava mada (Telugh). Api-api putih, Apia pi, Api-api hitam (Borneo), Paira Baen (Sundarbans of India)

Fig 1.14; Image of *Avicennia alba* from website http://oceangrow.septentriones.com

1.5.1 Botany

Avicennia alba Blume is a small tree (up to 5 meters, tall) The bark is slightly roughened and are somewhat brown in colour. The leaves are characteristically lanceolate, pointed at the apex and have a white undersurface. The Inflorescences are long spicate with flowers in distal units. The fruits are conical in shape extended into a pronounced beak, especially in the early stages of development. A frequent character is the development of a sooty mold on older stem parts.

The flowers are with 1.5 to 3 cm. long with 10 to 30 flowers per unit and the lower flowers pairs are usually quite distant, completely distinguished within the *Avicennia marina*. (Tomlinson, P., B., 1932).

1.5.2 Habitat

Avicennia alba Blume is distributed widely from India to Indochina, Malay Archipelago to the Philippines, New Guinea, New Britain, Northern Australia (Tomlinson, P., B., 1932) and the Southeast, East and West of Thailand (Smitinand, T, 1976).

1.5.3 Traditional uses

The barks and seeds are used as fish poison and the resin is used in birth control (Vadlapudi, V., *et al*, 2009).

1.5.4 Previous biological investigations

The ethanol extract of air-dried *Avicennia alba* leaves exhibited significantly decreased the physiological and histopathological alterations inducing by ethanol in the low dose (100mg/kg) and it inhibited those changes in rats in the high dose (300mg/kg). In 2001, reported by Itoigawa *et al* that naphthoquinones and their analogs from *Avicennia* plant including *Avicennia alba* showed the cancer chemopreventive activity. Six natural and four synthetic naphoquinone and five of their analogs were investigated the inhibitory activity against Epstein-Barr virus early antigen activation. The isolated compounds, 1, 4-Furanonaphthoquinone and analogs having a hydroxyl group on the dihydrofuran ring showed the most potential in the activity. Suntar *et al* reported in 2010 revealed that the naphthoquinones and

some flavonoids displayed remarkable wound healing and anti-inflammatory activities and in the present the extract of *Avicennia alba* has evaluated for its effects against ethanol induced gastric mucosal damage in female rats (Al-Attar, A.M., 2011). The methanol extract of *Avicennia alba* showed activity against *Streptococcus mutans* (MIC 5mg/ml). The chloroform and methanol extracts were active against plant pathogenic fungus, *Rhizoctonia solanic* and *Lactobacillus acidophilus*. Its plant extracts can be used to treat infectious diseases caused by resistant pathogenic microorganisms (Vadlapudi, V., *et al*, 2009).

1.5.5 Previous chemical investigations

Three new naphthoquinones, namely acequinone A(I), B(II), C(III) and avicenol A, B and C and analogs have been isolated from the stem bark of *Avicennia alba* Blume (Chihiro, Ito, *et al*, 2000). Sringaldehyde and some sugars were detected in the bark (Shinoda, Y, *et al*, 1987). Glutamic, aspartic acids, malic, citric oxalic, glycolic and succinic acids were detected in *Avicennia alba* Blume leaf (Bharucha, F., R., *et al*, 1957). Taraxerol, β-amyrin, taraxerone, betulin, betulinic acid and triacontanal have been isolated from *Avicennia alba* Blume bark and leaves. (Ghosh, M., S., *et al*, 1979) (Table 1.14-1.16).

Table1.14 Previous quinones isolated from *Avicennia alba* Blume

Compounds	Part used	References
Avicequinone A (A1)	stem barks	Chihiro, I., 2000
Avicequinone B (A2)	stem barks	Chihiro, I., 2000
Avicequinone C (A3)	stem barks	Chihiro, I., 2000
Avicenol A (A4)	stem barks	Chihiro, I, 2000
Avicenol B (A5)	stem barks	Chihiro, I, 2000
Avicenol C (A6)	stem barks	Chihiro, I, 2000

Table 1.15 Previous triterpenoids isolated from *Avicennia alba* Blume

Compounds	Part used	References
Taraxerol (A7)	barks and leaves	Ghosh, M., S., 1979
β-amyrin (A8)	barks and leaves	Ghosh, M., S., 1979
Taraxerone (A9)	barks and leaves	Ghosh, M., S., 1979
Betulin (A10)	barks and leaves	Ghosh, M., S., 1979
Betulinic acid (A11)	barks and leaves	Ghosh, M., S., 1979
Triacontanal (A12)	barks and leaves	Ghosh, M., S., 1979

Table 1.16 Previous miscellaneous isolated from *Avicennia alba* Blume

Compounds	Part used	References
Lignin	bark	Shinoda, Y, 1987
Syringaldehyde	bark	Shinoda, Y, 1987
Sugars	bark	Shinoda, Y, 1987
Glutamic	leaf	Bharucha, F., R., 1957
Aspartic acids	leaf	Bharucha, F., R., 1957
Malic	leaf	Bharucha, F., R., 1957
Citric oxalic	leaf	Bharucha, F., R., 1957
Glycolic	leaf	Bharucha, F., R., 1957
Succinic acids	leaf	Bharucha, F., R., 1957
Moisture	leaf	Bharucha, F., R., 1957

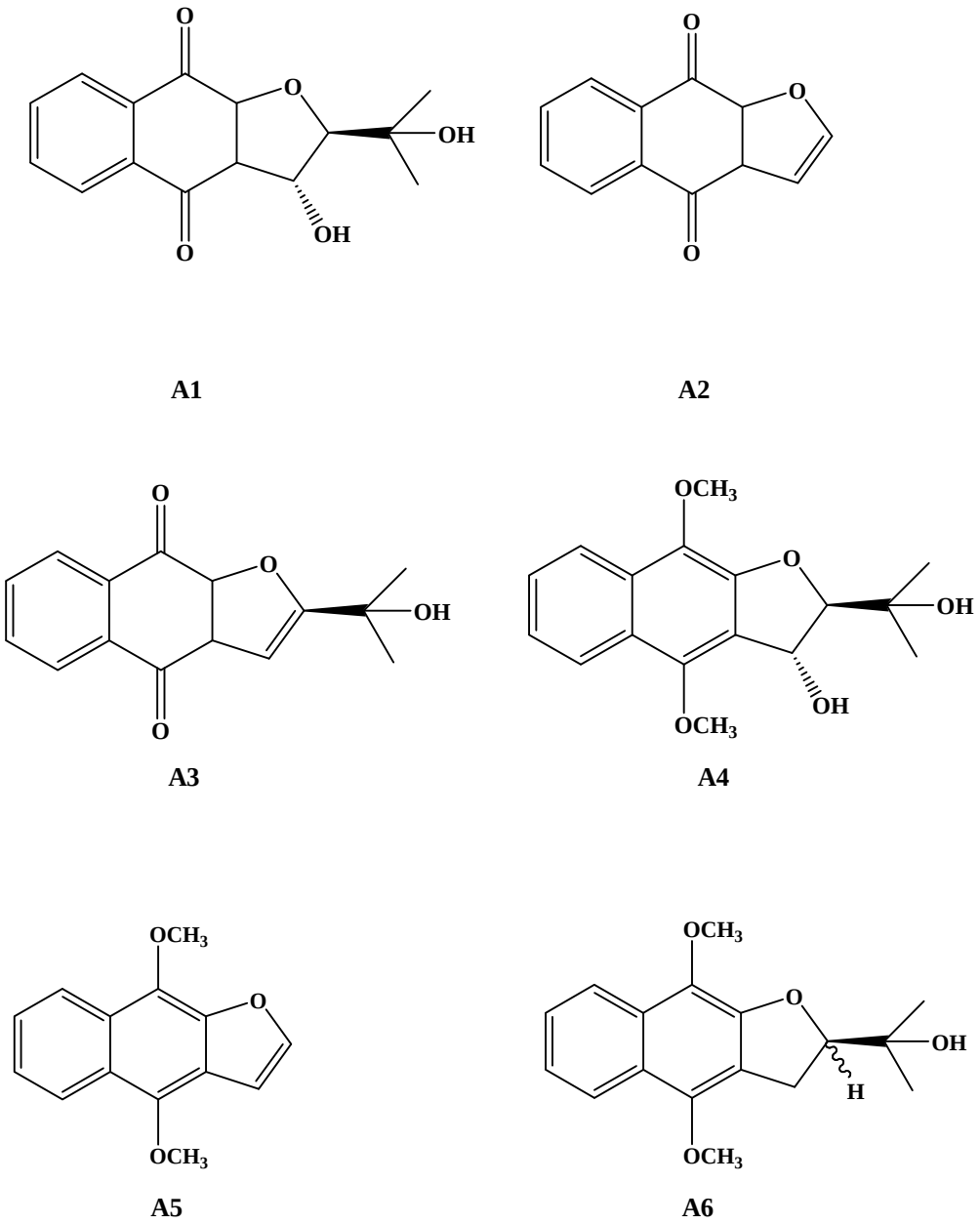
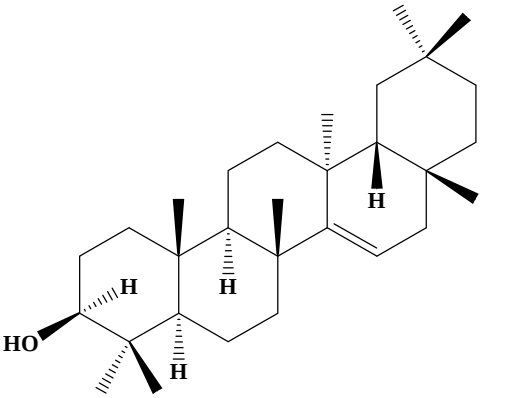
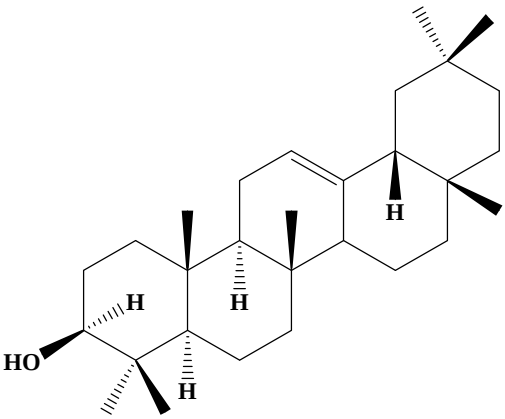


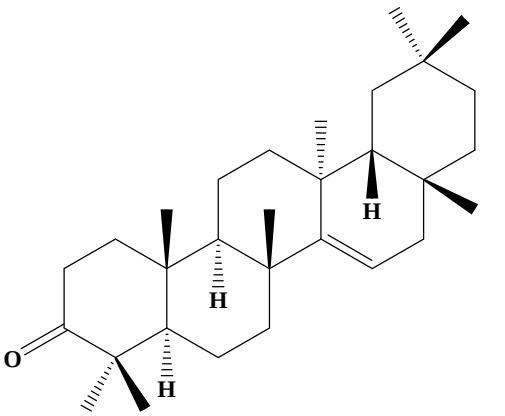
Figure 1.15 Quinone compounds isolated from *Avicennia alba* Blume.



A7

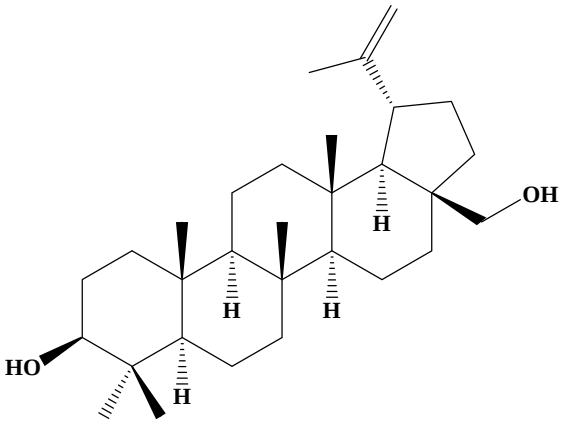


A8

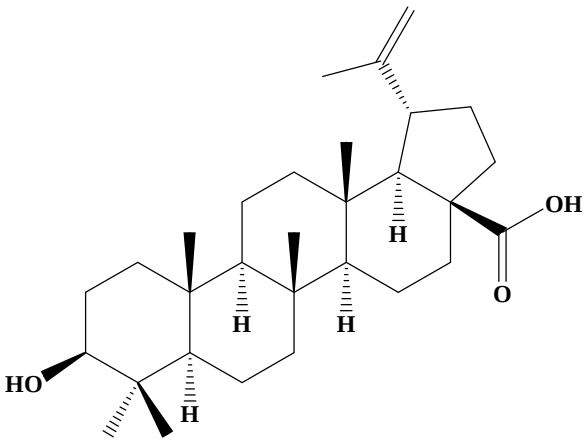


A9

Figure1.16 Triterpenoids isolated from *Avicennia alba* Blume.



A10



A11

Figure1.17 Triterpenoids isolated from *Avicennia alba* Blume.

1.6 Avicenniaceae Family

The avicenniaceae is known as the “black mangrove” family including *A. marina, A. australis, A. acutissima, A. anomala, A. ramphlina, A. intermedia, A. resinifera, A. alba, A. mindanaense, A. lantana and A. eucalyptifolia*. It is along with the “red mangrove” (*Rhizaphora mangle*), “white mangrove” (*Langancularia racemosa*) and “buttonwood” (*Conocarpus erctus*) in Southernmost coastal zone of Florida (Nelson, G, 1996). This family was classified into either Verbenaceae or Acanthaceae. (Schwarzbach, A., E., McDade, L., 2002). Recent phylogenetic studies suggest it derives from the Acanthaceae family (Watson, L and Dallwitz, M., J., 1992).

1.6.1 Botany

The family comprises small trees; reaches from 8 up to ca 20 m. Tree have long heavy roots growing extensions that aid in gas exchange. The branchlets are articulate; the leaves are opposite, leathery, simple with entire lamina.and have a daxial hypodermis present. The seed matures and germinates on the tree and then fall to the mud. The wood is parenchyma apotracheal. The inflorescence, floral, fruit and seed are morphology. Its flower aggregated in the inflorescences. The flowers are bracteate, small, cyclic and tetra cyclic (Watson, L and Dallwitz, M., J., 1992).

1.6.2 Distribution

This genus grows in dense stands on mud flats along the coast and estuaries, in brackish coastal swamps and a river banks along lower brackish parts. It generally grows along tropical and subtropical shores.

1.6.3 Habitat

Plants from this family are found in highly salted, low aerated of waste-logged soil and frequently changing water levels due to tidal cycles. Avicennia is considered in as a major “true mangrove” element because it is endemic to mangrove habitats, plays a predominant role in community structure. (Schwarzbach, A., E., McDade, L., 2002).

1.7 *Bruguiera gymnorrhiza*

Scientific name *Bruguiera gymnorrhiza* (L.) Savigny

Family Rhizophoraceae

Synonyms *Rhizophora gymnorrhiza* (1828), *Rhizophora conjugate* (1753), *Bruguiera cylindrical* Blume (1871), *Bruguiera capensis* Blume (1827) (Lewis, J., 1956).

English name Large-leafed mangrove, Oriental mangrove

Vernacular names Pangka Huasum (Thai), Orange mangrove (Northern Australia), yangach (Yap Island), denges (Palau), Jon (Marshall Islands), Swartwortelboon (Africa)

Fig 1.18; Images of *Bruguiera gymnorrhiza* from the book written by Allan, J., A. and Duke, N., C., 2006

1.7.1 Botany

Bruguiera gymnorrhiza is a glabrous tree with 6-20 m. tall, the bark is reddish-brown or grey in colour and stipules are often reddish. Leaves are dark green in colour. Flowers are in axilsre of upper leaves, solitary, red or pinkish red, 3 cm. Petals are about 12-14, 1.3-1.5 cm in size and their outer margin fringed with white silky hairs; sinus is bristles with 3 or 4 per petal, 2-3 cm. which distinctly exceeding petals. Petals ain white in colour but soon turn into brown (Lewis, J., 1956). Fruits are adnate to calyx tube, 2.5 mm. and persistent calyx on fruit or hypocolyls ribbed only apically. Hypocotyl is in cigar-shaped and slightly angular with the size of 15-25 cmx1.5-2 cm. in size (Haining, Q and Boufford, D., E., 1753).

It is found in the sea level attitude (Lewis, J., 1956), intertidal zones with 0-2 meters or the elevation range between mean sea level and highest tide with annual rainfall about 40-315 inches. (Allan, J., A. and Duke, N., C., 2006).

1.7.2 Distribution

Bruguiera gymnorrhiza is a common buttress tree found in mangrove forests. It is widely distributed in Thailand, other area of Asia, Southern and eastern Africa, Australia, Micronesia and Polynesia and China (Homhual, S., *et al*, 2006). It is also found in Potugal, Madagascar and Kenya. Lewis, J., 1956).

1.7.3 Uses

The bark is rich in tannin used for tanning leather, treat sails and fishing nets for long preservation. It is also used as condiment, adhesive and it provides orange to reddish brown dyes. Wood is used as firewood, charcoal and in the paper industry. The plant is used to prevent and combat erosion along coasts.

1.7.4 Traditional uses

Fruits of *Bruguiera gymnorrhiza* have been used to treat diarrhoea in China (Bamroongrugsa, B., 1999). The fruits are also used to treat shingles and eye diseases (Rudjiman, 1992). The bark is used as an astringent treatment for malaria. The roots and leaves are used to heal burns (Homhual, S., *et al*, 2006).

1.7.5 Previous biological investigations

The ethanol extract of *Bruguiera gymnorrhiza* roots exhibited the reduction of blood sugar in streptozotocin induced diabetic rats.This study indicated hypoglycemic and antihyperlipidemic effect of this plant (Karimulla, S. and Kumar, B. P., 2011). Brugierol isolated from the stem bark of *Bruguiera gymnorrhiza* has shown antibacterial activity against *Lactobacillus acidophilus* and *Bacillus subtilis*. Brugierol and isobrugierol showed termicidal activity against *Coptotermis formosanus*, a subterranean termite. The chloroform extract from its flowers was evaluated for cancer chemoprevention (Homhual, S., *et al*, 2006).

1.7.6 Previous chemical investigations

Compounds that have been isolated from *Bruguiera gymnorrhiza* include disulfides (Xiao-Ying, F., *et al*, 2010; Guo, Y.-W., *et al*, 2006), triterpenes (Homhual, S., *et al*, 2006; Sarkar, A., *et al*, 1978; Abu, R., S., M., *et al*, 1995), diterpenoids (Han, L., *et al*, 2005;Ham, L., *et al*, 2004; Mfilinge, P., *et al*, 2005; Sun, Y.-Q., 2004), aromatic compounds (Han, L., *et al*, 2007; Han, L., *et al*, 2005), lignans (Han, L., *et al*, 2007; Suisheng, S., *et al*, 2008), tannins (Liang-Zhen, F., *et al*, 2008; Achmadis, S., *et al*, 1994; Cunningham, G., E., *et al*, 1963; Silva, D., *et al*, 1950), steroids (Ghosh, A., *et al*, 1985), cyanidin (Seshadri, T., R., *et al*, 1959; Liang-Liang, Z., *et al*, 2008) and other miscellaneous compounds (Run-Zhen, F., *et al*, 2009; Jie, W., *et al*, 2008; Shinoda, Y., *et al*, 1987; Vang, L., *et al*, 2005; Misra, S., *et al*, 1987; Hogg, R., W., *et al*, 1984;Runzhen, F, *et al*, 2010) (Table 1.17-1.26).

Table 1.17 Sulfides isolated from *Bruguiera gymnorrhiza*

Compounds	Part used	References
Cis-3, 3'-dihydroxy-1, 5, 1', 5'tetrathiacyclodacane (B1)		
	-	Huang, X.-Y., 2009
Cis-3, 3'-dihydroxy-1, 5, 1', 5'-tetrathiacyclic decane (B2)		
	-	Huang, X.-Y., 2009
Gymnorrhizol (B3)	Fresh leaf	Liu, H.-L., 2008
		Fan, R.-Z., 2008
		Gong, J.-X., 2007
		Sun, Y.-Q., 2 004
		Sarkar, A, 1978
Neogymnorrhizol (B4)	Fresh leaf	Fan, R.-Z., 2008,
		Liu, H.-L., 2008
		Huang, X.-Y., 2009
Gymnorrhiza (B5)	-	Yaou, H., 2007
Bruguiesulfurol (B6)	Stem, Leaf, flowers	Huang, X.-Y., 2009
		Shen, X, 2008
		Yaou, H., 2007
		Homhual, S., 2005
		Fan, R.-Z., 2008
		Liu, H.-L., 2008

Table 1.18 Sulfides isolated from *Bruguiera gymnorrhiza* (continued)

Compounds	Part used	References
Brugierol (B7)	Fresh leafs	Fan, R.-Z., 2008 Liu, H.-L., 2008 Huang, X.-Y., 2009 Yaou, H., 2007 Homhual, S., 2005 Islam, M.S., 2006
Isobrugierol (B8)	Fresh leaf	Fan, R.-Z., 2008 Liu, H.-L., 2008 Huang, X.-Y., 2009 Yaou, H., 2007 Homhual, S., 2005 Islam, M.S., 2006 Mfilinge, P.L., 2005

Table 1.19 Terpenoids isolated from *Bruguiera gymnorrhiza*

Compounds	Part used	References
Ent-kaur-16-en-13-hydroxy-190-al (B9)		
	Outer layer of root bark	Subrahmanyam, 1999
15(S)-isopimar-7-en-15, 16-diol (B10)		
	Outer layer of root bark	Subrahmanyam, 1999
Ent-kaur-16-en-13, 19-diol (B11)		
	Outer layer of root bark	Subrahmanyam, 1999
Methyl-ent-kaur-9(11)-en-13, 17-epoxy-16-hydroxy-19-oate (B12)		
	Outer layer of root bark	Subrahmanyam, 1999
1β,15(R)-ent-pimar-8(14)-en-1,15,16-triol (B13)		
	Outer layer of root bark	Subrahmanyam, 1999
Eent-kaurane diterpenoids I, II, III (B14, B15, B16)		
	Stems	Han, 2004
Ent-beyerane diterpenoid (B17)		
	Stems	Han, 2004
Taraxerol (B18)	Wood	Takei, 1994
		Abu, 1995

Table 1.20 Terpenoids isolated from *Bruguiera gymnorrhiza* (continued)

Compounds	Part used	References
Lupeol stearate (B19) .	-	Abu, 1995
β-amyrin (B20)	-	Abu, 1995
Lupenone (B21)	-	Abu, 1995
lupeol (B22)	-	Abu, 1995
α-amyrin (B23)	Leaves	Ghosh, 1985
β-amyrin, lupeol (B24,B25)	Leaves	Ghosh, 1985
B-amyrin palmitate (B26)	Leaves	Abu, 1995
Ent-8(14)-pimarene-15R, 16-diol (B27)	-	Han, 2005
Ent-8(14)-pimarene-1α, 15R, 16-triol (B28)	-	Han, 2005
(5R, 9S, 10R, 13S, 15S)-ent-8(14)-pimarene-1-oxo-15R,16-diol (B29)		
	-	Han, 2005
Oleanolic acid (B30)	Fresh leaves	Ghosh, 1985
Bruguierin A, B, C (B31, B32, B33)	Flowers	Homhual, 2006
		Islam, 2006
		Han, 2005
Gymnorrhizol (B34)	-	Gong, 2007
	-	Sarkar, 1978

Table1.21 Flavonoids isolated from *Bruguiera gymnorrhiza*

Compounds	Part used	References
Total flavones	Pulverized leaves	Runzhen, 2010
Total flavonoids	Leaves	Zheng, 2008
4', 5', 7'-trihydroxy-3',5-dimethoxy flavone (B35)		
	Leaves	Raihan, 1994
Flavandiol	Barks	Cunningham, 1963
3', 4', 5'-trihydroxy-7-hydroxy-5methoxy flavone (B36)		
	-	Hu, 2007

Table 1.22 Lignan isolated from *Bruguiera gymnorrhiza*

Compounds	Part used	References
Lignin (B37)	Wood	Takei, 1994
	Barks	Shinoda, 1987
	Fresh leaves	Misra, 1987
Brugranin (B38)	-	Shang, 2008

Table 1.23 Tannin isolated from *Bruguiera gymnorrhiza*

Compounds	Part used	Ref.
Condensed tannin	Cylyx	Zhang, 2008
Condensed tannin	Barks	Achmadi, 1994
Tannin	Dried barks	Cunningham, 1963
Tannin	Barks	Silva, 1950

Table 1.24 Aromatic compounds isolated from *Bruguiera gymnorrhiza*

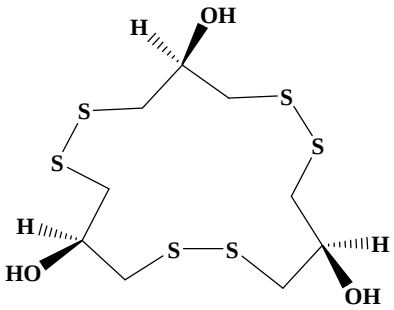
Compounds	Part used	References
BruguninA (B39)	Branches	Han, 2007
2, 3-dimethoxy-5propylphenol (B40)	Branches	Han, 2007
Bruguierol D (B41)	Branches	Han, 2007
4-hydroxydithiolane-1-oxides(B42)	-	Islam, 2006

Table 1.25 Steroids isolated from *Bruguiera gymnorrhiza*

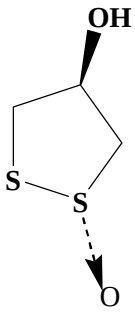
Compounds	Part used	References
β-sitosterol (B43)	-	Abu, 1995
	Wood	Takei, 1994
Sterols	Leaves	Hogg, 1984
Cholesterol (B44)	Leaves	Hayata, 1984
Campesterol (B45)	Leaves	Hayata, 1984
Stigmasterol (B46)	Leaves	Hayata, 1984
Sitosterol (B47)	Leaves	Hayata, 1984
Stigmast-7-en-3β-ol (B48)	Leaves	Hayata, 1984

Table 1.26 Miscellaneous compounds isolated from *Bruguiera gymnorrhiza*

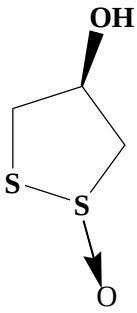
Compounds	Part used	References
30 volatile constituents and 23 fatty acids	Hypocotyl	Fan, 2009
Elementary chemical compositions	Fresh leaves	Fan, 2008
Quantitative analysed of abscisic acid, gibberellin	-	Wang, 2008
Proanthocyanidin	Calyces	Zhang, 2008
Anthocyanidin	Calyces	Zhang, 2008
Total lipids and fatty acids	Leaves	Mfilinge, 2005
(Z)-8-dodecan-10-ol (B50)		Vang, 2005
(E)-8-dodecan-1-ol, dodecan-1-ol (B51)		Vang, 2005
Carbohydrate complexes	Wood	Takei, 1994
Chemical compositions	Barks	Shinoda, 1987
Ash, vanilla, syring aldehyde, sugars		Shinoda, 1987
Hydrocarbon and wax esters	Fresh leaves	Misra, 1987
Normal, branch chain HC between C16-C36	-	Misra, 1987
Leucocyanidins	Barks	Seshadri, 1959
Fatty acids, hydrocarbons	Leaves	Hogg, 1984
Ten polymorphic microsatellite loci	-	Islam, 2006



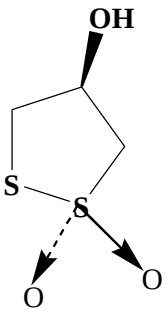
B3



B6

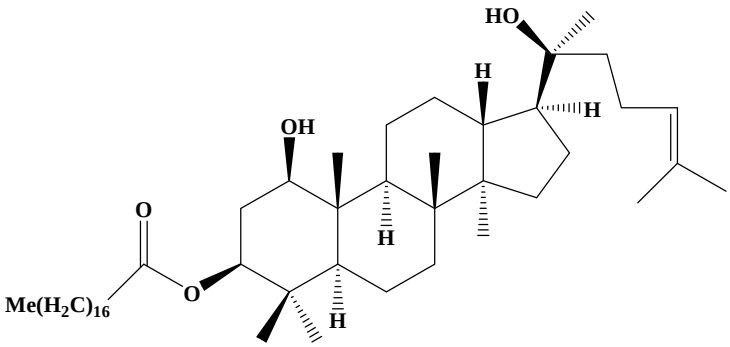


B8

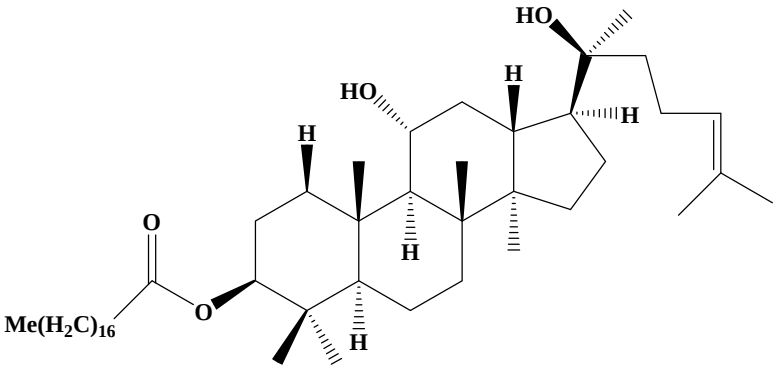


B9

Figure1.19 Sulfides isolated from *Bruguiera gymnorrhiza*

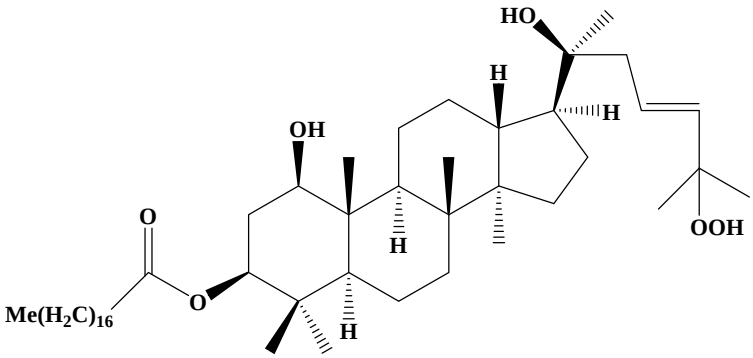


B31

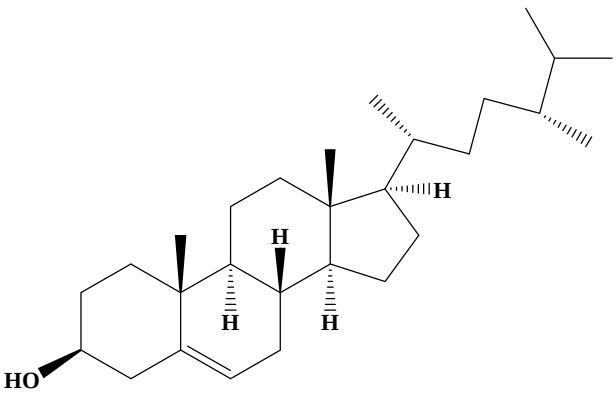


B32

Figure1.20 Triterpenoids isolated from ***Bruguiera gymnorrhiza***



B33



B46

Figure1.21 Triterpenoids isolated from ***Bruguiera gymnorrhiza***

1.8 Rhizophoraceace family

This family contains approximately 14 genera and 100 species of tropical trees, often mangrove trees (Wiart, C., 2006).

1.8.1 Botany

Rhizophoraceae comprises mangrove or inland trees or shrubs or climbers. Branches are swollen at the node. Leaves are usually opposite with interpetiolar stipulate; Inflorescence is a cyme or raceme, axillary. The flowers are regular and usually bisexual with hypothium, solitary or in cymes. Fruits are berry, baccate and drupaceous or capsule; seeds are often germinating while still attached to the parent plant (vivipary) before the fruits fall (Keng, H. and Keng, R.-S., L., 1987; Castro, I., R., 2006).

1.8.2 Distribution

Rhizophoraceae is confined to the tropics in moist mountain forests and totally flooded wetlands or mangrove communities (Castro, I., R., 2006).

1.8.3 Economic importance

The wood of some species in this family for example, *Rhizophora mucronata, Rhizophora candelaria and Bruguiera sexangula*, is used for firewood and charcoal production. The bark is a source of tannins and dyes used in the leather tanning industry (Castro, I., R., 2006).

1.8.4 Chemicals and Biological Investigations

Phytochemicals isolated from plants belonging to this family include tannin, series of pyrrolidine, pyrrolizidine and tropane alkaloids. A few of pharmaceutical investigations of plants have been carried out in this family. Some studies have revealed that plants from the family are active against the Human Immunodeficiency Virus. A study of *Rhizophora upiculata* bark extract and found that a polysaccharide showed MT-4 cells protection from the HIV-induced cytopathogenicity and blocking

the expression of HIV antigens. It was also exhibited completely inhibited binding of the viral to the cells (Premanathan, M., *et al*, 1999).

1.9 Aims and objectives

This study aimed to investigate some secondary metabolites from four selected medicinal plant species from Southeast Asia searching for active components against microbial pathogens and to corroborate some of the traditional uses of the medicinal plants.

The objectives of this project were to:

1) Extract, isolate and purify of some secondary metabolites from the plants by applying chromatographic procedures;
2) Identify the isolated compounds using various spectroscopic techniques;
3) Screen the isolated compounds obtained in sufficient yields against bacterial (*Stapylococcus aureus*) and fungal (*Candida spp.*) pathogens.

Chapter 2

Materials and Methods

2.1 Plant materials

Ground *Avicennia alba* stems, *Bruguiera gymnorrhiza* and *Cassia tora* leaves selected by Dr. Veronique Seidel from Strathclyde Institute of Pharmacy and Biomedical Sciences (SIPBS) collection.

Piper betel leaves were purchased from a Thai market by a commercial supplier (Bangkok) in October 2007. *Piper betel* was identified by Dr. Mark Newnan at Royal Botanic Garden, Edinburgh, Scotland as shown below.

FLORA OF THAILAND

PIPERACEAE

***Piper betle* L.**

Thailand, Saraburi, Muang District, Moo 3, Tambon Nongplalai. 14º 31’ 42’’ N 100º 54’ 35’’ E
Altitude: 10.516 m
Habitat: cultivated

Vine to 3 m tall, cultivated as follows, 1) cut 50 cm long stem including branches, leaves and roots from the original plant 2) put the bottom of stem that has roots in the soil 10 cm deep 3) tie the rest of stem with ropes to attach a small wooden pole and 4) water the plant daily.

Vernacular name: Plu (Thai)

Collector: Mr. Prawate Kluaypa
Coll. No.: 1
Date: March 2009

Cited in Khanittha Chawananorasest, 2012. Phytochemical and antimicrobial studies of some medicinal plants from South East Asia. PhD thesis, Strathclyde Institute of Pharmacy and Biomedical Sciences (SIPBS), University of Strathclyde, Glasgow, UK.

Please communicate new identifications to Royal Botanic Garden Edinburgh

2.2 Extraction

Ground *Cassia tora* leaves were extracted sequentially with hexane, ethyl acetate and methanol, using an Accelerated Solvent Extraction instrument. The extracts were filtered and concentrated to dryness in vacuo, using a rotary evaporator at 40^0C.

Piper betel leaves were cut into small pieces and extracted with 3L of ethanol by maceration at room temperature. The marc was extracted with ethanol for another two times to complete extraction. The extracts were filtered and concentrated to dryness in vacuo, using a rotary evaporator at 30^0C.

2.3 General

Stationary phases using silica gel 60, particle size 70-230 mesh ASTM (cat. No. 1.07734.1000), Merck and Florisil, particle size 100-200 US mesh, BDH, were used for open column chromatography.

Silica gel 60, particle size 90% ≤45 µm (cat. No. 1.07749.100), Merck, was used for vacuum liquid chromatography and preparative TLC.

Lipophilic *Sephadex*®, bead size 25-100 µm, Sigma-Aldrich, was used for gel permeation chromatography.

Silica gel 60 F254, size 20x20 cm, cat. No.1.05554.0001, Merck for TLC analysis.

AR grade solvents were used for chromatography and extractions, purchased from BDH, Riedel-de Haën, Fisher Scientific and Sigma-Aldrich.

Distilled water was used for extraction.

2.4 Isolation

2.4.1 Vacuum Liquid Chromatography (VLC)

Vacuum Liquid Chromatography is a form of column chromatography, useful for the fractionation of crude extracts. The column is contained in a Buchner funnel with the side-arm connected to the vacuum supply. The column size is about 12.5x10.5 cm. depending on the amount of the sample. The stationary phase is either normal or reverse –phase. TLC grade silica gel absorbent, packed under reduced pressure to about 6-7 cm. in height. Crude extracts are prepared by mixing with the absorbent in a ratio of plant extracts 1:1 or 2:1, depending on the extracts. The extract powder

obtained is packed on the top of VLC column. Mobile phases are poured on the top of the column under vacuum. Fractions are collected in a stepwise elution using solvents of increasing polarity and aliquots were collected in an Erlenmeyer flask and the vacuum was disconnected after each elution.

2.4.2 Open column chromatography

Column chromatography is a method to purify mixture of compounds. A column glass tube was used with a different diameter, depending on the amount of sample. The column was prepared by putting some cotton wool at the base of the column following by a small amount of sea sand a suitable mobile phase was poured to wet the column. Slurry was prepared with a stationary phase and a suitable mobile phase. The mobile phase was released with a proper flow rate during the application of the slurry. A sample was applied to the column after releasing the mobile phase to the same level as the front of the stationary phase. The sample was prepared by mixing with some stationary phase or dissolved with a suitable solvent. The elution was carried out under gravity using a gradient elution sequence with a mobile phase of increasing polarity of mobile phase.

2.4.3 Gel Permeation Chromatography

This is a separation technique based on hydrodynamic (size in solution), sometimes called gel filtration or size exclusion. This technique was discovered by Flodin and Porath in 1958, it is the cross-linked gel formed from the reaction of dextran with epichlohydrin. Sephadex, is one of material usually used, has a semirigid structure and wide range of pore sized to organic solvents (Braithwaite, *et al*, 1996). Samples are separated by their molecular sizes, according to porous media, the large molecules can not access into the pores and they are then eluted from the column with shorter retention times than smaller molecules. Separations are accomplished without interactions between stationary phases. One of the solvent systems usually used for gel filtration was 95% chloroform/hexane.

2.4.4 Thin Layer Chromatography

Thin Layer Chromatography is another technique for the separation of mixture of compounds. The stationary phase is a thin layer of adsorbent such as silica, alumina oxide, or cellulose on the aluminum sheets. The sample was applied to the plate by spotting with capillary tube, and then the plate was put to a glass tank containing saturated with suitable mobile phase. Mobile phase was drawn up via capillary action. The separation depended on the ability of the compounds to dissolve to mobile phase or to bind to stationary phase. Different compounds have different rates of travel, indicated by *Rf* value or Retention factor which is determined by the distance of each solute traveled divided by the distance of solvents traveled. The compounds with about different *Rf* value can be separated easier than those the same or a little different *Rf* value. The plate is visualized under UV light with multi band 254/366 nm. and detected of spray reagents.

2.4.5 Preparative Thin Layer Chromatography (PTLC)

This is a technique for the isolation of quantities of component (10-100mg). The thin glass plates are coated with stationary phase such as silica gel with thickness 1-5 mm. and 20x20 cm. dimensions. The sample is applied as a streak by a pasteur pipette or capillary tube. Multiple developments allow separation to be more effective. The components can be detected by UV light or colouration methods can be used by spray reagents only the narrow strip at the edge of plate, other areas are covered during spraying. The components with double bonds in their structures can adsorb iodine vapour and become yellow or brown bands (Braithwaite, A, *et al,* 1996). The marked band is scraped off the plate with carefully and then mixed with a suitable solvent, filtered and evaporated to dryness.

2.4.6 Liquid-liquid partition

Partition is a separation technique based on the partition of solutes between two immiscible liquid phases (i.e. one organic and one aqueous phase). A solvent system should provide a suitable partition coefficient which is preferably much greater or much less than unity. The density of the solvent system should be different. Low interfacial tension of organic solvent-water contact results in stable emulsions that

make separation more difficult. A separation funnel was used to shake and to separate a solute out of an aqueous phase with an organic solvent. Repeat shaking give a high yield recovery of solute. Different solvents are used in this technique, depending on the different polarities of solutes.

2.5 Detection

2.5.1 UV detection

TLC plates were visualized under UV light with multi bands 254-366 nm.

Compounds in with conjugated double bond were detected as blue under short wavelength, lighter blue, or orange-red under long wavelength. Normally TLC plates were visualized by UV light before spraying with any specific reagents.

2.5.2 Spray reagents

2.5.2.1 *p*-Anisaldehyde-sulfuric acid

For detection of phenols, sugars, steroids, and terpenes

The reagent was prepared by 0.5 mL *p*-anisaldehyde in 10 mL glacial acetic acid, 85 mL methanol and 5 mL concentrate sulphuric acid. After spraying the plate was heated at 105 C until spots show maximum visualization.

Results: Phenols, terpenes, sugars, and sterols turn to violet, blue, red, grey or green colours.

2.5.2.2 Dragendorff reagents

For detection of nitrogen compound, alkaloids and surfactants.

The reagents were prepared by taken 10 mL each solution shown below to mix with 20 mL glacial acetic acid and 100 mL of water.

Solution 1) 2.125 g of bismuth subnitrate, 25 mL of glacial acetic acid and 100 mL of water.

Solution 2) 40 g of potassium iodide in 100 mL of water.

Results: compounds containing nitrogen atom turn to orange colour.

2.5.2.3 Anthraquinone-specific spray reagent

For detection of antraquinone derivatives

The reagent was prepared by 5% potassium hydroxide in ethanol

Results: compounds containing anthraquinone turn to pink colour.

2.6 Spectroscopic Instrumentation

2.6.1 Infrared (IR) spectroscopy

The Infrared light (5,000-400 cm^{-1} range) was used, splitted into two beams. One beam is made to be a longer path than the other. Either one or both beams are passed through the sample. The detected signals from two paths are converted into a plot of absorption against wave number using computer programmed of Fourier transformation.

Infrared spectra were recorded on an ATI Mattson Genesis Series FT-IR spectrometer using KBr discs for samples in a solid state (amorphous powders and crystals) or as a film on disc of sodium chloride for liquid samples.

FT/IT-4200 series, Jasco, PIKE Technologies Spectroscopy Creactivity, 2901 Commerce Park Drive Maddison W153719.

Diamond is used for an optical window material in this experiment. It has a wide range of analysis such as acids, bases and oxidizing agents. It is also resistant to scratch and abrasion, but high cost and intrinsic absorption from 2300-1800 cm-1 limiting in the use of some transmissions.

The depth of penetration (dp) provides a relative of the intensity of spectrum and express by this equation presented below.

$$\mathbf{dp = \lambda/2\P\ (\eta_1^2 \sin^2\theta_1 - \eta_2^2)^{1/2}}$$

λ = width of light

η_1 = reflection index of the crystal

η_2 = reflection index of sample

θ_1 = angle of incidence of IR beam

2.6.2 Mass spectroscopy (MS)

Electro spray Ionisation (ESI) spectra were obtained from a LTQ Orbitrap spectrometer. The samples were applied to the small flow (1-10µL/min.) with a capillary needle. The different voltage 3-6 kV used between a cylindrical electrode and the end of the capillary make the sample liquid leave from the capillary as a spray. The spray consists of positive or negative charged particles depending upon the voltage applied to the capillary. The spray was evaporated by a dried gas across the spray and it became charged particle. The particles were carried to an ion analyzer (quadruple mass spectrometer using on appropriate electric field. A positive mode ESI produced $[M+H]^+$ while a negative mode ESI generated $[M-H]^-$ molecular ion. Positive and negative modes of ESI were used in this work and methanol was used as solvent to dissolve the samples.

2.6.3 Nuclear magnetic resonance (NMR)

Proton magnetic resonance (^{1}H NMR) spectra were obtained on a JEOL 400 MH_Z NMR spectrometer. J-modulated ^{13}C and 2D NMR (COSY, HMBC, HMQC and NOE) were measured on the same spectrometer at 100 MH_Z. The samples were dissolved in deuterated solvents such as $CDCl_3$, C_5D_5N, and D_2O for these experiments.

NMR spectroscopy was used in this study in order to characterize the isolated natural products. Ernst and Anderson pioneered the pulse-Fourier- transform or PFT spectroscopy in 1960s. In this technique, all nuclei in a sample (^{1}H, ^{13}C) are simultaneously ecited by a radiofrequency pulse and it enhances the sensitivities of low abundance nuclide of ^{13}C, ^{15}N, large molecules spectra and also spectra containing singlet signal such as ^{13}C by using selective pulses, complex pulsed sequence and pulsed field gradients. The signals of interferogram or free inductive delay (FID) are detected by quadrature detection. The Fourier transformation converts interferogram from analog form into digital form and the records are on a stored to computer (Becconsall, 2005).

2.6.4 Proton NMR (^{1}H NMR)

This technique was used to determine the structure of the natural products isolated in this study. The three important pieces of information contained in an ^{1}H NMR spectrrm are the chemical shifts (δ), the coupling constants (J) and the intensities (I) of various functional groups. This information giving some characteristic signals providing evidence of the structural feature or functional groups of the compounds and also confirming the proposed structure or the expected signals for proton in molecules.

2.6.5 Proton broad-band decoupling (^{1}H BB decoupling)

Proton coupled ^{13}C NMR spectra are complex and difficult to analyse. This technique provides single peaks for the all carbon nuclei in the spectra by decoupling proton signals during the recording time. The decoupling uses a continuous sequence of composite pulses to irradiate all protons signals, resulting in only single ^{13}C NMR signals in the spectrum (Beccousall, 2005).

2.6.6 Distortionless enhancement by polarization transfer (DEPT)

DEPT is a polarisation transfer method which enhances the intensity of low abundance spins (^{13}C) by transferring the greater population from high abundance spins (^{1}H) of their spin coupled partner (Claridge, 2006). This technique is important to distinguish the multiplicities of carbon nuclei among ^{13}C signals. This can identify the belonging of signals to quaternary, CH, CH_2 and CH_3 nuclei following the sequence given below.

^{1}H channel**:** $90^0_{x'}$ - τ - $180^0_{x'}$- τ - $\theta_{y'}$ - τ - BB decoupling

^{13}C channel: $90^0_{x'}$ - τ - 180- τ- FID (t_2)

The pulse angle is chosen to $\theta_1=45^0$, $\theta_2=90^0$, $\theta_3=135^0$ in three separate experiments. During this time, BB decoupling switched on to remove multiplicities of proton signals give all singlet signals in the spectrum.The experiments provide three sub-spectra with the intensities of CH, CH_2 and CH_3 signals depending upon the angle θ. As a result, DEPT 90^0 contains all signals of CH groups while DEPT 135^0 gives positive signals for CH, CH_3 groups and negative signals for CH_2 groups. DEPT 45^0 provides decoupling spectra including C, CH, CH_2 and CH_3 groups which is

compared to other DEPT spectra result in the signals of quaternary carbons nuclei (Becconsall, 2005).

2.6.7 Proton-proton correlated spectroscopy (^{1}H-^{1}H COSY)

This technique is useful to identify the correlation between A and X protons in AX system with coupling constant *J*AX.

The fourier transformation of the FID gives a two-dimensional spectrum with four groups, each of them containing four signals; two groups are diagonal peaks and the others are cross-peaks or correlation peaks. The cross peaks occur when protons A and X interact with each other through a scalar coupling. These peaks give direct evidence of the coupling between protons A and X (Claridge, 2006). One proton can couple to some other protons nearby, and give the diagonal peaks. This is identified as the resonance position of coupled nuclei. Long-range coupling in this technique is small, but it can be detected better by fixing the delay time (Becconsall, 2005).

2.6.8 The nuclear overhauser effect (NOE)

NOE produces a change in the intensity of one resonance when the other spin transitions are perturbed from their equilibrium populations. The perturbation can be done by saturation a resonance. The intensities change either positive or negative depending upon relaxation process. In W_2 relaxation process, spins are remove from ββ state and transferred to αα state giving positive NOE or increasing in the intensities. Likewise, W_0 relaxation process, transferred spins from βα state to αβstate result in negative NOE or decreasing in the intensities (Claridge, 2006). This depends on the distance between the dipolar-coupled nuclei which should be ideally less than $5A^0$or $3A^0$ (Becconsall, 2005).

These are some rules in this technique.

- The samples should not contain any impurities with paramagnetic.
- The sample should be dilute and low in viscosity.
- NOE experiments between CH_3 group and a single proton, should saturate CH_3 group to measure the intensity of the single proton because it give a small percent of NOE signal enhancement compared to the single one.

2.6.9 Heteronuclear single-bond correlation spectroscopy

The two techniques of heteronuclear single-bond correlation spectroscopy namely, heteronuclear multiple-quantum correlation (HMQC) and heteronuclear single-quantum correlation (HSQC), provide single-bond heteronuclear shift correlations. They are different in some details of consequence experiments. HMQC have favourably used in chemical community for small molecules while HSQC have preferred by biological spectroscopists for ^{1}H-^{15}C correlations in proteins. HMQC has more tend to robust to imperfection or miscalibrations of experiments whilst HSQC has more characteristics for very high-resolution experiments and more flexible to modification and extension of the sequence, which is now widely used in chemical laboratory (Claridge, T., D., W., 2006).

2.6.10 Heteronuclear multiple-quantum correlation (HMQC)

This technique is used for proton-detected single-bond correlation experiments and its 2D spectrum provides a simple map of the connectivity between two attached nuclei appeared as a cross peak correlations.

HMQC illustrates three significant features of experiments.

1) The ability to transfer known proton assignments onto the spectrum of heteronucleus, extending the characterisation of molecules.
2) Dispersion of the proton resonances, aiding the initial interpretation of proton spectrum.
3) Ability to identify structural assignments of *diastereotopic* geminal pairs.

The HMQC sequence comprises of four rf pulses for a simple ^{1}H-^{13}C spin pair. The sequence begins with proton excitation, followed by proton magnitisation under one-bond carbon-proton coupling ($^{1}J_{\text{C-H}}$), antiphase of proton magnitisation, transferring by subsequent of rf and first carbon pulse to generate proton-carbon multiple-quantum coherence as a pooling of transverse magnetization of coupled spins. The final carbon pulse reconverts the multiple-quantum coherence back to observed single-quantum proton magnetization, antiphase with respect to $^{1}J_{\text{C-H}}$. The result is a two-dimensional spectrum with ^{1}H shifts represented in f2 dimension, ^{13}C shift in f1 dimension and crosspeaks indicating one-bond connectivity (Claridge, T., D., W., 2006).

2.6.11 Heteronuclear single-quantum correlation (HSQC)

HSQC is differing from HMQC in only transverse single-quantum magnetization of the heteronuclear, generated by polarization from attached protons via INEPT sequence spin rather than ^{1}H-X multiple-quantum coherence. HSQC with improved resolution in the dimension is advantage over HMQC for small molecules. The disadvantage of HSQC over HMQC in intensity loss from rf inhomogeneity, pulse miscalibration or off-resonance excitation which can be minimized by careful probe tuning and using of composite 180^{0} pulses (Claridge, T., D., W., 2006).

2.6.12 Heteronuclear multiple-bond correlation (HMBC)

The ^{1}H-X heteronuclear correlation methods presenting the presence of a proton bound to heteroatom, but unable to assign of non-protonated centres and clear carbon assignments of proton resonances overlapping. The correlations between carbon and neighbouring protons more than one bond called *long-range* or *multiple bond* correlations involving proton-carbon connectivities through couplings over two or three bonds ($^{n}J_{CH}$, n=2,3), providing most powerful approach to define of organic compounds structure (Claridge, T., D., W., 2006).

2.6.12.1 The HMBC sequence

The HMBC experiments establishes multiple-bond correlations by taking advantage of the sensitivity associated with proton detection which is turned to detect correlations via small couplings by setting preparation Δ period. The sufficiently long time allows small long-range proton- carbon couplings to produce the antiphase displacement of vectors which generate heteronuclear multiple-quantum coherence. The Δ period at least 100ms ($1/2^{n}J_{CH}$) is applied when magnitude of ^{1}H-^{13}C couplings are smaller than one-bond couplings (<5 Hz). During the long Δ period homonuclear ^{1}H-^{1}H couplings which are similar magnitudes to the long-range heteronuclear couplings, evolve and introduce phase distortions to the observed crosspeaks (Claridge, T., D., W., 2006).

2.6.12.2 HMBC Applications

The long-range proton-carbon couplings over two or three bonds rarely exceed 25 Hz indicating that Δ should be 100ms or more. In larger molecules Δ is set to about 60-80ms and in smaller molecules tend to have slower relaxation thus longer delay can be used. HMBC was used to define the regiochemistry of a dipeptide antibiotic, Tü1718B isolated from *Streptomyces* cultures, by analysis of the long-range correlations between three protons to the carbonyl groups. The long-range of proton-silicon and proton-carbon correlations of HMBC were used to confirm the structure of an unexpected product from the rearrangement of epoxydisilanes (Claridge, T., D., W., 2006).

2.7 Anti MRSA activity

2.7.1 Microorganisms

Clinical MRSA, 120 isolates, were collected from the clinical laboratories of the New Royal Infirmary (Edinburgh, UK) from February to April 2006 (Vali et al., 2008).

The selected organisms were used for anti-MRSA activity (n=15) including the epidemic EMRSA-15 (LF17, LF98, LF81), EMRSA-16 (LF109, LF80, LF89). Methicillin-sensistive *S. aureus* (MSSA) (ATCC 9144), EMRSA-16 strain and *Acinetobacter baumannii* (ATCC 19606), control strains used in the typing of MRSA isolates. Methicillin-sensistive *S. aureus* (MSSA) (ATCC 9144) and *Pseudomonas aeruginosa* (ATCC 25668), control strains were selected for the anti-MRSA screening test (Raghukumar, R., *et al*., 2010).

2.7.2 Agar dilution assay

The anti-MRSA activity was evaluated by an agar dilution assay performed following BSAC guidelines (Andrews, 2005). The MRSA, MSSA and *Pseudomonas aeruginosa* strains were each cultured in nutrient broth (Oxford, Basingstoke, UK) at 37^0C, 18 hrs. Inocula were prepared by dilution of the broth with 0.9%w/v sterile saline solution to1x104 colony-forming units/mL. The stock solutions of isolated compounds (eugenol, 4-allyl pyrocatechol) were prepared with DMSO solvent at the required concentration before use.

The aliquots were placed in 10cm Petri dishes and to that cooled molten Iso-sensitest agar (Oxford, Basingstoke, UK), was poured to a final volume of 20 mL. The mixture was homogenized; sample concentrations were in the range of 1024 to 4 mg/L and allowed to set on a surface level. The antibiotics were used as positive controls including amplicillin, gentamicin, oxacillin, cefotaxime, cefuroxime, tetracycline, vancomycin and ciprofloxacin (sigma-Aldrich, Gillingham, UK) at different concentrations (128-0.016 mg/L) on the plates. The plates were inoculated subsequently with 2µl bacterial inoculum using a Denley multipoint inoculator (Denley, Billinghursi, UK), giving a final concentration of $2x10^2$ CFU/spot. The inoculated plates were incubated at 37^0C, 18 hrs.

The minimum inhibitory concentration (MIC), mg/L, defined as the lowest concentration of sample in the plate, resulting in complete inhibition of macroscopic growth for the given strain. The samples were each tested in duplicate and DMSO solvent was used at the highest concentration (5.4%v/v) and no deleterious effects observed (Raghukumar, R., *et al*., 2010).

Chapter 3

Results and Discussion

3.1 Phytochemical studies

3.1.1 Characterisation of KC-1 as physcion

The ^{1}H NMR data of compound KC-1 (Table 3.1) showed two sharp singlets of chelated hydroxyl groups at δ 12.12 and 12.32 ppm which are characteristic of an antraquinone moiety. The data (Table3.1) showed one aromatic methyl group at δ 2.45 ppm and an aromatic methoxy group at δ 3.93 ppm. The broad singlets at δ 7.08 and δ 7.63 ppm were attributed to the meta-coupling while two other H-2 and H-4 protons of ring A. Aromatic protons showing at δ 7.36 and δ 6.69 ppm indicated the other meta-coupling protons H-5 and H-6, respectively. From the data given above and by comparison with the literature (Motoyoshiya, 2006 and Meselhy, 2003), this compound was identified as physcion (Figure 3.1 and 3.2).

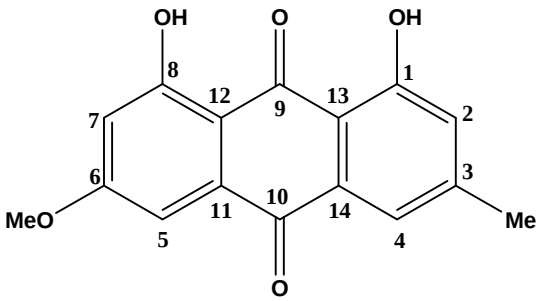


Fig.3.1; Structure of KC-1

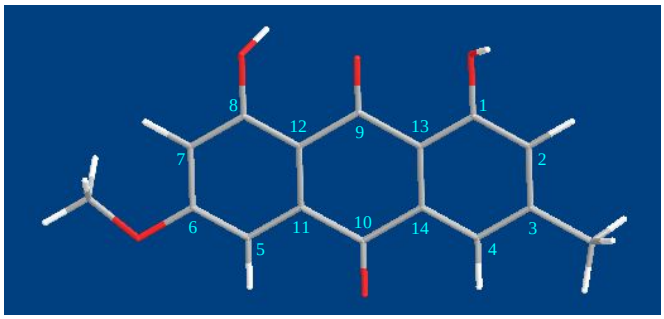
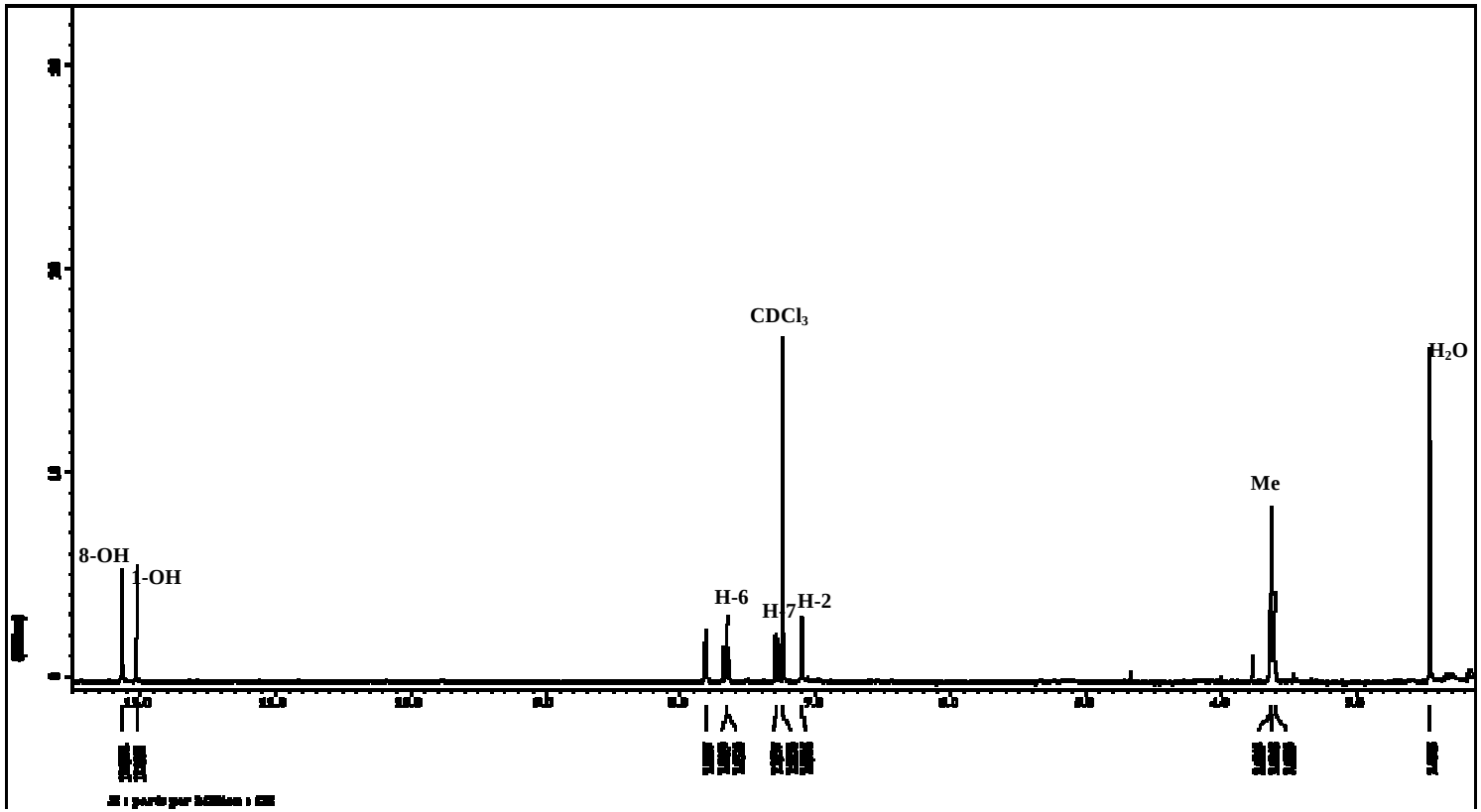


Fig.3.2 3D Structure of KC-1

Table 3.1 ^{1}H NMR data for compound KC-1

^{1}H	δ H (J H$_Z$) (KC-1)	δ H (J H$_Z$) (Meselhy, 2003)	δ H (J H$_Z$) (Motoyoshiya, *et al*., 2007)
1-OH	12.12, *s*	12.10, *s*	12.11, *s*
8-OH	12.32, *s*	12.31, *s*	12.31, *s*
3-Me	2.45, *s*	2.45, *s*	2.45, *s*
6-OMe	3.93, *s*	3.50, *s*	3.94, *s*
H-2	7.08, *brs.*	7.08, *brs.*	7.09, *s*
H-4	7.63, *brs.*	7.62, *brs.*	7.63, s
H-5	7.36, *d* (3.08)	7.36, *d* (2.6)	7.37, *d* (2.78)
H-7	6.69, *d* (3.08)	6.69, *d* (2.6)	6.69, *d* (2.52)

All spectra were recorded in $CDCl_3$, 400 M H$_Z$ and coupling constants are in brackets.



Spectrum3.1; ^{1}H (400MH_z) NMR spectrum of KC-1 in $CDCl_3$

3.1.2 Characterisation of KC-2 as chrysophanol

The ^{1}H NMR data of compound KC-2 (Table 3.2) showed two sharp singlets at δ 12.02 and δ 12.13 characteristic for two chelated hydroxyl groups. It also showed five aromatic protons, including two protons in meta position on ring A at δ 7.10 and δ 8.66, respectively and three protons on ring C, presenting an ABC system at δ 7.81, 7.80 and 7.28 ppm. The spectrum also revealed one aromatic methyl group at δ 2.46 ppm. On the basis of the data given above and by comparing to previously reported data (Garcia-Sosa, 2003 and Meselhy, 2006), this compound was identified as chrysophanol (Fig. 3.3 and 3.4).

Table 3.2 ^{1}H NMR data for compound KC-2

^{1}H	**δ H (J Hz)** (KC-2)	**δ H (J Hz)** (Meselhy, 2003)	**δ H (J Hz)** (Garcia-Sosa *et al.* 2006)
1-OH	12.02, *s*	11.99, *s*	11.97, *s*
8-OH	12.13, *s*	12.10, *s*	12.04, *s*
3-Me	2.46, *s*	2.46, *s*	2.40, *s*
H-2	7.10, *brs.*	7.08, *brs.*	7.04, *d* (0.4)
H-4	8.66, *brs.*	7.64, *brs.*	7.59, *d* (0.4)
H-5	7.81, *d* (7.88)	7.81, *brd.*(8.4)	7.76, *dd* (0.76, 7.52)
H-6	7.80, *d* (8.36)	7.67, *d* (8.4)	7.50, *d* (8.1)
H-7	7.28, *dd* (8.36, -)	7.27, *dd* (1.1, 8.4)	7.23, *dd* (0.74, 8.4)

All spectra were recorded in $CDCl_{3,}$ 400 MH_Z and coupling constants are in brackets.

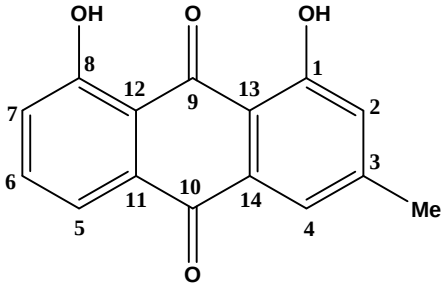


Fig.3.3; Structure of KC-2

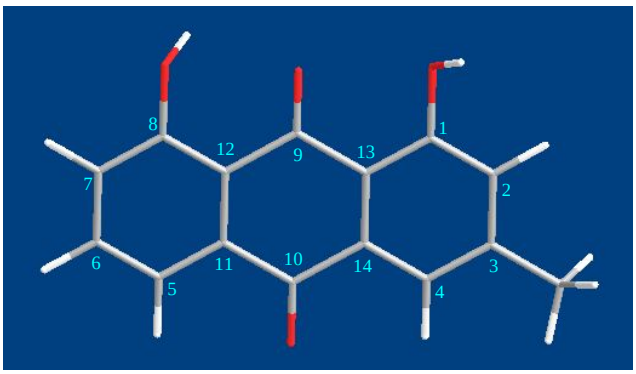
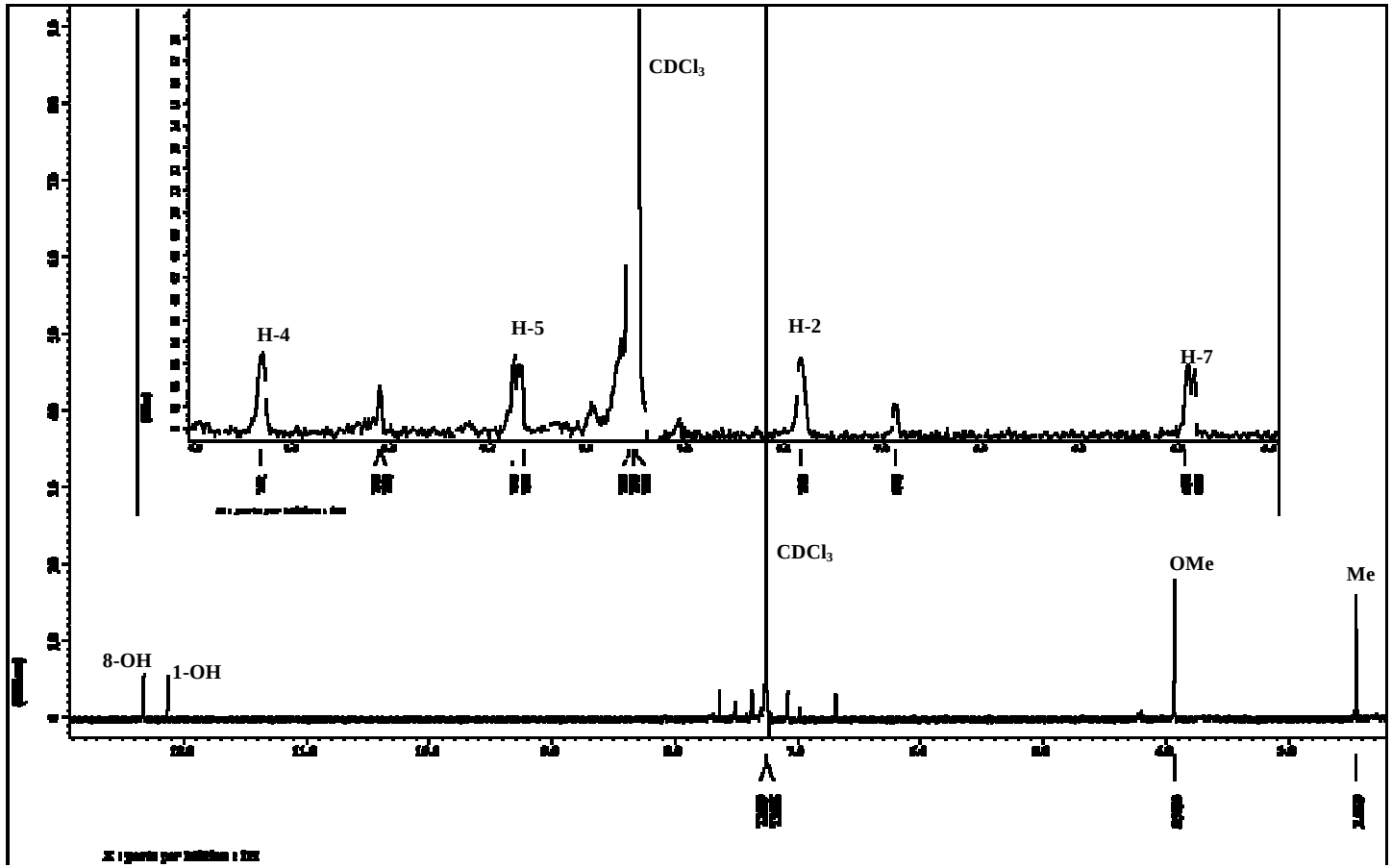


Fig.3.4; 3D structure of KC-2



Spectrum 3.2; ^{1}H (400MH_z) NMR spectrum of KC-2 in $CDCl_3$

3.1.3 Characterisation of KC-3 as a β-sitosterol (KC-3a) and stigmasterol (KC-3b) mixture

Compound KC-3 (Spectrum 3.3) showed some axial proton H-3 attached to an oxygen bearing carbon (*δ*3.5, *m*), olefinic proton, H-6 at *δ* 5.3, *d* (*J*=5.28 H_Z), six methyl groups at *δ* 0.66, (*s*), 0.68, (*s*), *δ* 0.77, (*d*), (*J*=7.04 H_Z), *δ* 0.81, (*d*, *J*=1.76 H_Z), *δ* 0.82, (*t*, *J*=6.16 H_Z), and *δ* 1.00, (*d*, *J*=7.48 H_Z), methylene and methyl protons (*δ* 0.90-2.31 ppm).Two olefinic protons at (*δ* 4.97, (*dd*, *J*=8.8 H_Z) and *δ* 5.1, (*dd J*=7.48, 8.8 H_Z) for protons H-22 and H-23. The evidence given above revealed that the compound had a steroid nucleus. Comparison with some authentic samples indicated that the compound was a mixture of β-sitosterol (Fig.3.5 and 364) and stigmasterol (Fig.3.7 and 3.8) in a ratio 1:1.

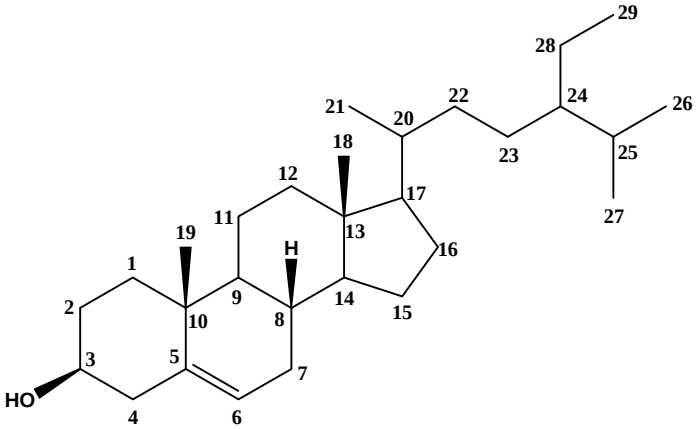


Fig.3.5; Structure of KC-3a

Fig.3.6; 3D Structure of KC-3a

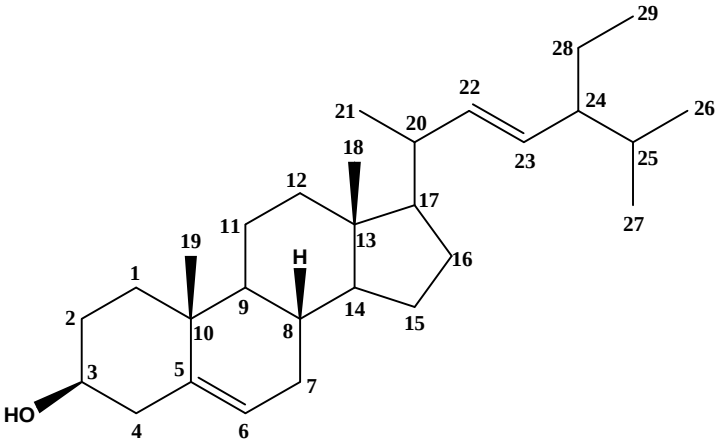


Fig.3.7; Structure of KC-3b

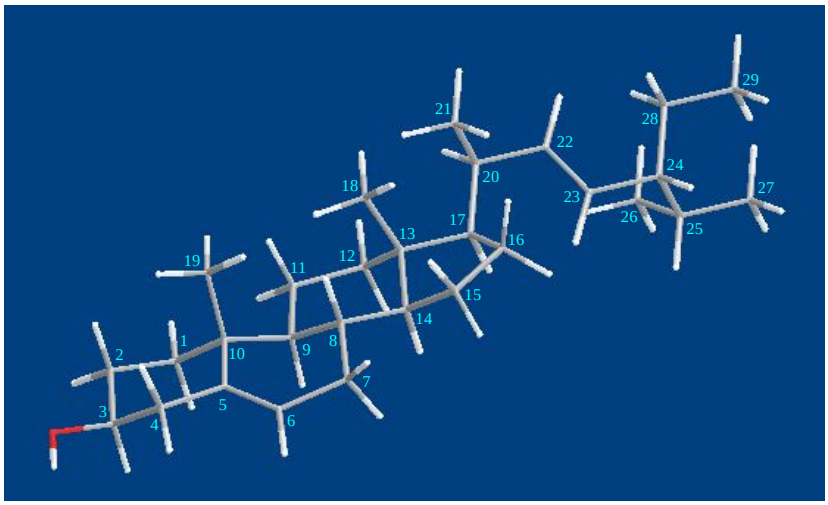
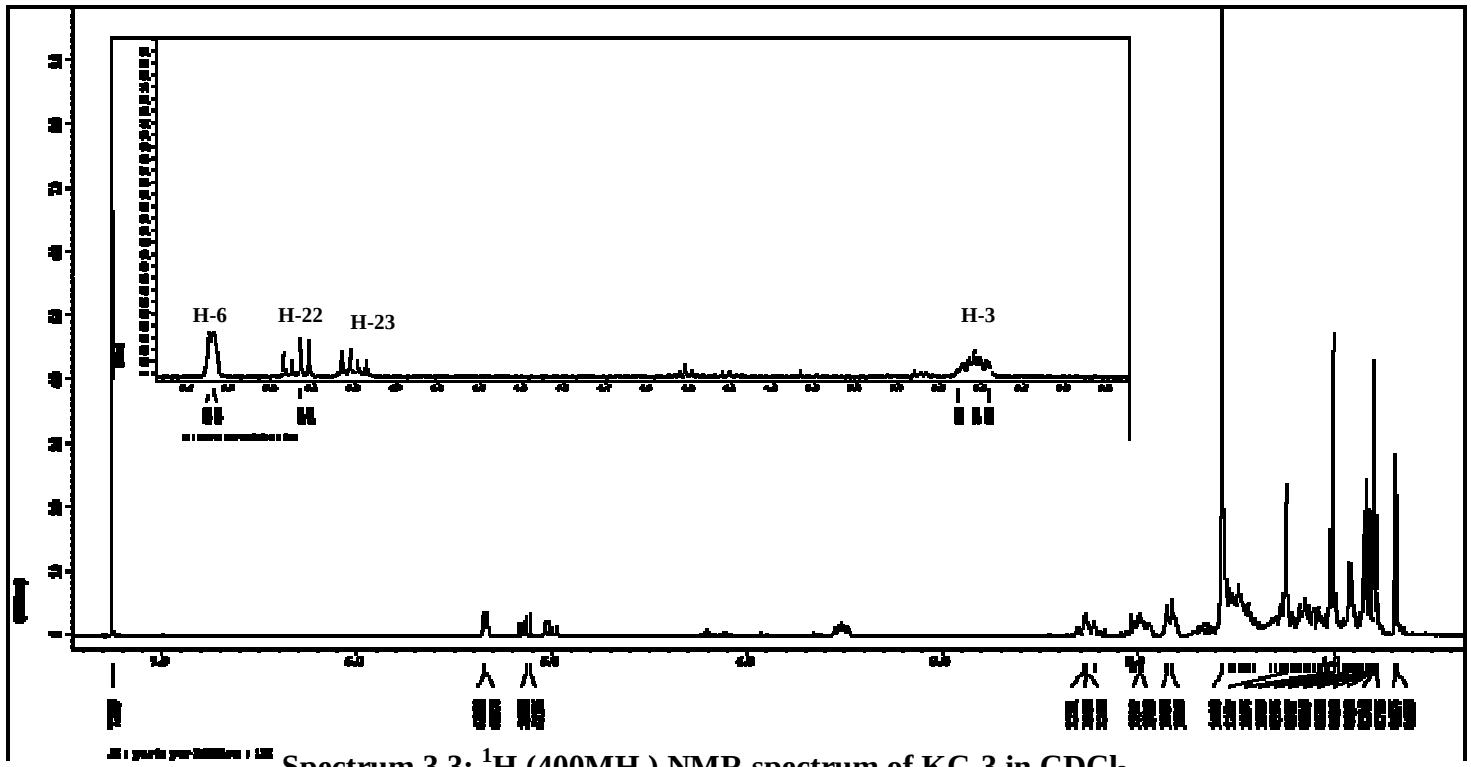


Fig.3.8; 3D Structure of KC-3b



Spectrum 3.3; ^{1}H (400MH_z) NMR spectrum of KC-3 in $CDCl_3$

3.1.4 Characterisation of KC-4 as eugenol

The IR spectrum indicated O-H stretching (3386cm^{-1}), C=C stretching (1652 cm^{-1}), 1436, 1406 and C-O bending (1012cm^{-1} , 905 cm^{-1}).

The MS experiment showed fragments of 164 (100) [M^+], 163(54), 133(100), 132(38), 105(17), 75(10), 41(6) and 29(12), indicating for a molecular ion at m/z 164.09 suggesting for a molecular formula of $C_{10}H_{12}O_2$.

This compound was isolated from Caryopylli flos (Ogata, M., *et al*, 2000), found from the dry flowers of *Syzigium aromaticum* (Ayoola, G., A., 2008) and the stems of *Piper tiwanense* (Chen, Y.-C., *et al*, 2004).

The ^{1}H NMR (Table 3.3) showed signals for benzylic protons H-1' (δ 3.32 ppm.) and exomethylene protons H-3' (δ 5.07 and 5.10 ppm.). An olefinic proton at 5.98 ppm showed *cis* coupling to one of the exomethylene proton H-3' at 5.07 ppm (J=10.12 H_Z) and *trans* coupling to H3' (J=16.68 H_Z). It also coupled to H-1' (J= 7.04 H_Z). The ^{1}H NMR showed an ABX system for an aromatic ring with the other H-6, H-5 and H-2 signals at δ 6.70, 6.81, and 6.83 ppm, a phenolic proton (δ 5.76 ppm) and methoxy protons (δ 3.87 ppm). The COSY spectrum showed that the olefinic proton at 5.98 ppm correlated to H-3', H-5 correlated to H-6 of aromatic ring and exomethylene proton H-3' linked to benzyllic proton H-1'. The DEPT 135 revealed that C-1' and C-3' were methylene groups. Selective NOE on the methoxy group indicated that it corected to the proton H-5 (Fig. 3.10). The ^{13}C NMR given in Table 1 showed aliphatic carbons (δ 39.74 and 56.12 ppm) and aromatic carbons (δ 119.98, 133.56, 110.86 ppm). On the evidences given above, this compound was identified as eugenol (Fig. 3.9), a known compound isolated from *Caryopylli flos* as an example (Ogata *et al.*, 2000).

Table 3.3 ^{1}H (400MHz) and ^{13}C (100MHz) NMR data for compound KC-4

^{1}H	δ H (J Hz)	^{13}C	δC
OMe	3.87, *s*	OMe	56.12
OH	5.76, *brs.*	C-1	133.55
H-2	6.83,*d* (1.76)	C-2	110.86
H-6	6.70, *dd* (1.76, 8.36)	C-3	145.12
H-5	6.81, *d* (8.36)	C-4	145.67
H-1’	3.32, *d* (7.04)	C-5	115.08
H-2’	5.98, *ddt* (17.16, 10.12, 7.04)	C-6	119.98
H-3’	5.07,*dm* (9.68, 1.76)	C-1’	39.74
	5.10, *dq* (16.72, 1.76)	C-2’	137.82
		C-3’	115.64

Spectra were recorded in $CDCl_3$, 400 MHz and coupling constants are in brackets.

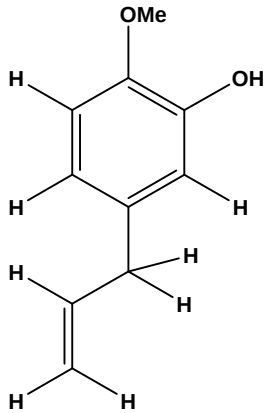


Fig.3.9; Structure of KC-4

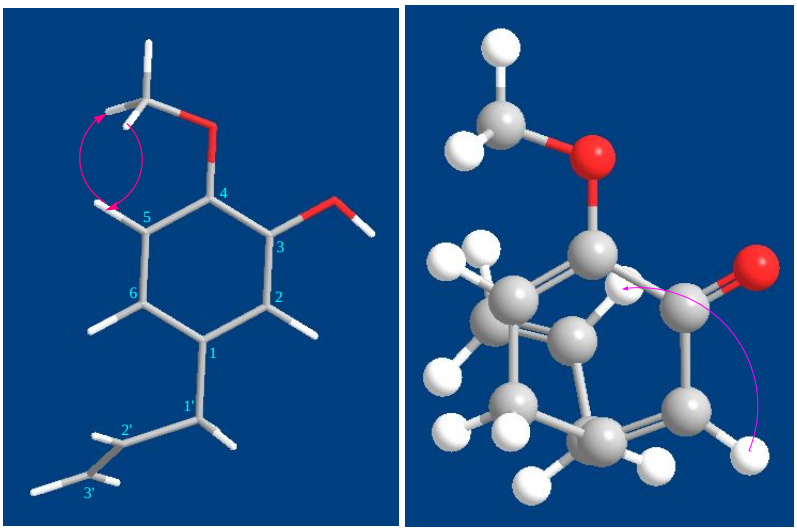
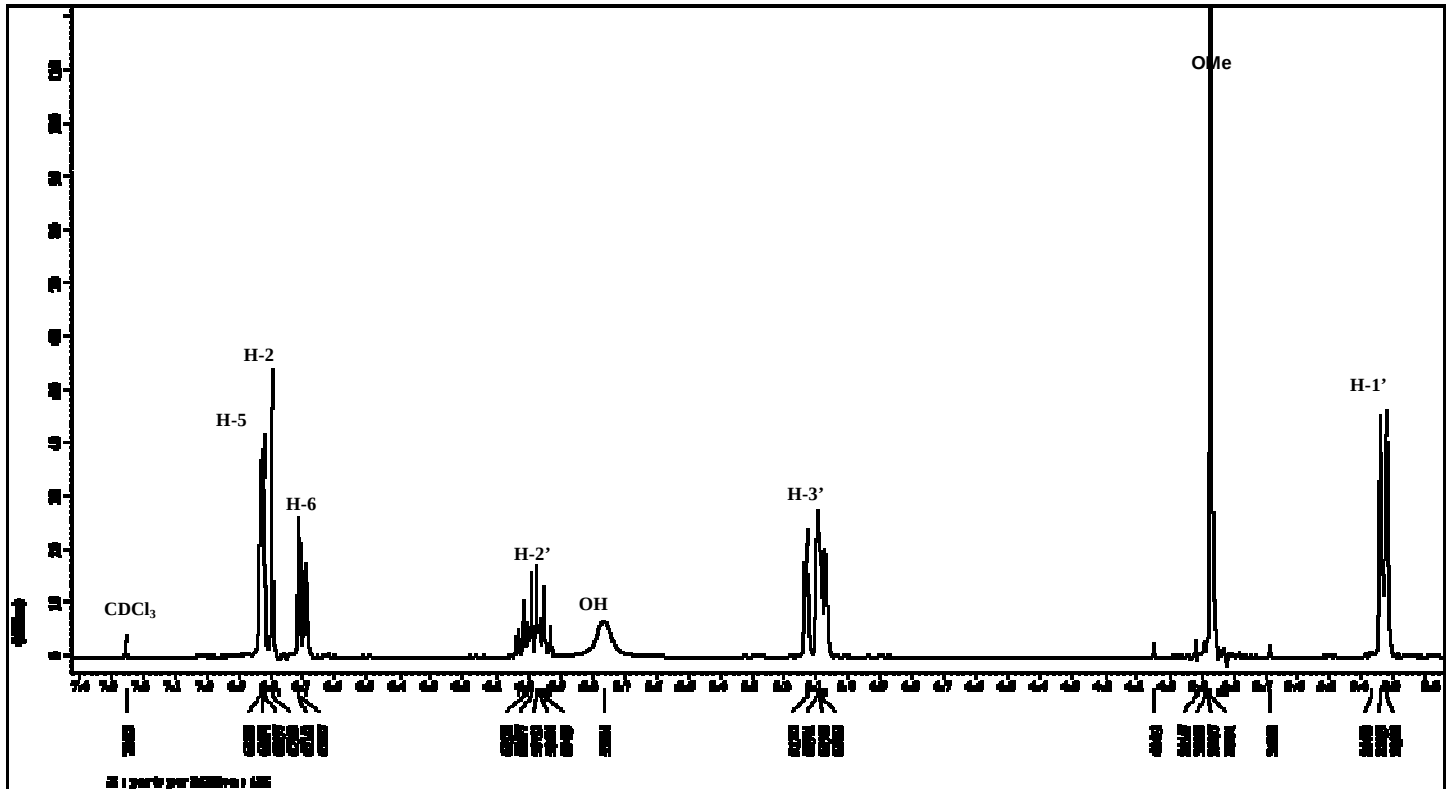
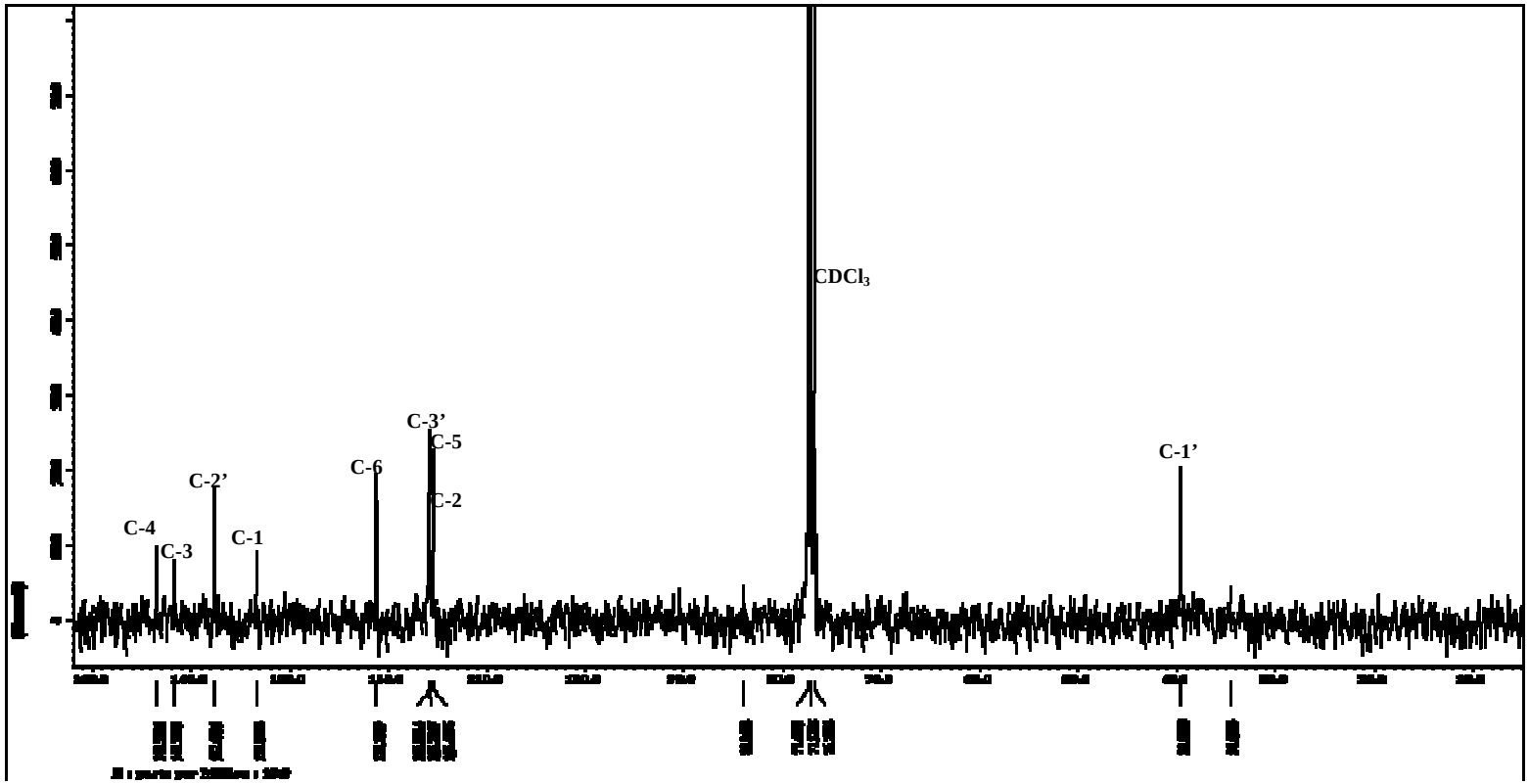


Fig.3.10; 3D Structure and ↷ NOE correlations observed for KC-4



Spectrum 3.4; ^{1}H (400MH_z) NMR spectr um of KC-4 in $CDCl_3$



Spectrum 3.5; ^{13}C (100MH_z) NMR spectrum of KC-4 in $CDCl_3$

3.1.5 Characterisation of KC-5 as 4-allylpyrocatechol

The IR spectrum indicated the absorption of O-H stretching (3241.8cm^{-1}), C=C stretching (1604.0, 1528.3cm^{-1}), 1443.0, 1345.6, 1253.5, 1185.5, C-O bending (1109.4, cm^{-1}, 812.8 and 787.8 cm^{-1}).

The MS spectrum presented m/z 150.1 (Cadogan, J., I., G., *et al*, 1996) suggested a molecular formula of $C_9H_{10}O_2$.

The ^{1}H NMR data (Table 3.5) showed an ABX system of aromatic protons at δ6.76 (H-2), δ6.68 (H-5) and δ6.57 (H-6), some benzylic protons H-1'(δ 3.20), exomethylene protons H-3'(δ 5.02 and δ5.05) and olefinic protons H-2'(δ5.85). The olefinic proton showed *cis* coupling to one exomethylene proton H-3' (J=10.56HZ) and *trans* coupling to the other exomethylene proton (J=16.72 HZ). The latter also coupled to methylene protons H-1' (J=6.56). The DEPT 135 experiment showed methylene groups C-3' and C-1' (δ 115.82 and δ 39.59). Selective NOE on H-1' showed the correlations to δ 6.76 (H-2), δ 6.57 (H-6) and δ 5.85 (H-2') (Fig. 3.12). The evidence given above enables the identification of KC-5 as 4-allyl pyrocatechol (Fig. 3.11), a known compound already isolated from *Piper betel* L. (Savaspun *et al.*, 2003)

This compound has previously been isolated from the leaves of *Piper betel* (Bhattacharya, S., *et al*, 2007, Ali, I, *et al*, 2010) and found from the stems of *Piper Tiwanense* (Chen, Y.-C., et al, 2004).

Table 3.4 ^{1}H (400MH_Z) and ^{13}C (100MH_Z) NMR data for compound KC-5

^{1}H	δ H (J H_Z)	^{13}C	δC
H-2	6.76, *d* (7.92)	C-1	133.39
H-5	6.68, *d* (1.76)	C-2	115.46
H-6	6.57, *dd* (1.32, 8.36)	C-3	141.74
H-1’	3.20, *d* (6.56)	C-4	143.35
H-2’	5.85, *ddt* (16.72, 10.56, 6.56)	C-5	115.71
H-3’	5.02, *dm* (9.24, 1.32)	C-6	121.16
	5.05, *dq* (7.04, 1.32)	C-1’	39.59
		C-2’	137.69
		C-3’	115.82

Spectra were recorded in $CDCl_3$, 400 MHz and coupling constants are in brackets.

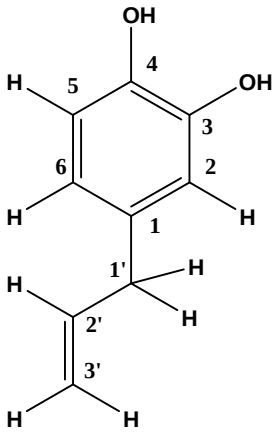


Fig.3.11; Structure of KC-5

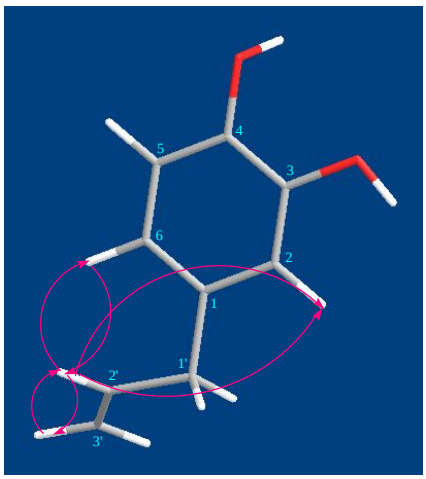
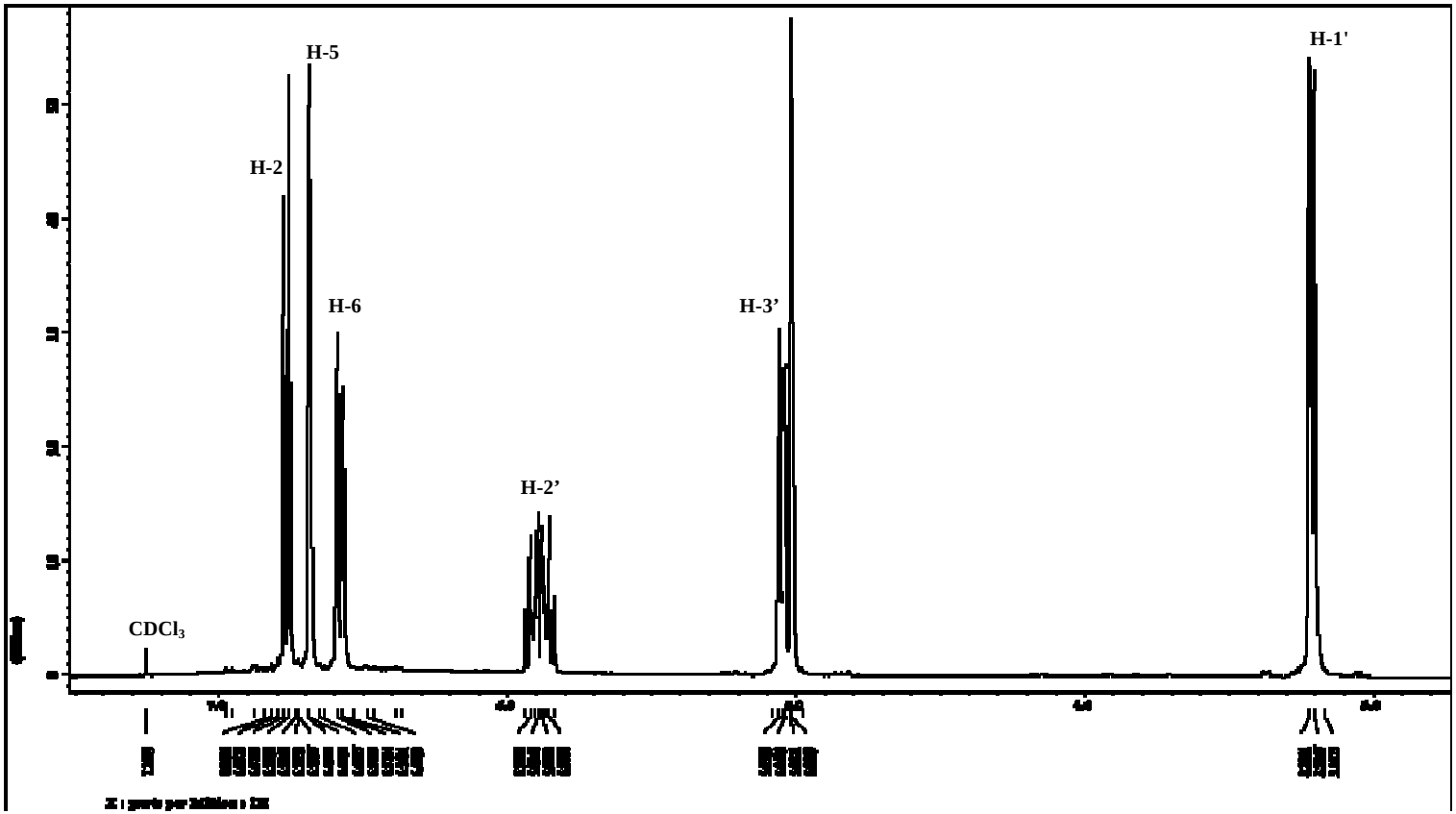
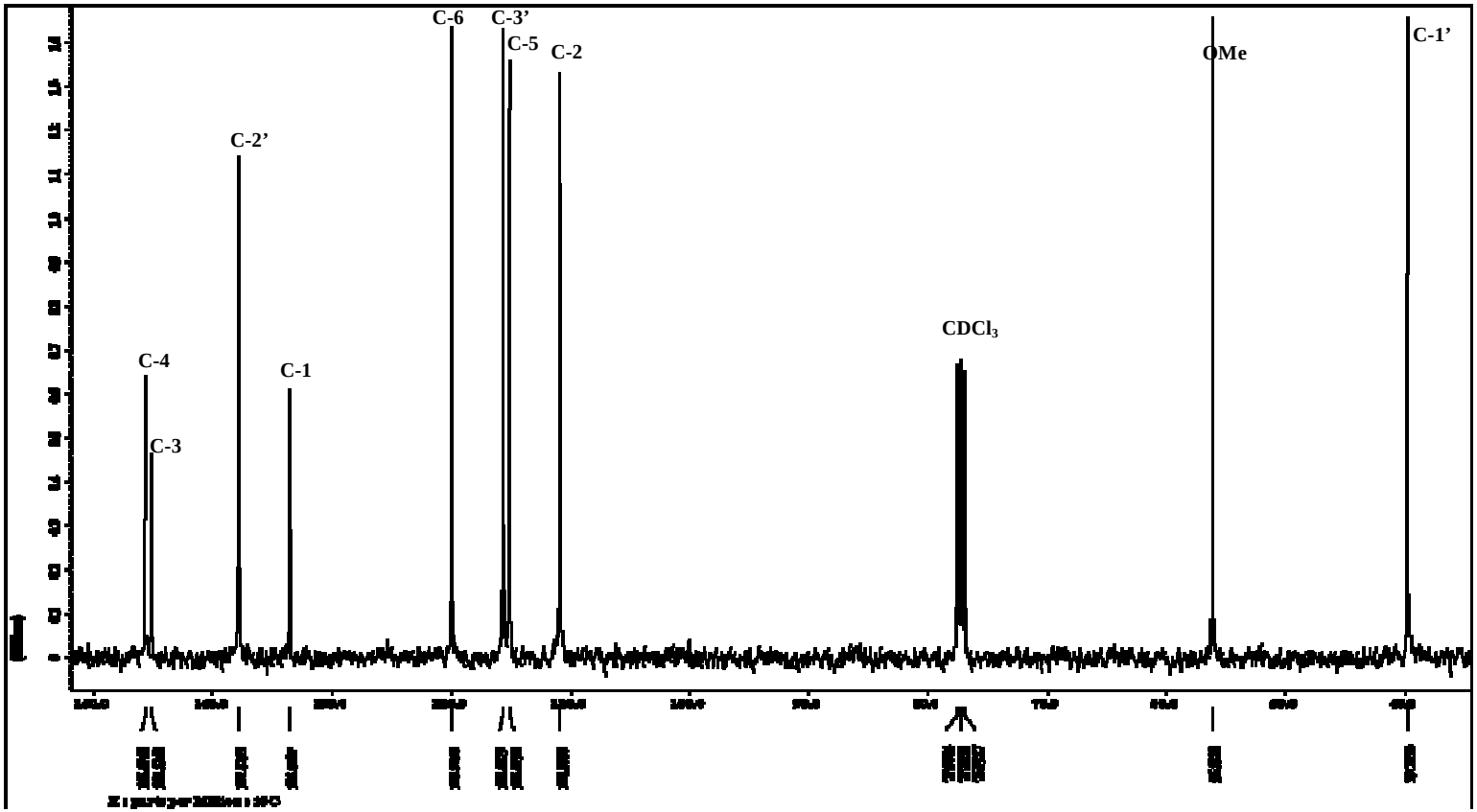


Fig.3.12; 3D Structure and NOE correlations observed for KC-5



Spectrum 3.6; ^{1}H (400MH_z) NMR spectrum of KC-5 in $CDCl_3$



Spectrum 3.7; ^{13}C (100MH$_z$) NMR spectrum of KC-5 in $CDCl_3$

3.1.6 Characterisation of KC-6 as a mixture of trans-calamenene (KC-6a) and β-elemene (KC-6b)

KC-5 was obtained as colourless oil from *Piper betel* dried leaves. It was active under short wavelength UV light and it showed a colourless spot on TLC after spraying with *p*-anisaldehyde-sulfuric acid followed by heating at 120^0 C.

The EIMS spectrum of KC-6a presented a molecular ion at m/z 202[M^+](9), 202, 159, 144, 129, 115, 105, 91; HR-EIMS found m/z 202.1696[M^+] suggesting for a molecular formula of $C_{15}H_{22}$ requires 202.1722.(Nakashima, K., *et al*, 2002).

The ^{1}H NMR spectrum data of KC-6a (Table 3.6) presented doublets at δ 6.94 (J =7.88 H_Z) and δ 7.11(J=7.92 H_Z) and a singlet at δ 7.01 suggesting the presence of aromatic system of three protons, H-2, H-3 and H-5, respectively. The spectrum also showed multiplet between δ 2.72 and δ 2.67, integrating for two protons suggesting for the presence of methine groups.

The COSY spectra also showed the presence of an isopropyl group with correlations between proton at δ 2.23 (H-11) to two methyls at δ 0.70 (H-12) and δ 0.99(H-13). The methyl at δ1.25 (Me-14) showed its correlation to the proton at δ 2.71 (H-10) confirming its situation at position 10. The correlation between two methylenes at δ 1.93 (H-8) and δ 1.59 (H-9) and the aromatic protons at δ 6.94 (H-2) coupled to δ 7.11 (H-3) presenting this compound possesed two six membered rings which one of them was an aromatic ring.

The ^{13}C NMR spectrum data of KC-6a showed 15 carbon signals, suggesting a sesquiterpene. Six aromatic carbons were found at δ 140.1, δ 140.0, δ 134.8, δ 126.2, δ 126.8 and δ 128.8. Four methyl carbons were present at δ17.4, δ 21.5, δ 22.4 and δ 21.3. Two methylene signals showed at δ 21.1 and δ 30.9 and two methane carbon signals were seen at δ 43.8 and δ 32.5.

The COSY spectrum showed correlation between protons at δ1.59 and δ1.93 suggesting for methylenes H-8 and H-9, respectively. The cross peak between δ0.70 and δ0.99 to δ 2.23 suggesting for two isopropyl methylenes Me-12 and Me-13, respectively.

The HMBC spectrum of KC-6a, aromatic protons at δ6.94 (H-2) showed ^{2}Jcorrelation to δ140.1(C-1) and at δ7.11 (H-3) to δ 134.5 (C-4). The proton at δ7.01 (H-5) presented ^{2}Jcorrelation to δ140.0 (C-6) and ^{3}Jcorrelation to δ21.3 (C-15). The methyl at δ 1.25 (Me-14) showed ^{2}J and ^{3}J correlation respectively to δ32.5 (C-10) and δ140.1 (C-1) (Fig. 3.14). These suggested this compound had a six membered aromatic ring with connections to another ring.

On the basis of the above results and by comparison with previous reported data of this compound which has previously been isolated from *Chamaecyparis nootkatensis* (Anderson, N., H., *et al*, 1972) and the flowering aerial parts of *Rosa abyssinica* (Kheyrodin, H., 2009), KC-6a was identified as *trans*-calamenene (Fig. 3.13) and this is the first report of its isolation from the dried leaves of *Piper betel*.

The IR spectrum of KC-6b indicated the presence of O-H stretching (2956.8, 2930.3, 2870.0cm^{-1}), C=C stretching (1717.3, 1669.6, 1646.9 cm^{-1}), 1449.2, 1385.1, 1367.8 and C-O bending (884.2 cm^{-1}) (Wong, K., *et al*, 2005).

The MS spectrum of KC-6b presented fragmentations of 53, 61, 81, 93, 107, 121, 133, 147, 161, 175, 189 and 204 indicated for m/z 204 and a molecular formula of $C_{15}H_{24}$ (Wong, K., *et al*, 2005).

The ^{1}H NMR spectrum of KC-6b (Table 3.7) revealed the presence of three exomethylenes with protons at δ 5.82 (J=10.98, 17.14 H$_z$), δ 4.91(J=5.8 H$_z$) and δ 4.84(J=6.6 H$_z$) and two singlets at δ 4.58 and δ 4.81. Three methyl signals occurred as singlets at δ 1.74, δ 1.70 and δ 0.98.

The ^{13}C NMR spectrum sgowed fifteen carbon signals suggesting a sesquiterpenes including three methines at δ 52.8, δ 45.7 and an exomethine at δ 150.4, three exomethylene signals at δ 109.9, δ 112.1 and δ 108.3 and three quaternary carbon signals at δ 39.9, δ 150.4 and δ 147.8, three methylenes at δ 32.9, δ 26.9, δ 39.9 and three methyl groups at δ 21.1, δ 24.8 and δ 16.7.

In COSY spectrum, the exomethylene protons showed the correlations between δ5.82 (H-1) and δ4.90 (H-2) and another correlation at δ4.81 (H-3) to δ1.70 (Me-14). The isopropyl proton at δ 1.74 (Me-13) exhibited the correlation to an exomethylene δ 4.84 (H-12). These results confirmed that this compound three exomethylene groups as its side chains and two of them were isopropylene groups.

The HMBC spectrum (Fig. 3.11), the olefinic methine at δ5.82 (H-1) showed ^{3}Jcorrelation to carbon at δ39.9 (C-9) and ^{2}Jcorrelation to signal at δ39.9 (C-10). The exomethylene proton at δ5.72 (H-2) represented ^{2}Jcorrelation and ^{3}Jcorrelation to δ150.4 (C-1) and δ39.9 (C-10), respectively. Another exomethylene proton at δ 4.81(H-3) had ^{3}Jcorrelation to δ52.8 (C-5) and δ24.8 (Me-14). The methylene proton at δ 1.59 presented ^{2}Jcorrelations to δ52.8 (C-5) and δ1.93 (H-7) and also showed long range coupling to the methyl carbon at δ21.1 (Me-13) (Fig. 3.16). These suggested the presence of isopropenyl side chains connecting to a cyclohexane ring.

From these results and compared to previous published data, this coumpound was isolated from the roots and stems of *Curcuma wenyurijin* (Wong, K., *et al*, 2005). KC-6b was identified as β-elemene (Fig. 3.15) and this is the first report of its isolation from the dried leaves of *Piper betel*.

Table 3.5: ^{1}H (400MH_Z) in $CDCl_3$ and ^{13}C (100MH_Z) in $CDCl_3$ NMR data of KC-6a (trans-calamenene)

Position	δ_H	δ_C	COSY	Selected HMBC correlations
KC-6a				
1	-	140.1		
2	6.94(1H, *d*, 7.88)	126.8	H3	C1
3	7.11(1H, *d*, 7.92)	126.2	H2	C4
4	-	134.5		
5	7.01(1H, brs)	128.8		C15, C6
6	-	140.0		
7	2.67(1H, *m*)	43.8		
8	1.59(1H, *m*)/1.82(1H, *m*)	21.1	H9α	
9	1.93(1H, *m*)/1.33(1H, *m*)	30.9	H8α	
10	2.71(1H, *m*)	32.5	Me14	
11	2.23(1H, *m*)	32.0	Me12, Me13	
12	0.70(3H, *d*, 7.04)	17.4		
13	0.99(3H, *d*, 7.04)	21.5		
14	1.25(3H, *d*, 6.60)	22.4		C1, C10
15	0.99(3H, *s*)	21.3		

Chemical shifts (*ppm*.), Coupling constants (H_Z)

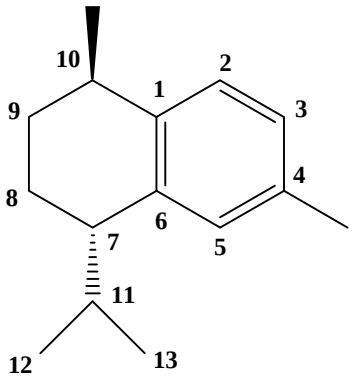


Fig.3.13; Structure of KC-6a

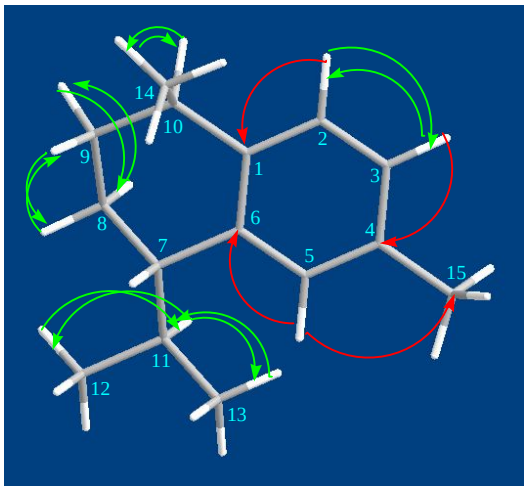
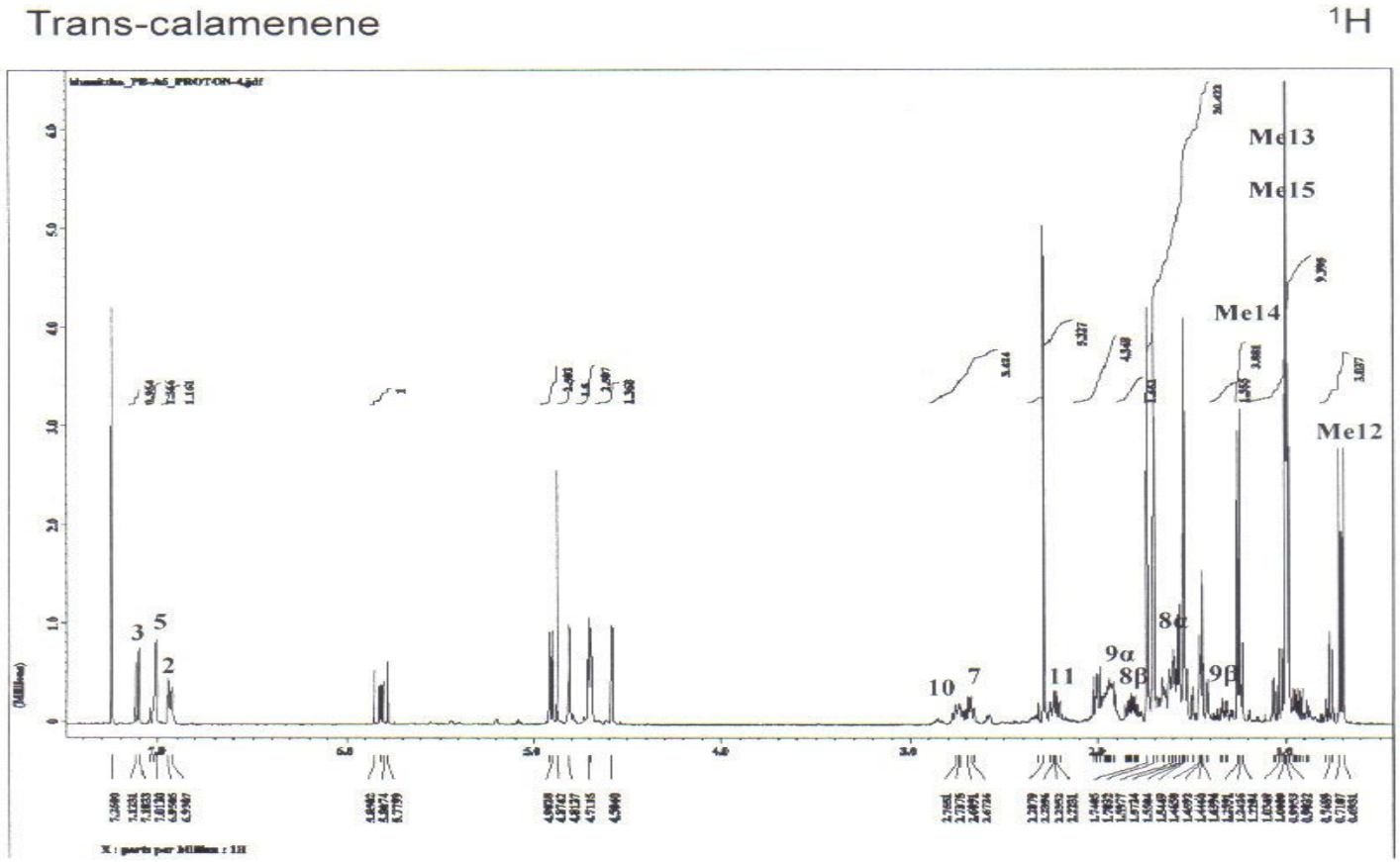
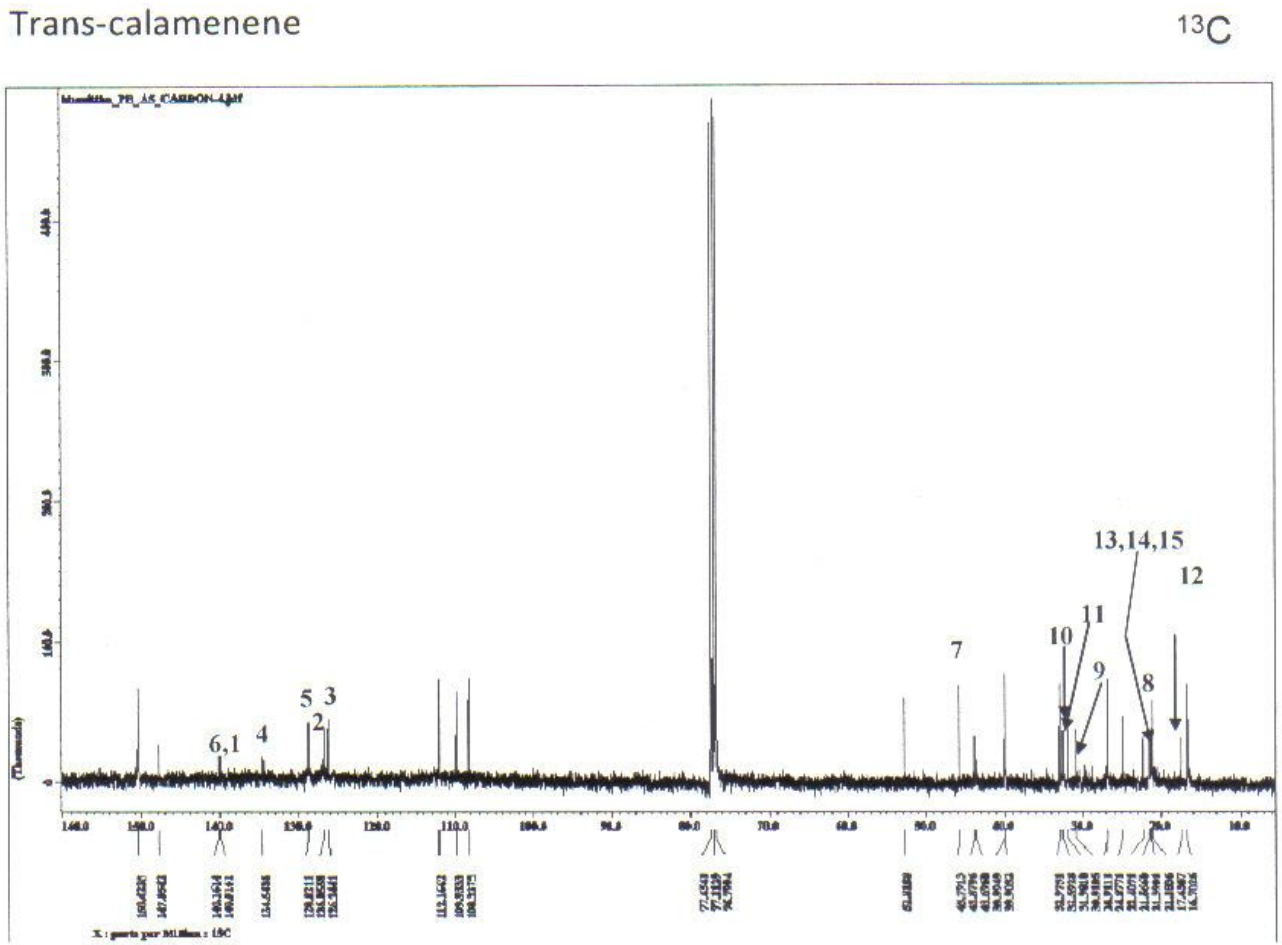


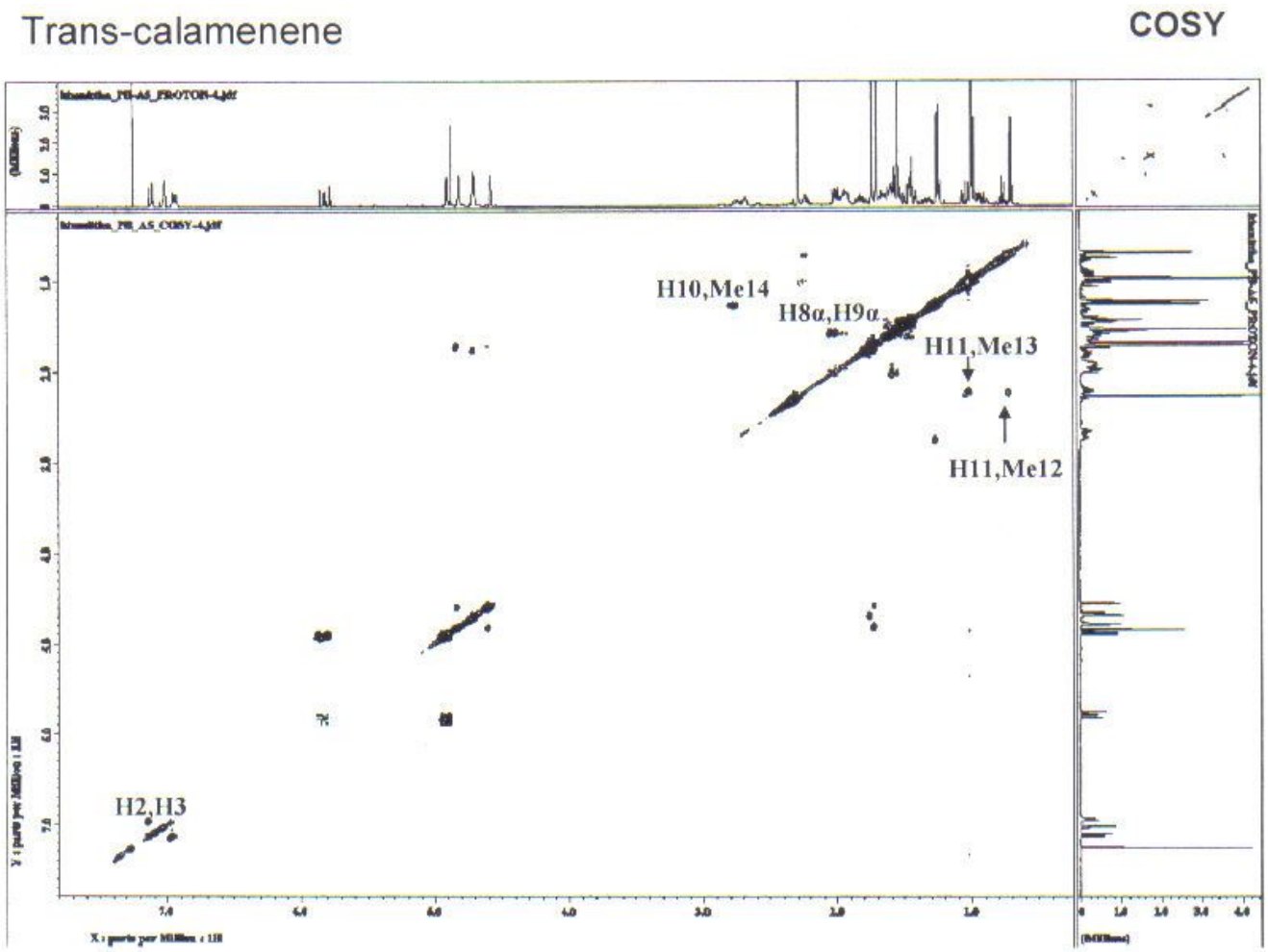
Fig.3.14; 3D Structure of KC-6a with representing for HMBC correlations and for COSY correlations



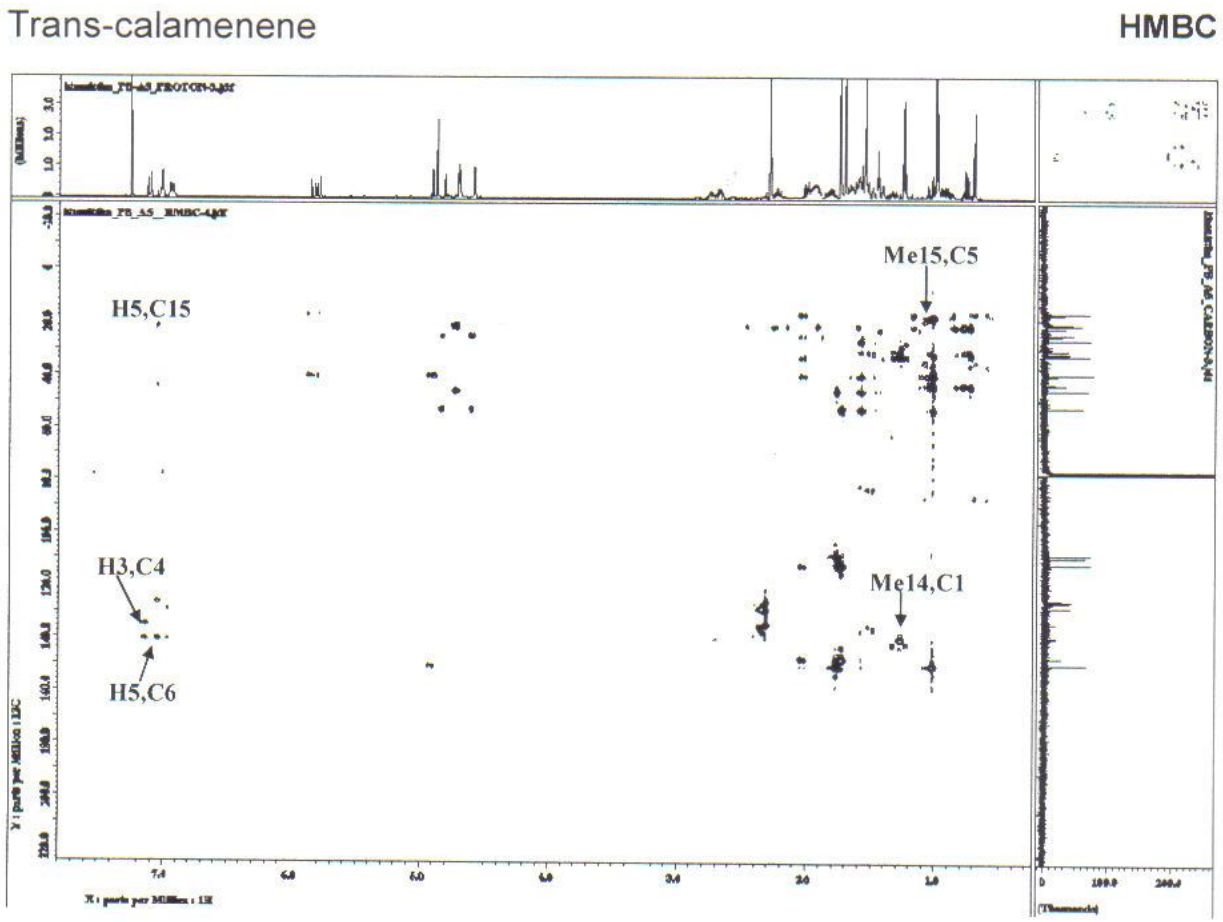
Spectrum 3.8; ^{1}H (400MH_z) NMR spectrum of KC-6a $CDCl_3$



Spectrum3.9; ^{13}C (100MH$_z$) NMR spectrum of KC-6a in $CDCl_3$



Spectrum 3.10; COSY correlations spectrum of KC-6a in $CDCl_3$



Spectrum 3.11; HMBC correlations spectrum of KC-6a in $CDCl_3$

Table 3.6: ^{1}H (400MH_Z) in $CDCl_3$ and ^{13}C (100MH_Z) in $CDCl_3$ NMR data of KC-6b

Position	δ_H	δ_C	COSY	Selected HMBC correlations
1	5.82(1H,*dd*,10.98,17.14	150.4	H2	C10,C9
2	4.9,5.721(2H,*d*,5.72)	109.9	H1	C1,C10
3	4.58,4.81(2H,*d*,7.92)	112.1	Me14	C14, C5
4	-	147.8		
5	2.00(1H*m*)	52.8		Me15, C10
6	1.59(2H,*m*)	32.9		C7,C5
7	1.93(1H,*m*)	45.7		Me13
8	1.46(2H,*ddd*,2.64,2.64,2.64))	26.9		
9	1.43(2H,*dd*,2.64,3.04)	39.9		
10	-	39.9		
11	-	150.5		
12	4.84(2H,*d*,6.6)	108.3	Me13	C7
13	1.74(3H,*s*)	21.1	Me12	C12,C7
14	1.70(3H,*s*)	24.8		C5
15	0.98(3H,*s*)	16.7		C1,C5,C10

Chemical shifts (*ppm*.), Coupling constants (H_Z)

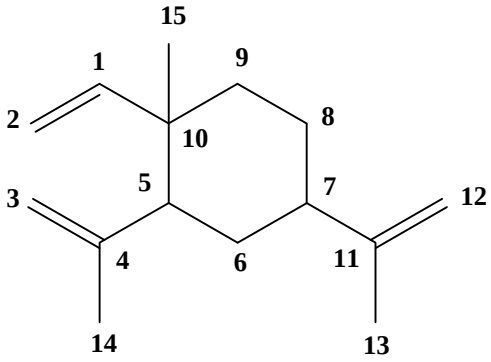


Fig.3.15; Structure of KC-6b

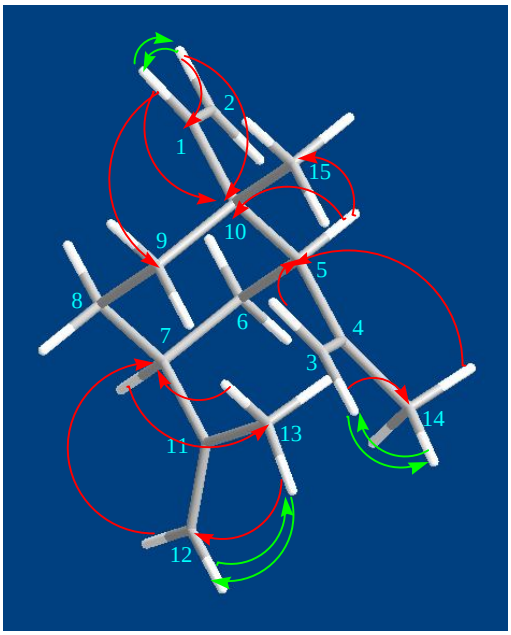
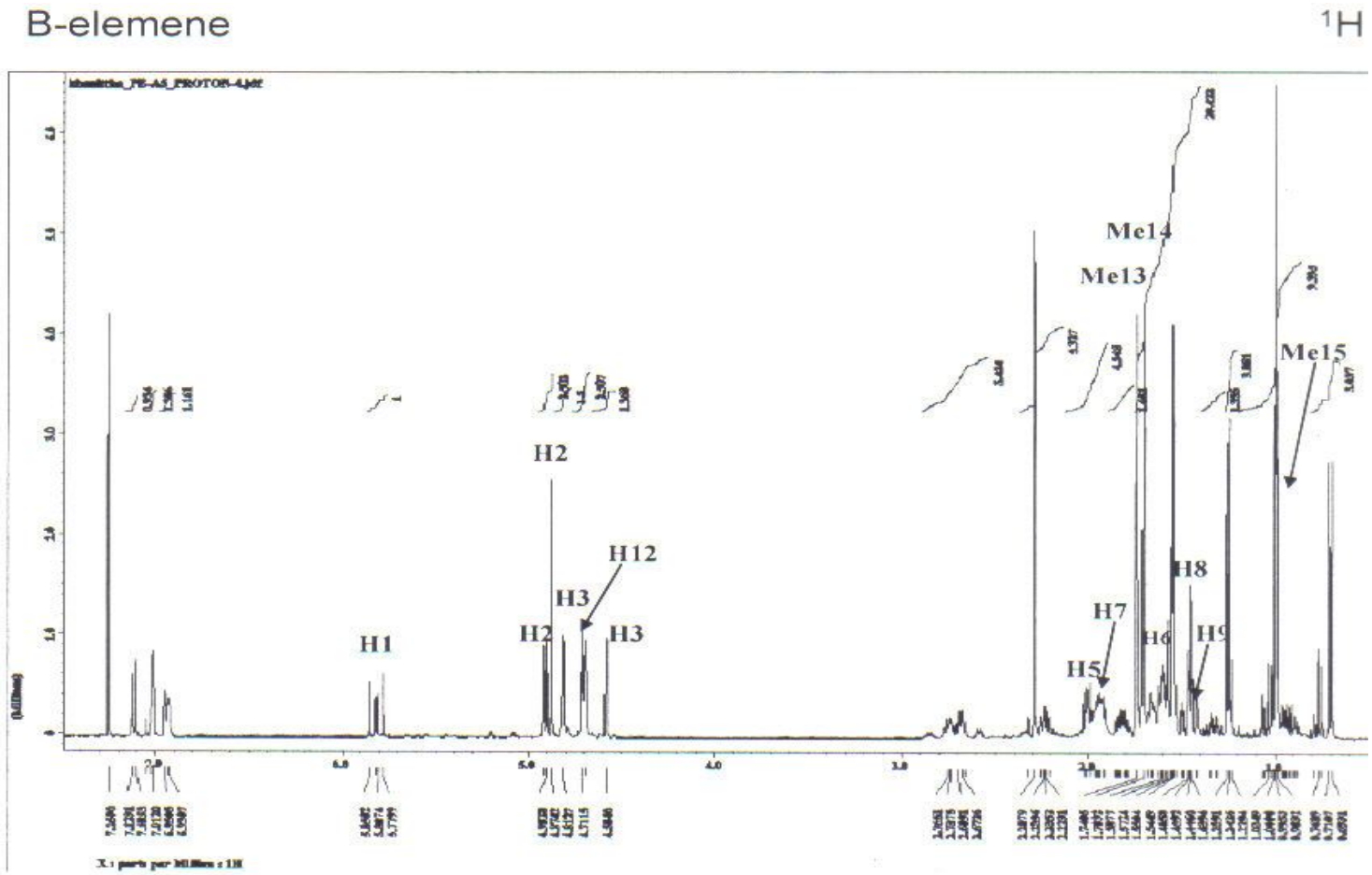
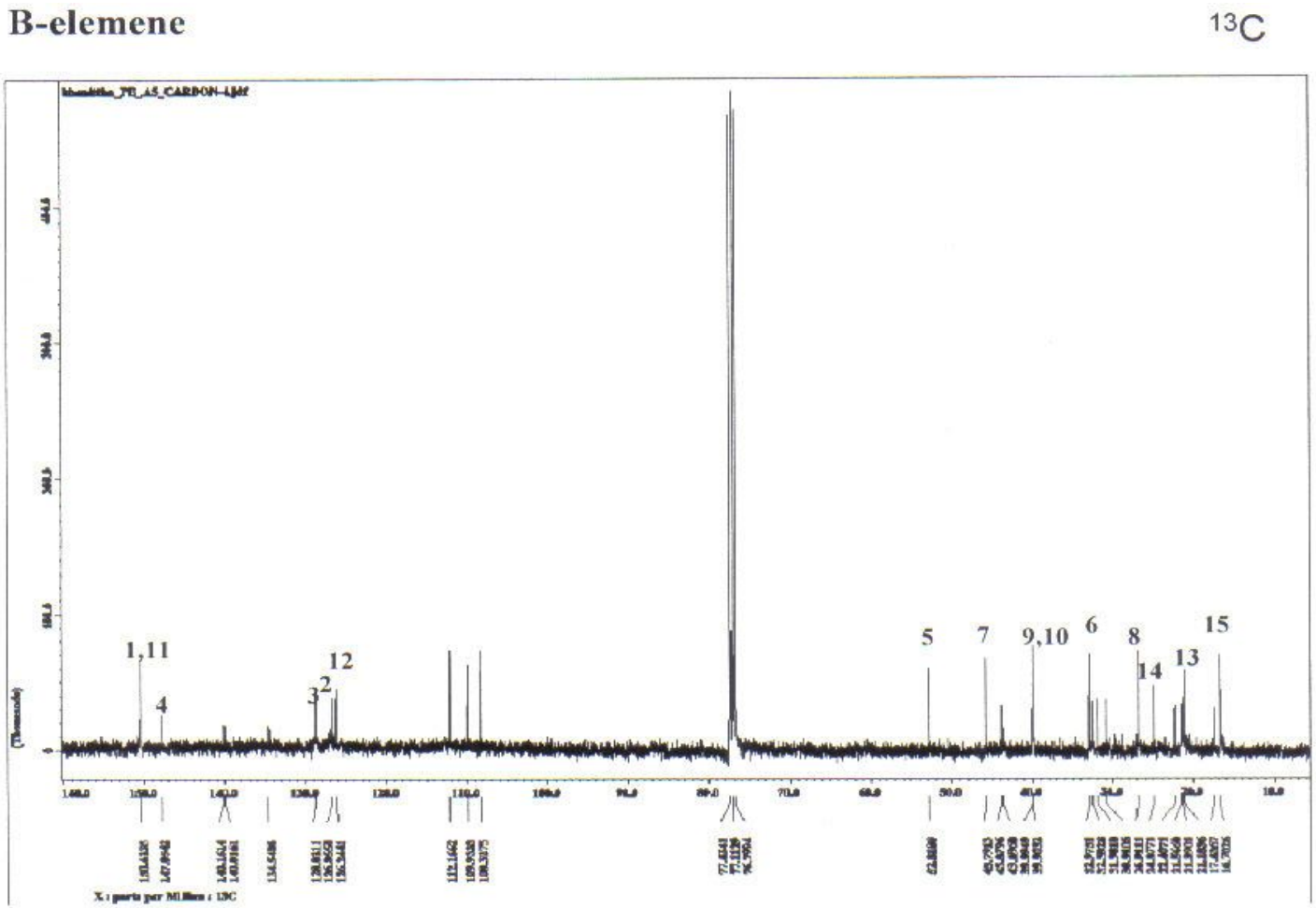


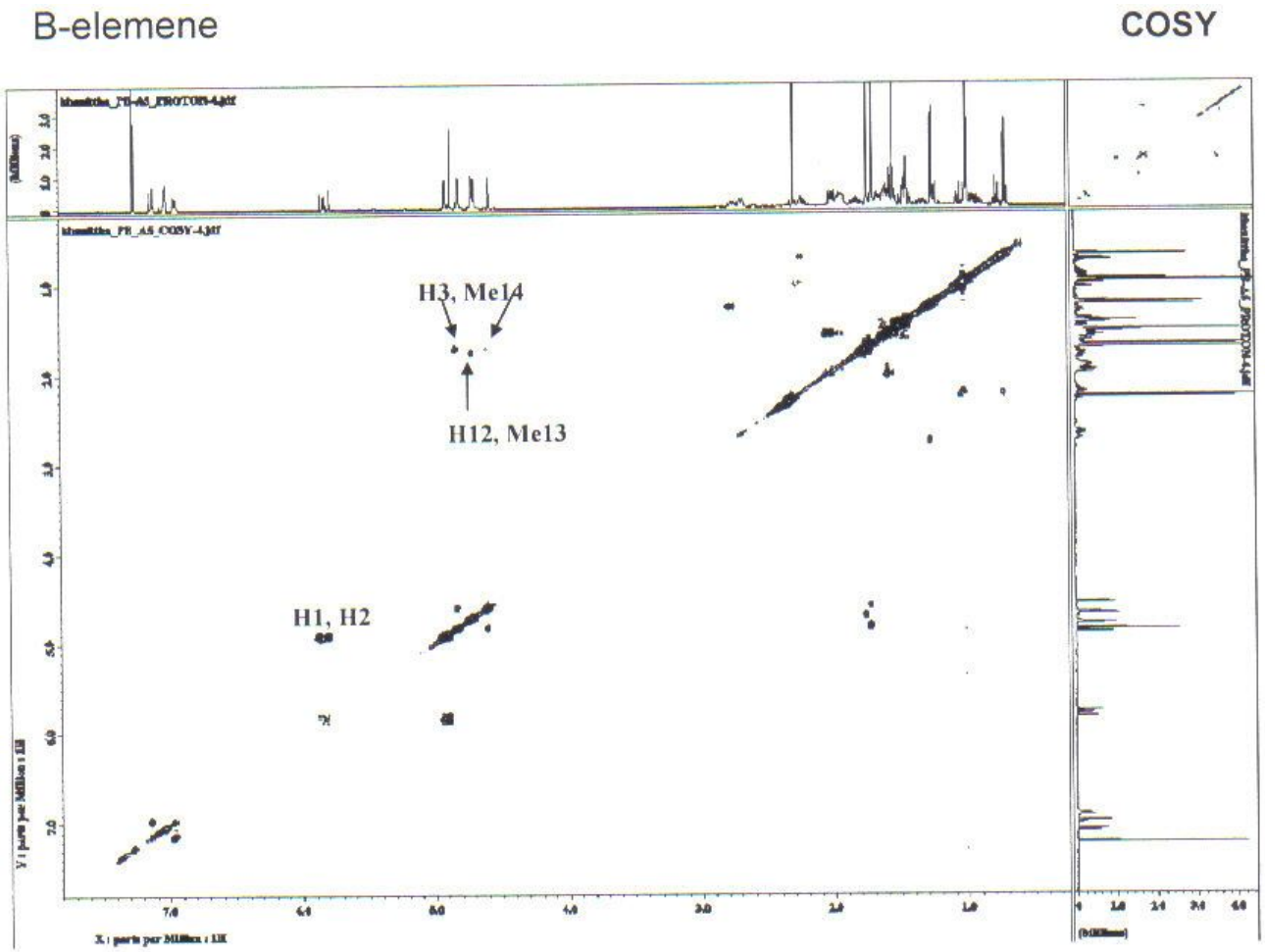
Fig.3.16; 3D Structure of KC-6b with representing for HMBC correlations and for COSY correlations



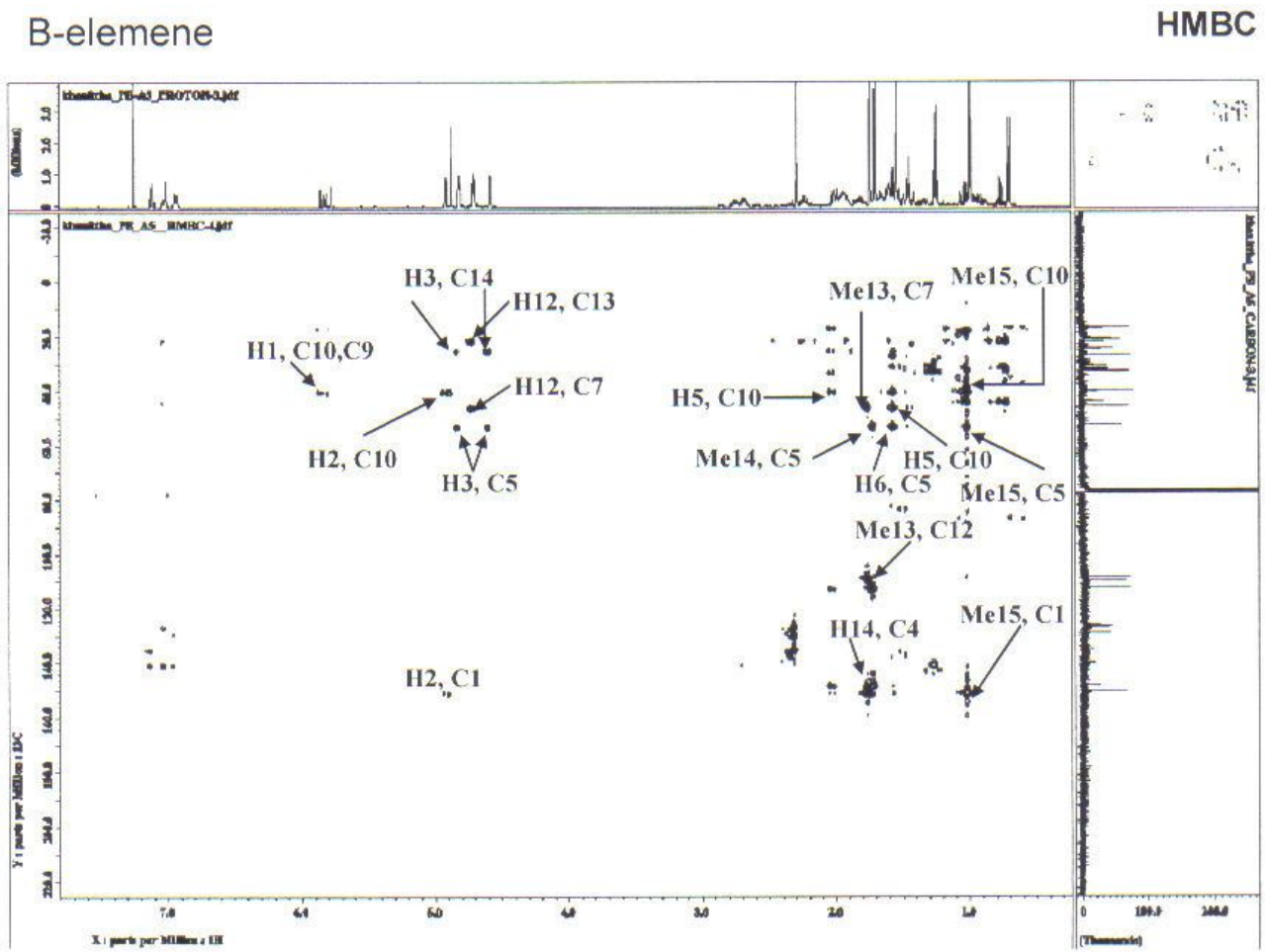
Spectrum3.12; ^{1}H (400MH$_z$) NMR spectrum of KC-6b in CDCl$_3$



Spectrum 3.13; ^{13}C (100MH_z) NMR spectrum of KC-6b in $CDCl_3$



Spectrum 3.14; COSY correlations spectrum of KC-6b in $CDCl_3$



Sp ectrum 3.15; HMBC correlations spectrum of KC-6b in $CDCl_3$

3.1.7 Characterisation of a mixture of KC-7 as γ-muurolene

KC-7 was obtained as colourless oil from a hexane phase, partitioned from ethanol extract of *Piper betel* dried leaves. The compound was active under short wavelength UV light and it showed a colourless spot on TLC after spraying with *p*-anisaldehyde-sulfuric acid followed by heating at 120^0 C**.**

The MS spectrum presented fragmentations of 41, 55, 67, 81, 91, 105, 119, 133, 147, 161 and 204 indicated for m/z 204 and a molecular formula of $C_{15}H_{24}$ (Leitậo, S., G., et al, 2008).

The ^{1}H NMR spectrum data (Table 3.11) represented an olefinic proton at *δ*5.56 (*d*, J =4.5H_Z), an exomethylene group at *δ*4.60. A signal at *δ*2.55 (J =13.2 H_Z) presented a cis coupling between *δ*2.55 (H5) and *δ* 5.56(H4). Two doublets at *δ*0.92 (J =7.04 H_Z) and *δ*0.79 (J =7.04 H_Z) were assigned for *δ*0.92 (Me12) and *δ*0.79 (Me13) coupled to a methine *δ*1.67 (H11), respectively presented an isopropanyl group in this structure. An olefinic methyl showed at *δ*1.69.

The ^{13}C NMR spectrum data showed fifteen carbon signals, assigned by DEPT experiment presented as two quaternary carbons at δ 133.91 and 154.34, four methines at δ39.78, δ44.8, δ43.58 and δ 23.9, an olefinicmethine at δ 124.62, four methylenes at δ 25.4, δ 31.68, δ 25.90, δ 30.91, and an exomethylene carbon signal at δ106.61, three methyl signals at δ 21.7, δ 15.5 and δ 26.7

The HMBC spectral data, olefinic proton at *δ*5.56 showed ^3J correlations to allylic protons at *δ*39.78 and *δ*31.68. The methyl proton at *δ* 1.69 presented ^3J correlations to two carbons at *δ*31.68 and *δ*124.62 and ^2J correlations to the carbon signal at *δ*133.91. The methyl proton at *δ*0.92 displayed ^3J correlations to carbons at *δ*15.5. The methyl signal at *δ*0.97 showed ^3J correlations to *δ*21.7 ans *δ*44.8. The olefinic proton at *δ*4.60 presented ^3J correlations to carbon signals at *δ*30.91 and *δ*43.58 and a long range coupling to the carbon at *δ*31.68. The allylic proton at *δ* 2.70 exhibited ^3J correlations to the carbon at*δ* 44.8. These can be suggested that this compound

obtained an isopropanyl group situated at C-6, a methyl group located at C-3 and the exomethylene connected between C-8 and C-10 (Fig. 3.18).

The evidence showed above and by comparison with previous reported data was found this compound from the leaves and the flowers of *Lippia lacunose* Mart&Schauer and *Lippia rotundifolia* Cham (Verbeneceae) (Leitậo, S., G., et al, 2008). Thus, KC-7 was identified as gamma-muurolene (Fig. 3.17) and it is the first time for its isolation the dried leaves of *Piper betel*.

Table 3.7: ^{1}H (400MH_Z) in $CDCl_3$ and ^{13}C (100MH_Z) in $CDCl_3$ NMR data of KC-7

Position	δ_H	δ_C	Selected HMBC correlations
1	2.00(2H,*m*)	25.4	
2	2.09(2H,*m*)	31.68	
3	-	133.91	
4	5.56(1H, *d*, 4.48)	124.62	C2, C5
5	2.55(1H,*d*,13.2), 1.95(1H,*m*)	39.78	
6	1.42(1H,*m*)	44.8	
7	0.99(1H,*m*)	25.9	
8	2.70(1H, *dq*, 2.2, 3.08)	30.91	C6
9	-	154.34	
10	2.38(1H, *m*)	43.58	
11	1.67(1H, *m*)	23.9	
12	0.92(3H,*d*,7.04)	21.7	C13, C6
13	0.79(3H,*d*, 7.04)	15.5	C12, C6
14	4.60(1H,*s*), 4.66(1H,*s*)	106.61	C8, C10, C2
15	1.69(3H,*s*)	26.7	C2, C4, C3

Chemical shifts (*ppm.*), Coupling constants (H_Z)

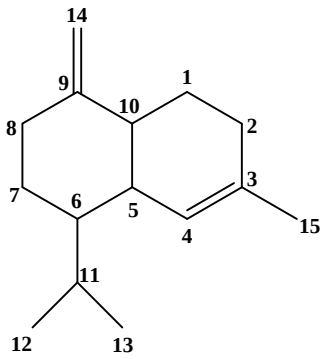


Fig.3.17; Structure of KC-7

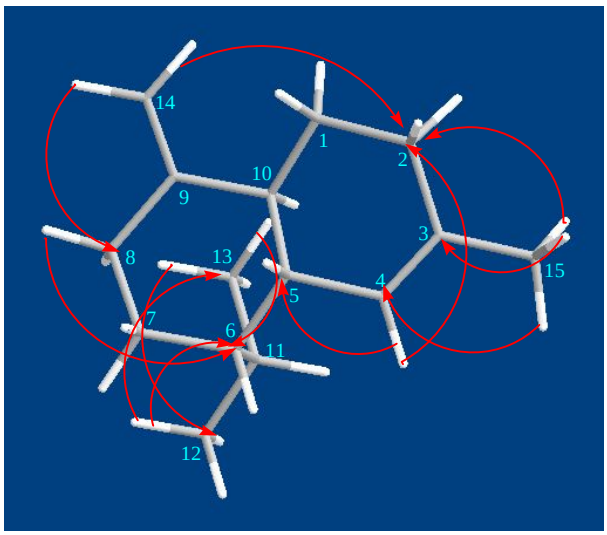
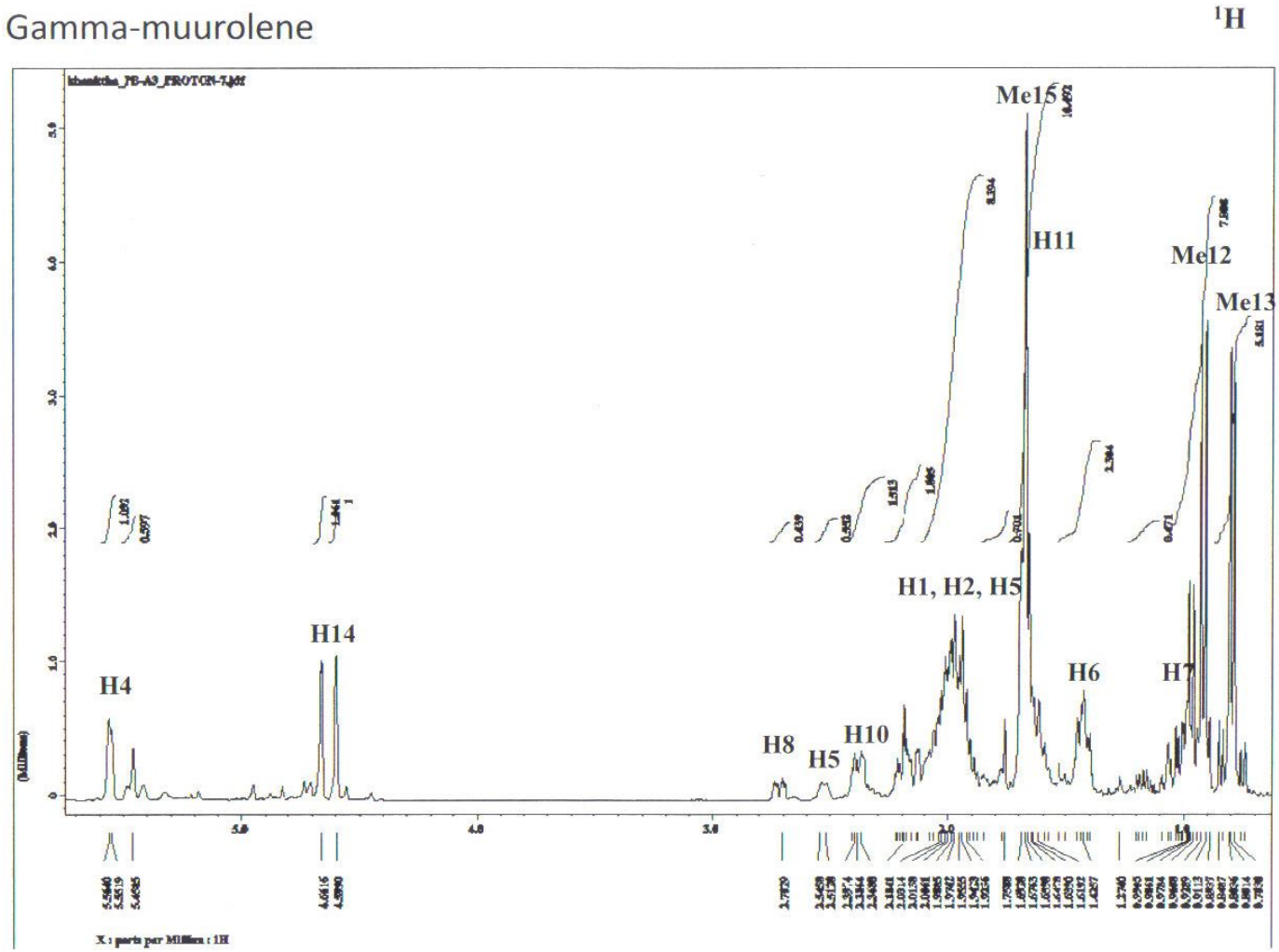
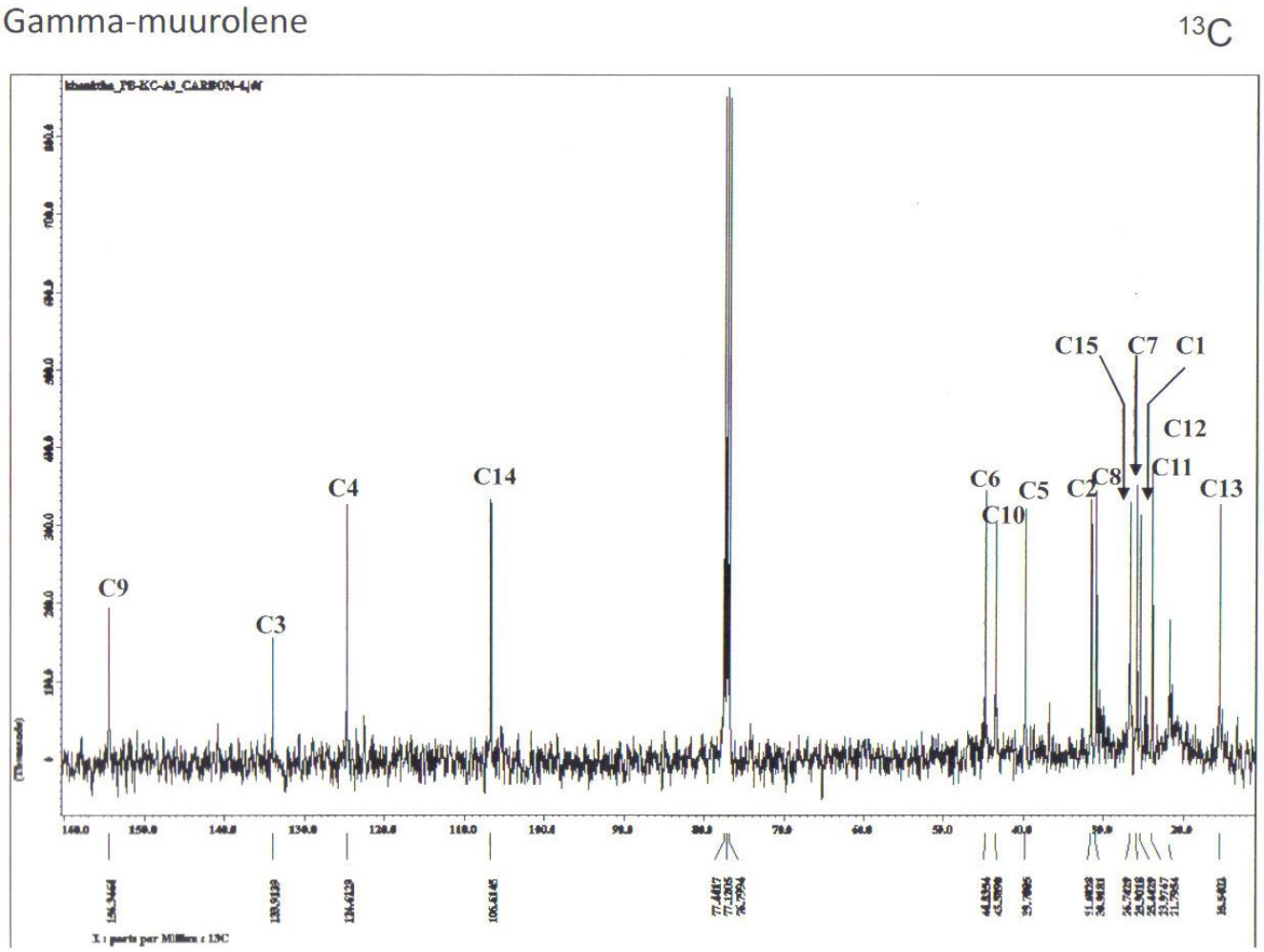


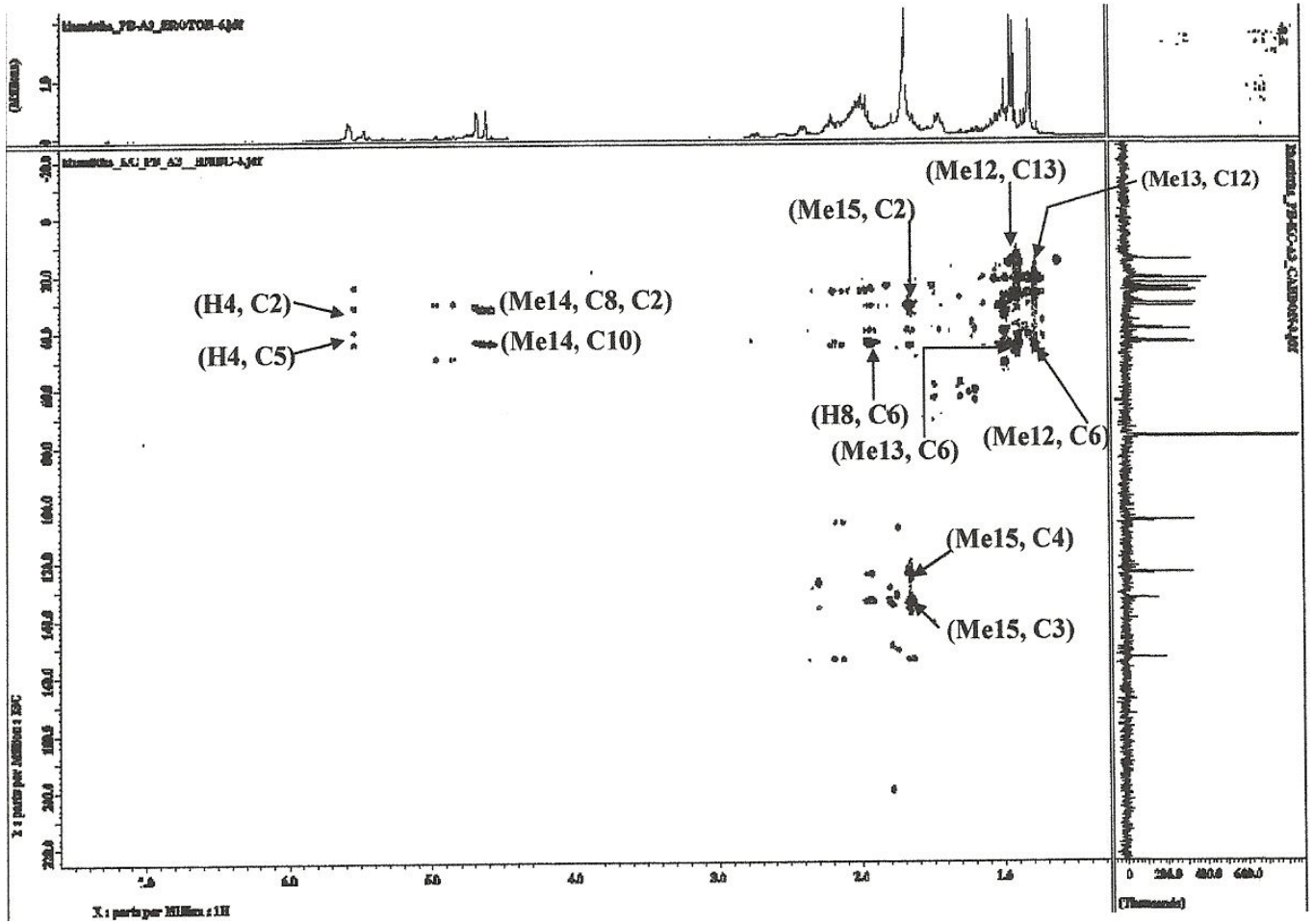
Fig.3.18; 3D Structure of KC-7 with representing for HMBC correlations



Spectrum 3.16; ^{1}H (400MH$_z$) NMR spectrum of KC-7 in CDCl$_3$



Spectrum 3.17; ^{13}C (100MH_z) NMR spectrum of KC-7 in $CDCl_3$



Spectrum3.18; HMBC correlations spectrum of KC-7 in $CDCl_3$

3.1.8 Characterisation of triterpenes

Seven triterpenes were isolated in this study. All isolated compounds were active under short wavelength UV light and displayed a purple-pink colour on TLC after spraying with *p*-anisaldehyde-sulfuric acid followed by heating.

The NMR analysis of the taraxerene-type triterpenes revealed similar features which were the presence of eight methyl signals resonating as singlets in the range δ 0.81-1.12, one olefinic signal (δ_H 5.55 and δ_C 117) and one quaternary carbon centre (δ_C 157.8) .

3.1.9 Characterisation of KC-8 as a mixture of taraxerol (KC-8a) and cis-careaborin (KC-8b)

KC-8 was obtained as a white solid from the ethyl acetate extract of *Bruguiera gymnorrhiza* leaves.

The ^{1}H NMR spectrum data (Table 3.12) showed the signals of typical triterpenes mentioned above associated with the signals observed at δ 7.60 (2H, *d*, *J*=8.4 H_Z) and δ 6.77 (2H, *d*, *J*=8.8H_Z) indicated the presence of a para-disubstituted aromatic ring. The signals found at δ 5.80(1H, *d*, *J*=12.7 H_Z) and δ 6.80(1H, *d*, *J*=13.2 H_Z) attributed for a double bond H-2' and H-3'. The low field double doublet signal at δ 4.50 (1H, *J*=3.1, 7.9 H_Z) presented the esterified oxymetine proton at C-3. The oxygen-bearing carbon centred at δ 3.19 (1H, *dd*, *J*=4.4, 11 H_Z). The olefinic proton observed at δ 5.52 (1H, *dd*, *J*=3.08, 7.92H_Z) and the methyl signals at between δ 0.80-1.09 were found with double of their integrations. These suggested this compound was a mixture of careaborin and taraxerol.

3.1.10 Characterisation of KC-8a as taraxerol

The ^{1}H NMR signals described above indicated the presence of typical pentacyclic triterpene skeleton. The oxygen-bearing carbon with a proton centred at δ 3.19 (1H, *dd*, *J*=4.4, 11 H_Z) assigned for the oxymethine proton at C-3 with its large coupling with the vicinal methylene protons suggested a β orientation. The olefinic group showed at δ 5.52 (1H, *dd*, *J*=3.08, 7.92H_Z) and the methyl signals presented at

between δ 0.80-1.09. The data of this compound was close to taraxerone, except the resonace at δ 3.19 of the oxymethine proton C-3 which was absent in taraxerone.
From the data given and by comparison to those of taraxerone **(KC-11)** also isolated in this study, KC-8a was identified as β-taraxerol (Fig. 3.19 and 3.20).

3.1.11 Characterisation of KC-8b as careaborin

KC-8b was obtained as a white solid from the ethyl acetate extract of *Bruguiera gymnorrhiza* leaves parts.

The IR spectrum exhibited the presence of hydroxyl group (3360 cm^{-1}), C-H stretching (2935, 2855 cm^{-1}), carbonyl (1704 cm^{-1}), C=C stretching (1604 cm^{-1}), 1513, 1454, C-H bending (1375 cm^{-1}) and C-O stretching (1167 cm^{-1}) (Jutiviboonsuk, A., 2006).

The LRESIMS experiment showed a pseudo-molecular ion *m/z* at 571 $[M-H]^-$ indicating for a molecular ion at *m/z* 572 supporting a molecular formula of $C_{39}H_{56}O_3$ (Jutiviboonsuk, A., 2006).

The ^1H NMR spectral data (Table 3.12 and 3.13) showed signals typical for a taraxerene-type triterpenoid as aforementioned. The presence of signals at δ 7.60 (2H, *d*, *J*=8.4 H_Z) and δ 6.77 (2H, *d*, *J*=8.8H_Z) indicated the presence of a para-disubstituted aromatic ring.The first signal resonated lower field than the later because of the anisothopic effect from by α,β-unsaturated carbonyl nearby. The signals found at δ 5.80(1H, *d*, *J*=12.7 H_Z) and δ 6.80 (1H, *d*, *J*=13.2 H_Z) were attributed to olefinic protons with the small coupling constant indicating a cis-configuration. The low field signal at δ 4.50 (1H, *dd*, *J*=3.1, 7.9 H_Z) was attributed to an esterified carbinolic proton at C-3, and with its small coupling constant, assigned to the configuration.

The ^{13}C NMR spectrum showed 39 signals. The multiplicity determined by HMQC techniques presented 8 methyl groups, 10 ethylene groups, 10 methine groups and 11 quaternary groups. A lower field signal resonating at δ 81.1 suggested that position

C-3 was esterified and also observed another carbon signal at δ 79.2 inferring oxymethine carbon. These defined the compound as a mixture of compounds. The carbon signal which showed at δ167.5 was assigned to the carbonyl group. The double bond signals appeared at δ158.1 and δ116.9. The para hydroxyl substituted carbons observed at low field resonating at δ130.0 affected by the α,β-unsaturated carbonyl group suggesting two aromatic carbons C-5 and C-9. The other aromatic carbon signals showed at δ127.4 and δ115.6.

The COSY spectrum data presented this cross peak between δ5.52 and δ1.90, assigned for a trisubstituted double bond. Other strong peaks revealed the connections at δ6.31 (H-2') to δ7.60 (H-3') of an α, β-unsaturated carbonyl group.

The HMBC spectrum data, the α-carbonyl proton at δ5.80 (H-1') showed ^{2}J correlation to the carbon signal at δ 167.5 and ^{3}J correlations to the signal at δ 127.4 suggesting an unsaturated double bond connected between the carbonyl and aromatic groups. The carbonyl proton at δ6.80 presented ^{3}J correlation to two signals at δ132.4 confirming its connection to aromatic ring. The aromatic protons at δ7.6 showed ^{2}J correlation to the signal at δ127.4 and ^{3}J correlation to signals at δ130.0. Other aromatic protons at δ7.60 presented ^{3}J correlation to the carbon at δ127.4. These indicated the having of the the phenolic group.

The methyl protons at δ0.80 and δ1.09 presented ^{3}J correlations to the carbon signal at 158.1 suggesting the double bond situated at C-14 and C-15. The germinal methyls protons at δ0.90 and δ0.94 showed ^{3}J correlations to the carbon signals at δ81.1 and δ55.7 and only the proton at δ0.90 presented a three bonds coupled to the carbon at δ16.8 (Fig. 3.22). These can be referred they bonded at quaternary carbons C-4.

From the evidence given above and also compared to the reported data, this compound was isolated from the fruits of *Rhizophora mucronata* (Jutiviboonsuk, A., 2006) and found from the leaves of *Barringtonia maunwongyathiae* (Jutiviboonsuk,

A., 2007). Thus KC-8a was identified as careaborin (Fig. 3.21) and it is the first report of its isolation from the leaves of *Bruguiera gymnorrhiza*.

Table 3.8: ^{1}H (400MH_Z) and ^{13}C (100MH_Z) NMR data of KC-8b in $CDCl_3$

Position	δ_H	δ_C	Selected HMBC correlations
1		36.7	
2		23.8	
3	3.19(1H,*dd*, 4.4, 11), 4.50(1H,*dd*, 5.95, 10.43)	81.1	
4		36.7	
5		55.7	
6		18.8	
7		33.8	
8		39.8	
9		48.8	
10		37.6	
11		17.6	
12		35.1	
13		38.0	
14		158.1	Me26, Me27
15	5.53(1H, *dd*, 3.52, 8.32)	116.9	
16	1.91(1H, *d*, 14.96)	37.5	
17		35.8	
18		49.3	
19		41.2	
20		28.8	
21		33.1	
22		33.1	
23	0.90(3H,*s*)	28.1	C-3, C-5, C-24
24	0.94(3H,*s*)	16.8	C-3, C-5
25	0.97(3H,*s*)	15.6	C-5
26	1.09(3H,*s*)	26.0	C-8
27	0.80(3H,*s*)	29.8	C-18
28	0.89(3H,*s*)	29.9	C-18
29	0.94(3H,*s*)	33.4	
30	0.89(3H,*s*)	21.4	

Chemical shifts (*ppm*.), Coupling constants (H_Z)

Table 3.9: ^{1}H (400MH_Z) and ^{13}C (100MH_Z) NMR data of KC-8b in $CDCl_3$ (continued)

Position	δ_H	δ_C	Selected HMBC correlations
1’	-	158.1	
2’	5.80(1H, *d*, 12.7)	116.3	C-1’, C-4’
3’	6.80(1H, *d*, 13.2)	144.1	C-5’, C-9’
4’	-	127.4	
5’,9’	6.77(1H, *d*, 8.8)	130.0	
6’,8’	7.60(1H, *d*, 8.4)	115.9	C-4’
7’	-	157.6	

Chemical shifts (*ppm.*), Coupling constants (H_Z)

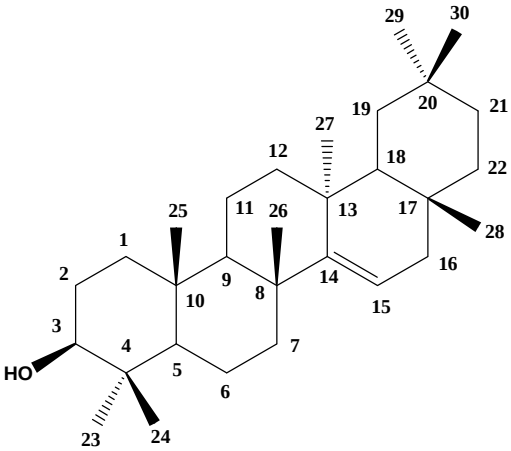


Fig.3.19; Structure of KC-8a

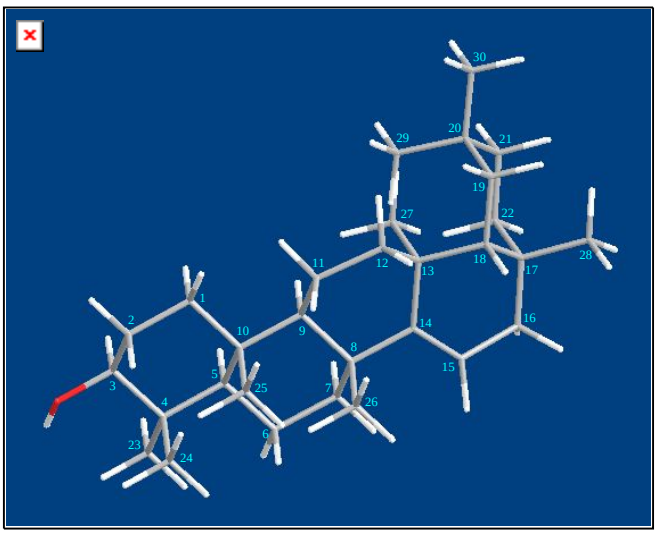


Fig.3.20; 3D Structure of KC-8a

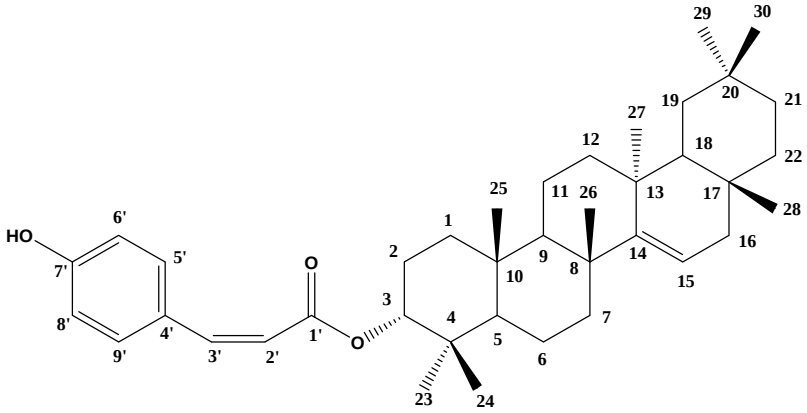


Fig.3.21; Structure of KC-8b

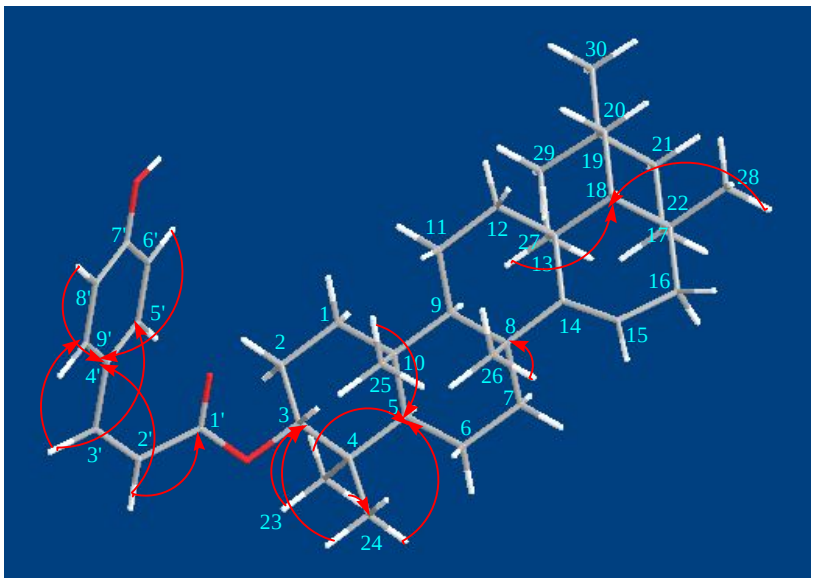
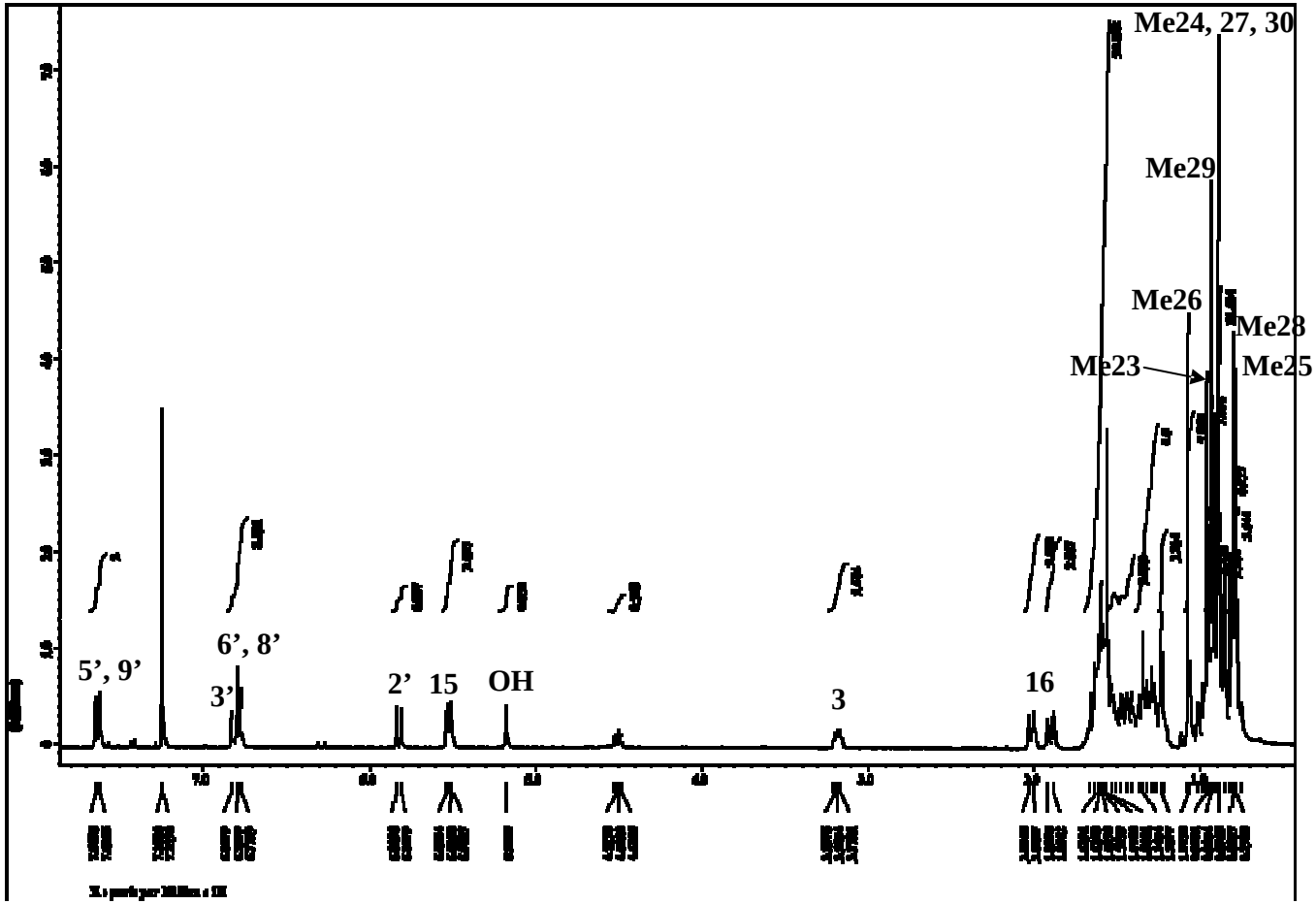
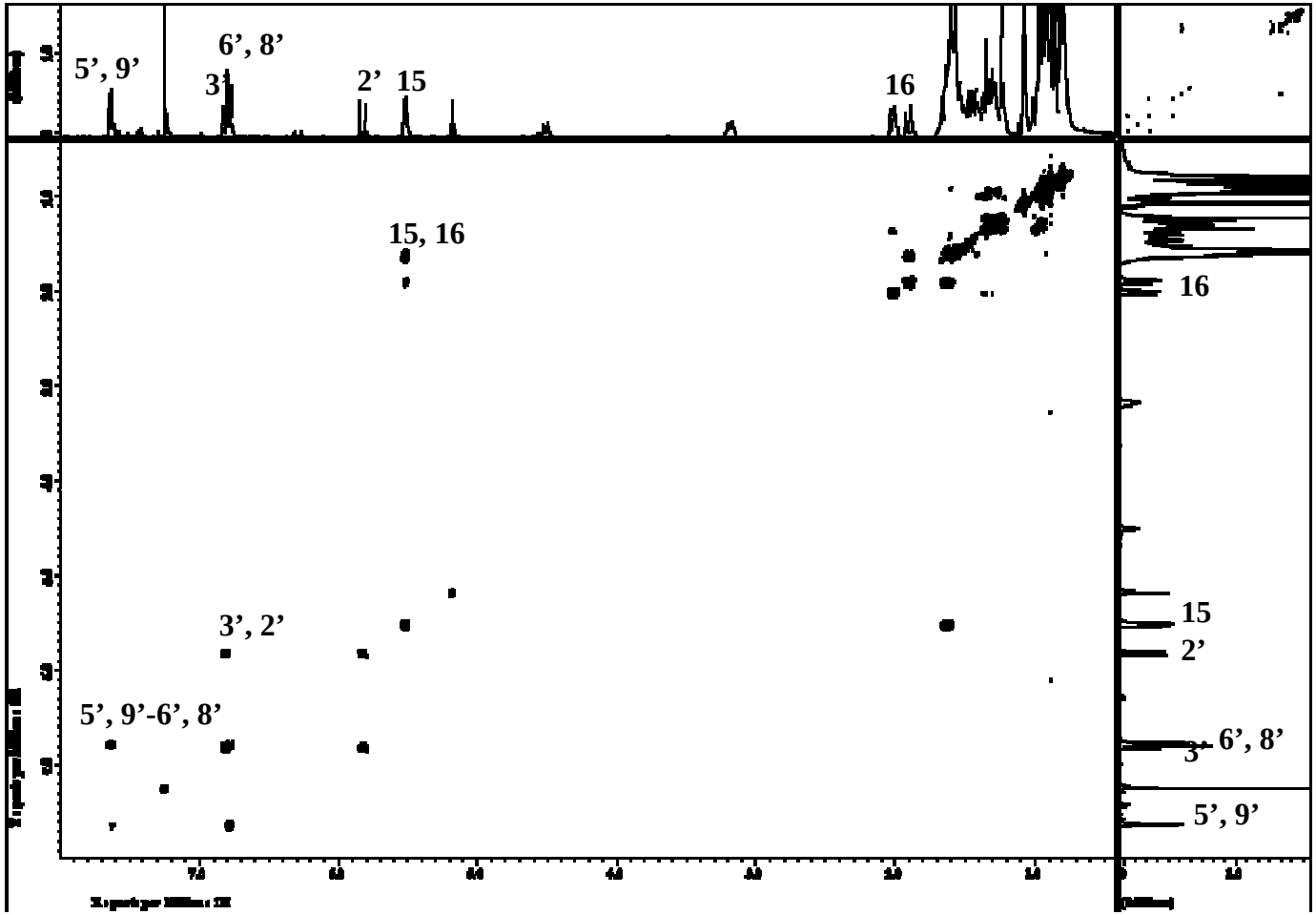


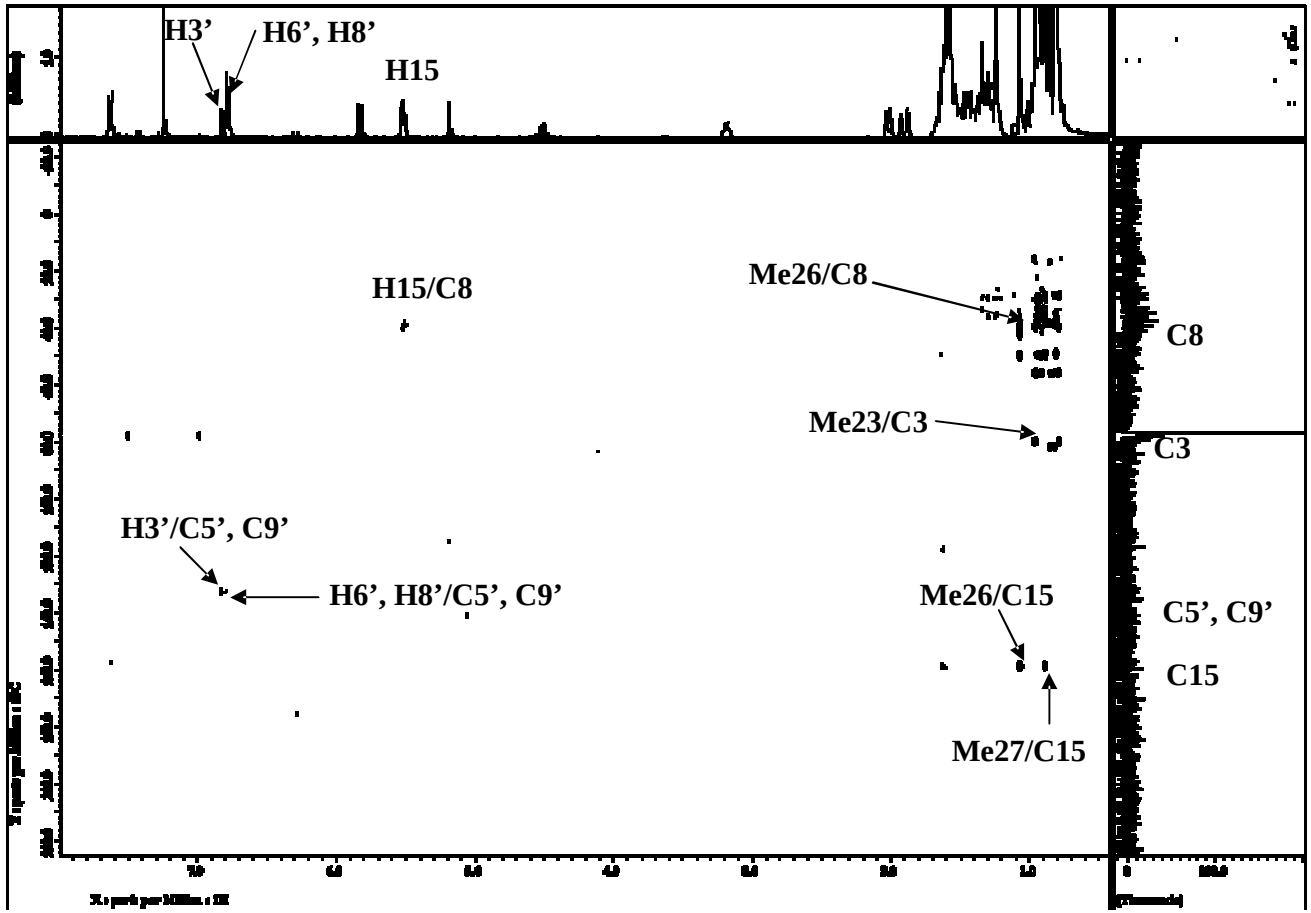
Fig.3.22; 3D Structure of KC-8b with representing for HMBC correlations



Spectrum3.19; ^{1}H (400MH_z) NMR spectrum of KC-8b in $CDCl_3$



Spectrum3.20; COSY correlations spectrum of KC-8b in $CDCl_3$



Spectrum 3.21; HMBC correlations spectrum of KC-8b in $CDCl_3$

3.1.12 Characterisation of KC-9 as 3β-*E*-*p*-coumaroyl taraxerol

KC-9 was isolated as white solid from an ethyl acetate extract of *Bruguiera gymnorrhiza* leaves.

The IR spectrum ($CHCl_3$) exhibited absorption band of hydroxyl group (3360 cm^{-1}), C-H stretching (2939, 2863 cm^{-1}), carbonyl (1681 cm^{-1}), C=C stretching (1604 cm^{-1}), 1513, 1452, C-H bending (1373 cm^{-1}) and C-O stretching (1169 cm^{-1}).

The LRESIMS experiment showed m/z 571 $[M-H]^-$ indicating for a molecular ion 572 supported a molecular formula of $C_{39}H_{56}O_3$ (Jutiviboonsuk, A., 2006).

The spectrum data (Table 3.14 and 3.15) showed that they were very similar to careaborin (KC-7b) accepted for the ^1H NMR signals at δ 6.29(1H, *d*, *J*=15.8 H_Z) and δ 7.59(1H, *d*, *J*=15.8 H_Z) assigned for the coupled between H-2' and H-3'. Due to the larger coupling constant than those of careaborin suggested for its *trans*-configuration. Also their location resonated at lower field than that in careaborin. The low field shift of aromatic protons at δ 7.42 indicating the *E*-conformation of the aromatic ring located opposite side to the carbonyl group and the anisothopic effect from α, β-unsaturated carbonyl group increased their magnetic resonances. On the other hand, the aromatic ring of *Z*-conformation of careaborin was situated in the same side of α, β-unsaturated carbonyl group which their manetic resonance decreased by the anisothopic effect. The esterified carbinolic proton signal centred at δ 4.58 (1H, *dd*, *J*=6.84, 10.34H_Z) with the large coupling constant with the vicinal methylene protons can be suggested for β orientation. Thus this compound was confirmed as 3β-*E*-*p*-coumaroyl taraxerol (Fig. 3.23). It is reported for the first time from the leaves of *Bruguiera gymnorrhiza*.

Table 3.10: ^{1}H (400MH_Z) and ^{13}C (100MH_Z) NMR data of KC-9 in $CDCl_3$

Position	δ_H	δ_C	Selected HMBC correlations
1		36.7	
2		21.4	
3	3.19(1H, *dd*, 4.4, 11.0)	79.2	
4		35.8	
5		49.3	
6		18.8	
7		33.7	
8		39.0	
9		48.8	
10		37.7	
11		17.5	
12		35.1	
13		38.0	
14		157.9	Me26, Me27
15	5.51(1H, *dd*, 3.0, 8.0)	116.9	C8
16	1.90(2H, *d*, 14.52)/ 2.03 (2H, *d*, 12.76)	37.7	
17		35.1	
18		48.8	
19	2.01(2H, *d*, 12.76)	41.3	
20		28.8	
21		30.0	
22		33.1	
23	0.97(3H, *s*)	28.0	
24	0.89(3H, *s*)	21.4	C-4
25	0.79(3H, *s*)	15.5	C-5
26	1.08(3H, *s*)	25.9	C-14, C-8
27	0.89(3H, *s*)	29.7	C-14, C-18
28	0.81(3H, *s*)	29.8	C-16
29	0.94(3H, *s*)	33.4	C-21
30	0.89(3H, *s*)	21.4	C-20

Chemical shifts (*ppm*.), Coupling constants (H_Z)

Table 3.11: ^{1}H (400MH_Z) and ^{13}C (100MH_Z) NMR data of KC-9 in $CDCl_3$ (continued)

Position	δ_H	δ_C	Selected HMBC correlations
1’	-	167.5	
2’	5.80(1H, *d*, 13)	117.7	
3’	6.80(1H, *d*, 13)	143.0	C-9’, C5’
4’	-	127.5	
5’,9’	7.60(1H, *d*, 8.6)	132.4	
6’,8’	6.77(1H, *d*, 8.6)	115.0	C5’, C9’
7’	-	158.0	

Chemical shifts (*ppm.*), Coupling constants (H_Z)

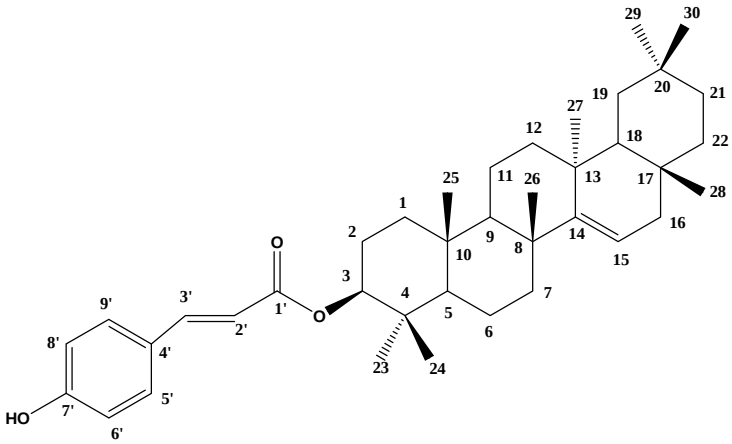


Fig.3.23; Structure of KC-9

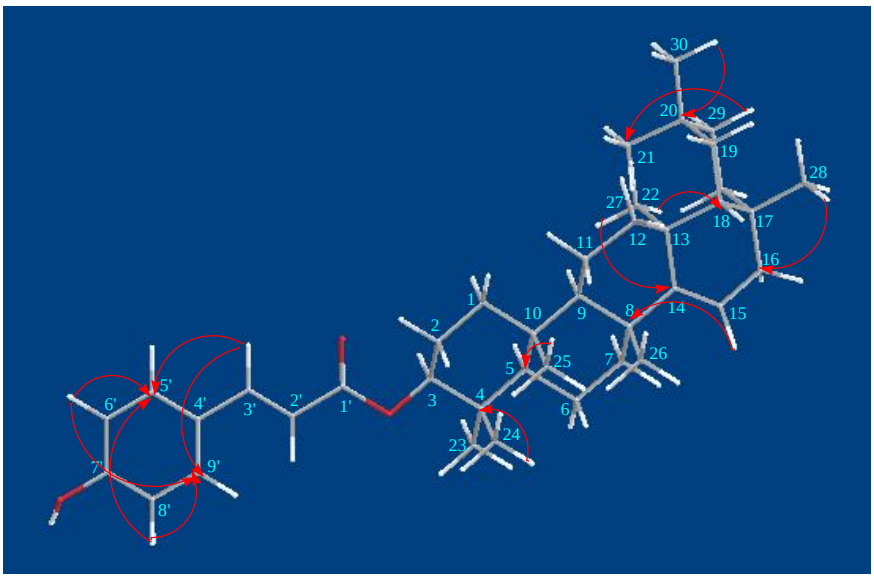
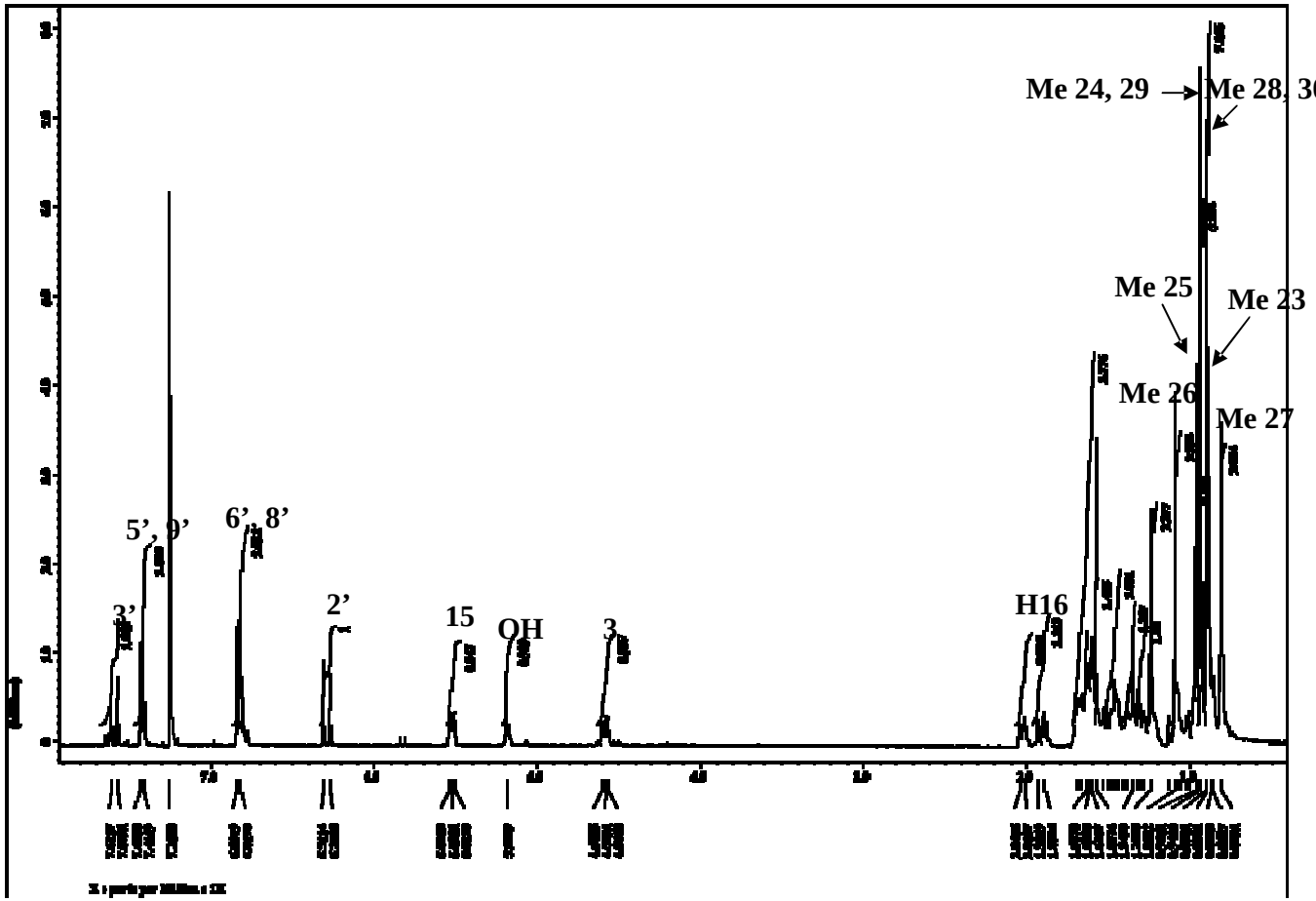
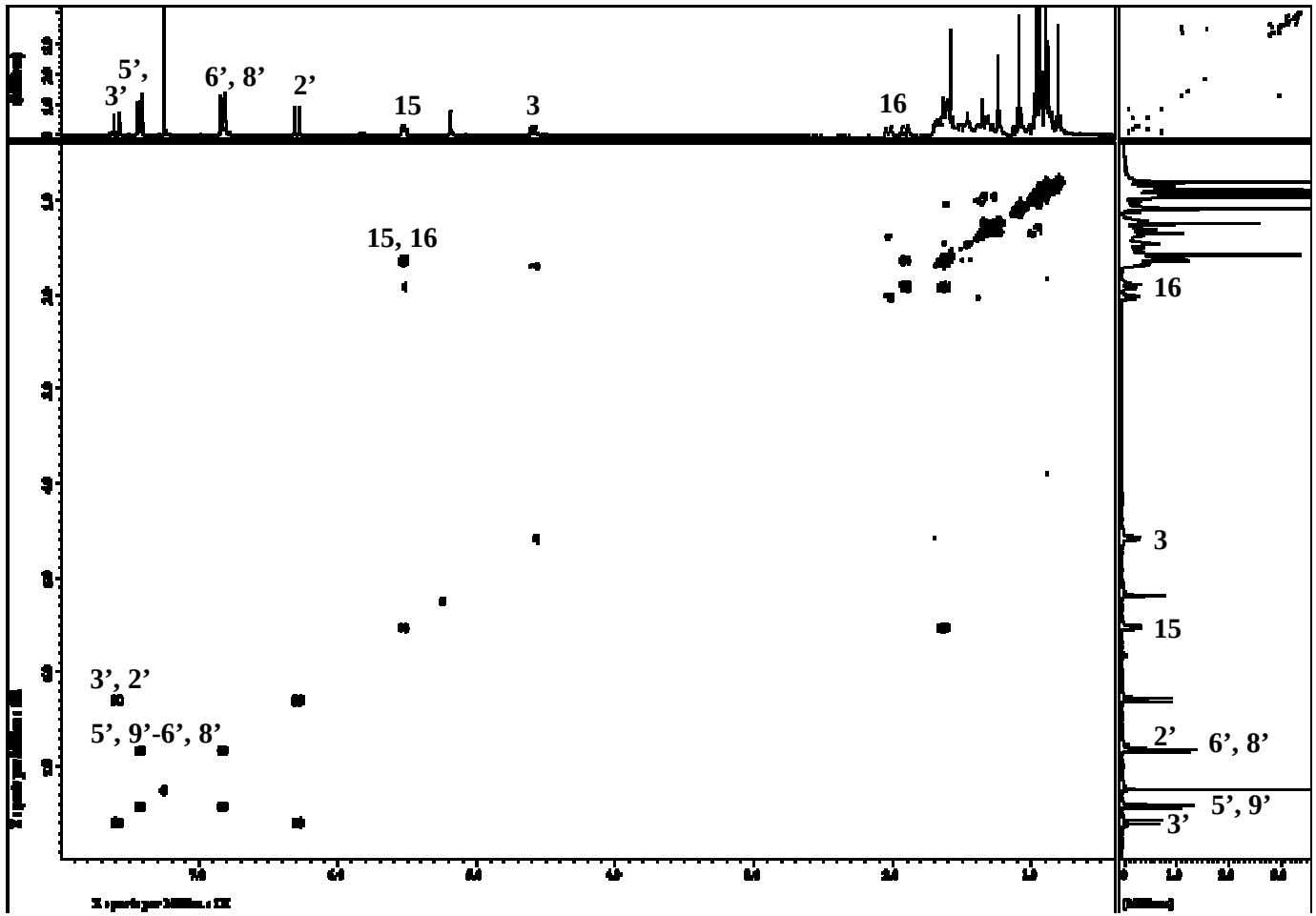


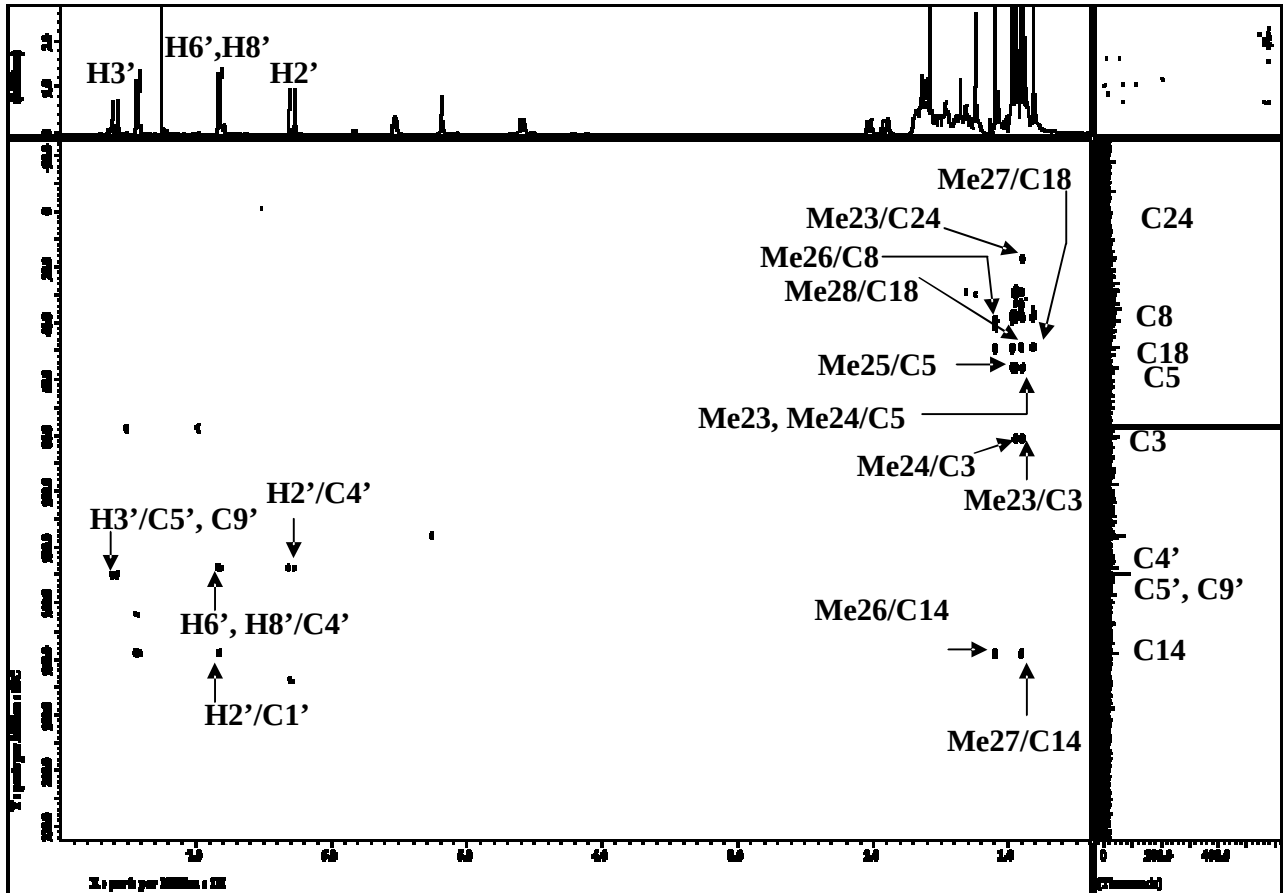
Fig.3.24; 3D Structure of KC-9 with representing for HMBC correlations



Spectrum 3.22; ^{1}H (400MH_z) NMR spectrum of KC-9 in $CDCl_3$



Spectrum3.23; COSY correlations spectrum of KC-9 in $CDCl_3$



Spectrum3.24; HMBCcorrelations spectrum of KC-9 in $CDCl_3$

3.1.13 Characterisation of KC-10 as 3*β*-lauryl-*β*-amyrin

KC-10 was isolated as a yellow solid from the ethyl acetate extract of *Bruguiera gymnorrhiza* leaves. This compound was active under short wavelength UV light and it had a deep purple-pink in colour on TLC after spraying with *p*-anisaldehyde-sulfuric acid followed by heating.

The positive mode ESI MS data obtained $[M+H]^+$ at *m/z* 609 suggested for a molecular formula of $C_{42}H_{72}O_2$.

The IR spectrum indicated the absorption of C-H stretching (2914, 2850 cm^{-1}), C=C stretching (1728 cm^{-1}), carbonyl (1707 cm^{-1}), 1472, 1468, C-H bending (1378 cm^{-1}) and C-O bending (1173 cm^{-1}).

The FTMS+p-ESIMS experiment presented m/z 611.3192 $[M+H]^+$ indicated for a molecular ion 610.3192, according to the positive mode MS maybe plus H^+ in its procedure more than one time, in this case it might possible had three times of plus H^+, therefore the actual mass m/z of this compound was 608 $[M+3H]^+$, suggesting for a molecular formula of $C_{42}H_{71}O_2$.

The NMR spectrum displayed typical signals for an olean-12-ene triterpenoid with an olefinic proton at *δ*5.15 (brs) and carbon signals at ca. *δ* 121.7 and *δ* 145.3.
The ^1H NMR spectrum data (Table 3.16 and 3.17) showed a signal at *δ* 2.27(2H, *t*, *J*=7.5 H_Z) suggesting methylene protons located at the α-position of a carbonyl group. The spectrum also showed a large envelope of methylene groups at *δ*1.24, together with a terminal methyl at *δ* 0.86 indicating the presence of a an aliphatic fatty acid moiety.

The ^{13}C NMR spectrum data presented 42 carbon signals. Their multiplicities, determined by DEPT and HMQC experiments, included nine methyls, twenty one methylenes, five methines and seven quaternary carbons. The carbon signal at *δ* 80.6 was attributed to an esterified C-3. The signal at *δ*173.70 was assigned to an ester

carbonyl group.The α methylene signals showed at δ34.93, a methyl group at δ14.23 and methylne groups at δ 29.82- δ 29.17 suggested for a long chain fatty acid.

The HMBC spectrum data, with an oxymethine proton at δ 4.49 showed ^{3}J correlations to the carbonyl at δ 173.7 and two geminal methyls at δ28.1 and δ15.6 indicating the presence of a carbonyl group at C-1' and it was esterified at C-3. The oxymethine proton also presented the long range coupled to the carbon at δ34.93; this revealed the presence of the side chain. The germinal methyls protons at δ28.1 and δ15.6 presented ^{3}J correlations to methine carbon at δ55.3 indicated their substitution at quaternary carbon C-4. The methyl proton at 1.09 showed ^{2}J correlations to the carbon at δ145.3 suggested Me-27 located at quaternary carbon C-13. (Fig. 3.26).

From the evidence given confirmed that this compound was 3β-lauryl -β-amyrin (Fig. 3.25). This was the first report of this compound from the leaves of *Bruguiera gymnorrhiza*.

Table 3.12: ^{1}H (400MH_Z) and ^{13}C (100MH_Z) NMR data of KC-10 in $CDCl_3$

Position	δ_H	δ_C	Selected HMBC correlations
1	-	38.3	
2	1.59 (2H,*m*)	27.0	
3	4.49(1H, *dd*, 6.4, 8.9)	80.6	C-2', Me-23, Me-24, C=O
4	-	37.8	
5	-	55.3	
6	-	18.4	
7	-	32.7	
8	-	39.9	
9	-	47.6	
10	-	36.9	
11	-	23.6	
12	5.15(2H, *t*, 3.30)	121.7	
13	-	145.3	
14	-	41.8	
15	-	26.1	
16	-	26.2	
17	-	32.6	
18	-	47.3	
19	-	46.9	
20	-	31.2	
21	-	32.0	
22	-	37.2	
23	0.85(3H, *s*)	28.1	C-5
24	0.86(3H, *s*)	15.6	C-5
25	0.95(3H, *s*)	16.9	
26	0.81(3H, *s*)	22.8	

Chemical shifts (*ppm*.), Coupling constants (H_Z)

Table 3.13: ^{1}H (400MH_Z) and ^{13}C (100MH_Z) NMR data of KC-10 in $CDCl_3$ (continued)

Position	δ_H	δ_C	Selected HMBC correlations
27	1.09(3H, *s*)	16.9	C13
28	0.86(3H, *s*)	28.5	
29	0.94(3H, *s*)	33.4	
30	0.86(3H, *s*)	23.8	
- $CH_2\underline{C}OO$-	-	173.7	
- $\underline{C}H_2COO$-	2.27(2H, *t*, 7.5)	34.93	
- $(\underline{C}H_2)_n$-	1.24(18H, *m*)	29.82-29.2	
- $(CH_2)_n\ \underline{C}H_3$	0.88(3H, *t*,)	14.23	

Chemical shifts (*ppm*.), Coupling constants (H_Z)

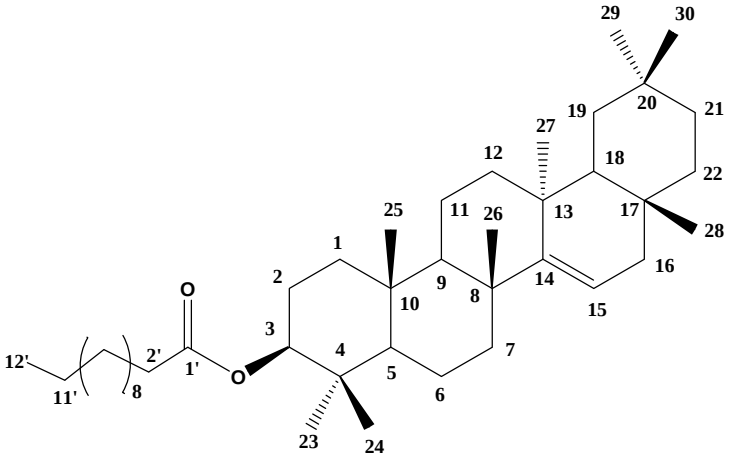


Fig.3.25; Structure of KC-10

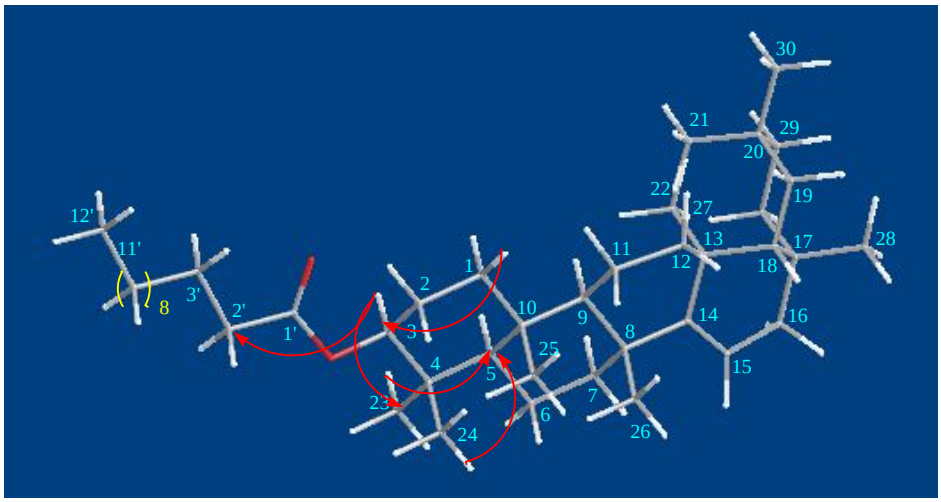
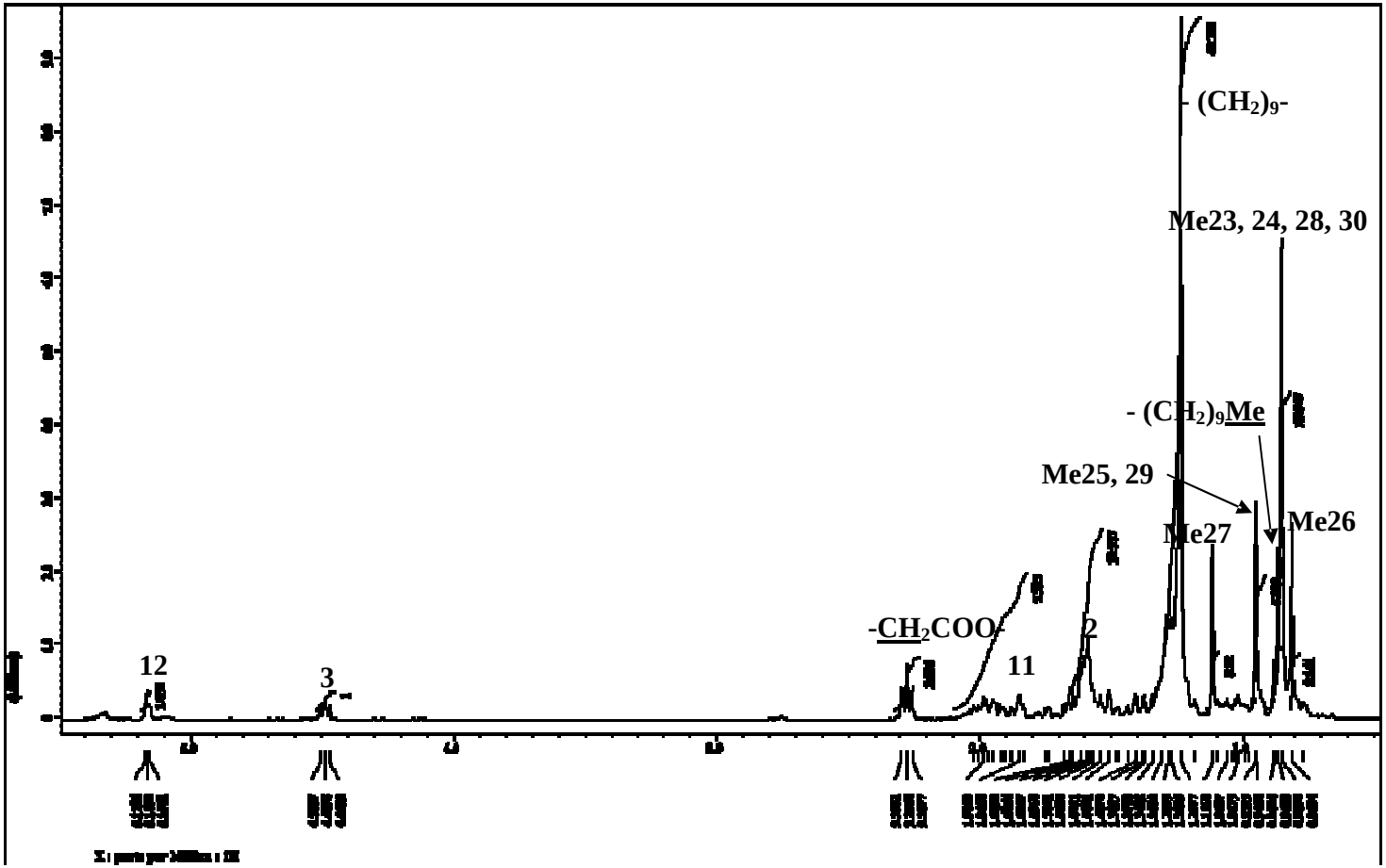
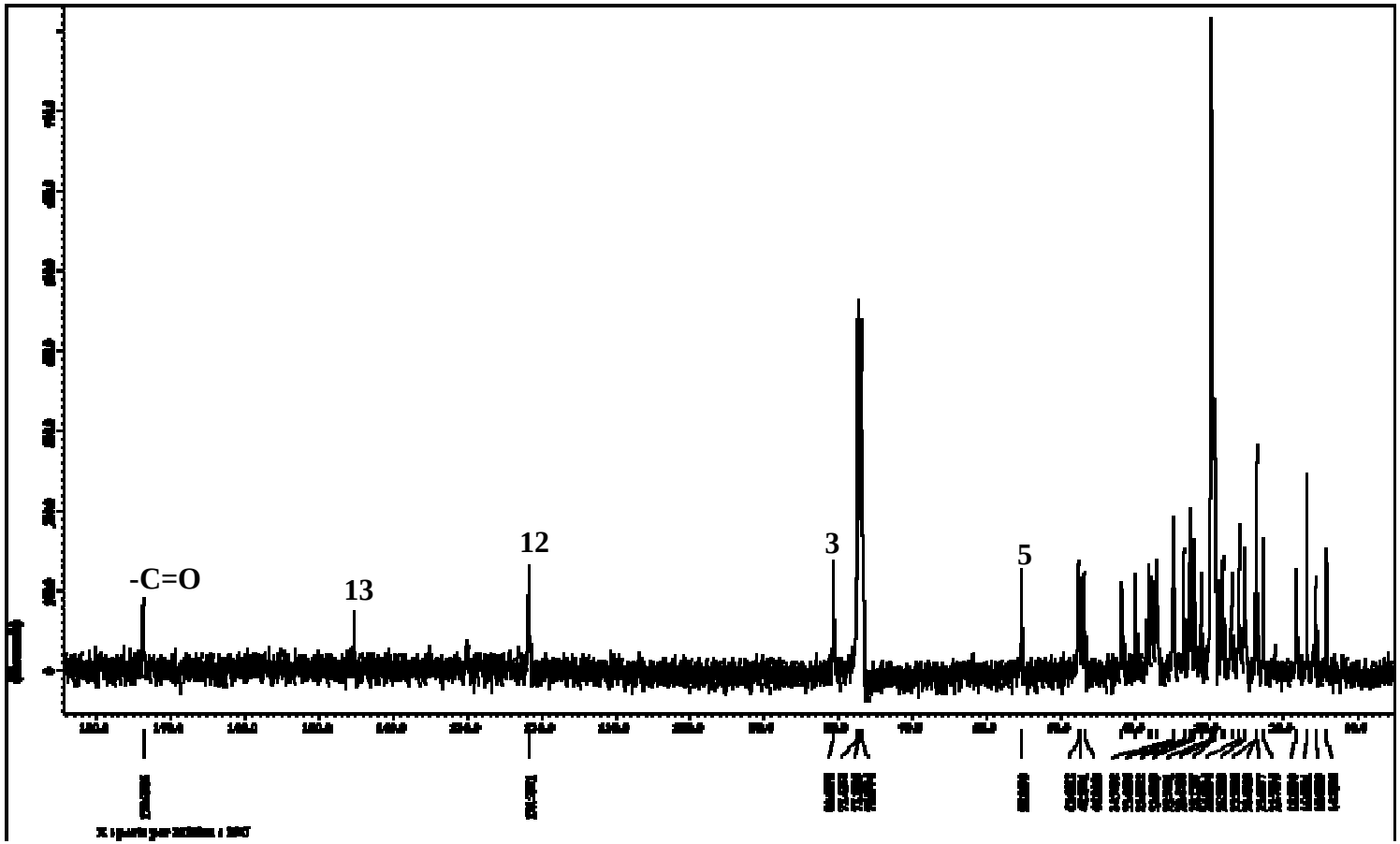


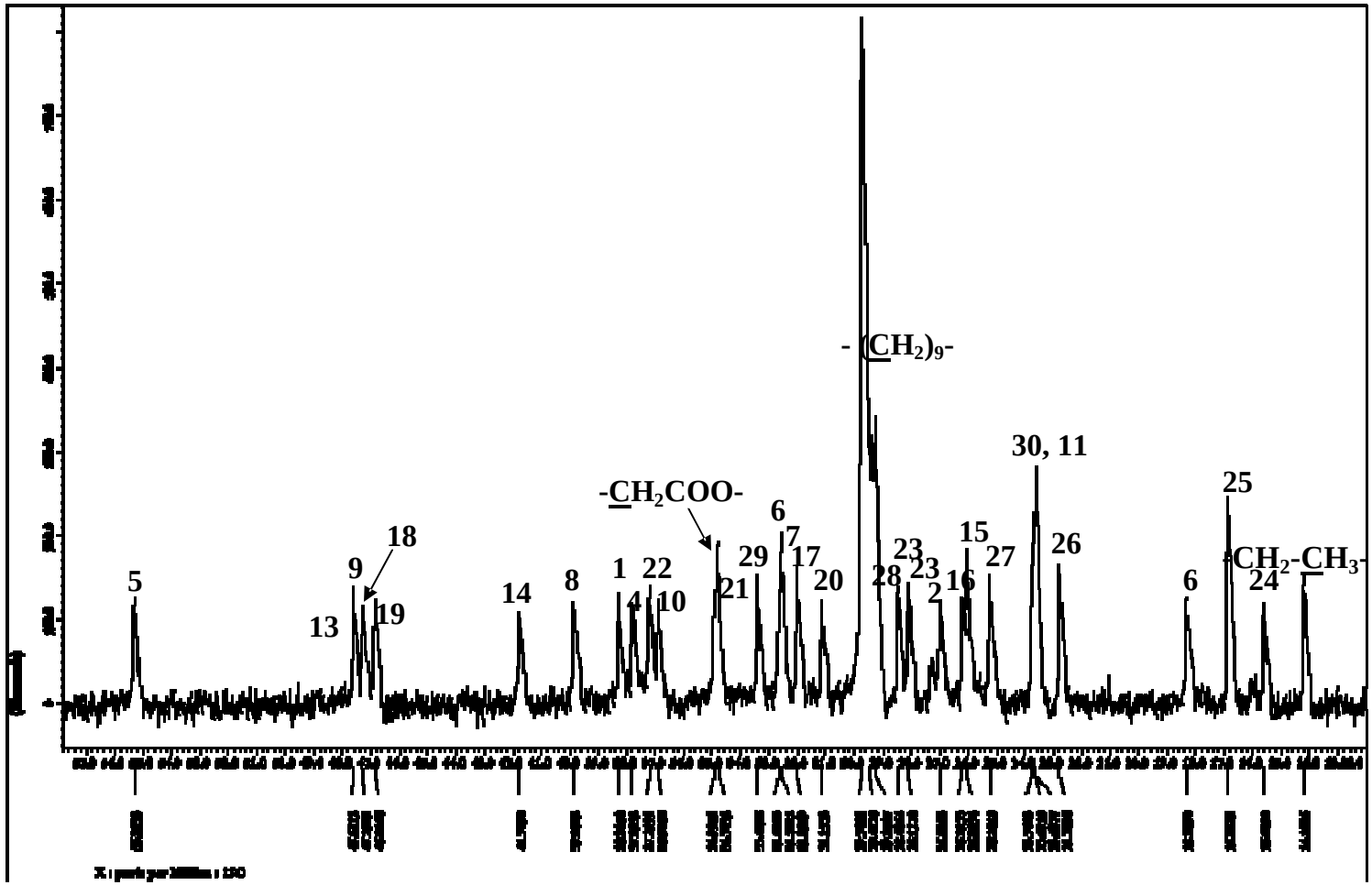
Fig. 3.26; 3D Structure of KC-10 with representing  **for HMBC correlations**



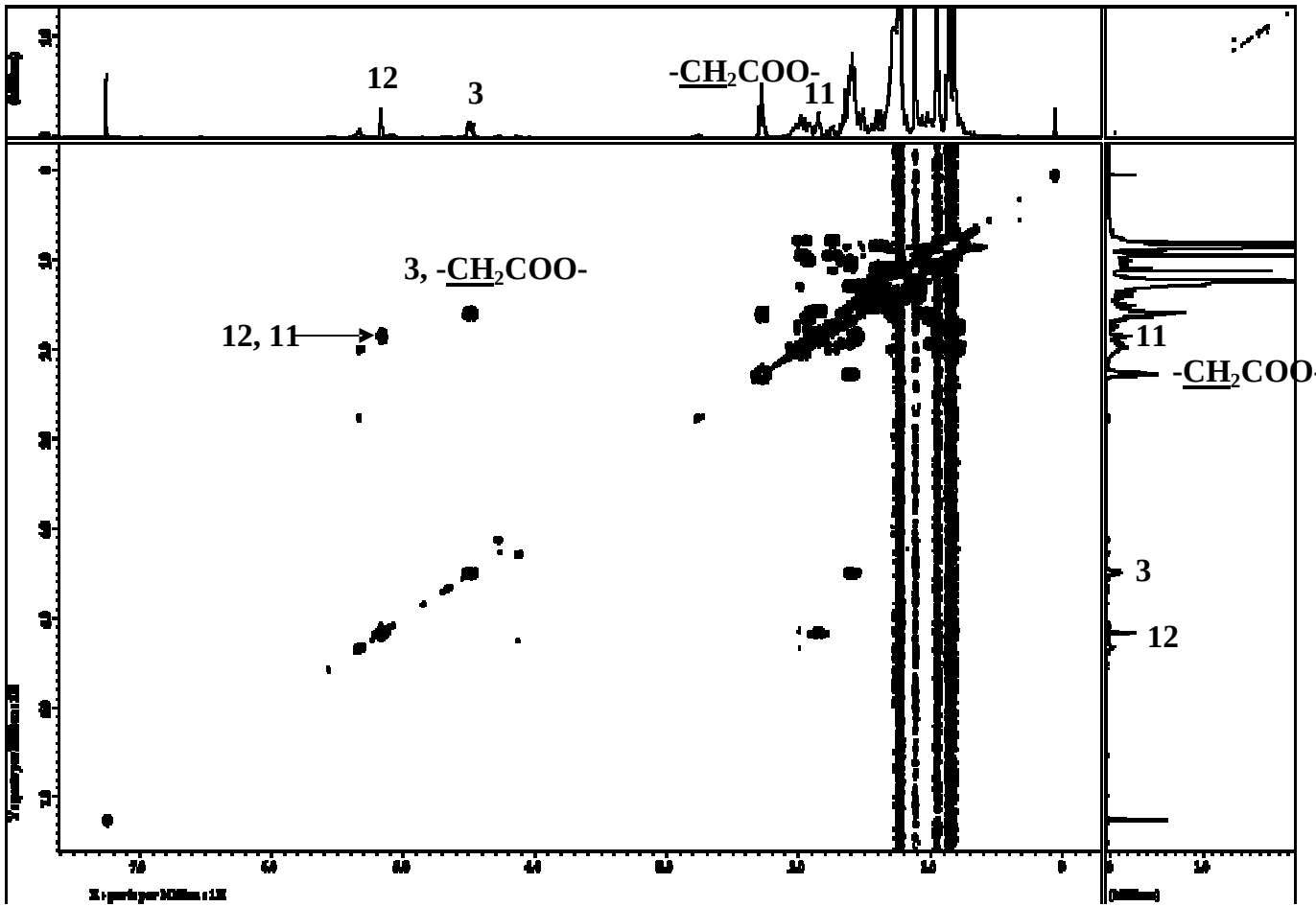
Spectrum 3.25; ^{1}H (400MH_z) NMR spectrum of KC-10 in $CDCl_3$



Spectrum 3.26; ^{13}C (100MH_z) NMR spectrum of KC-10 in $CDCl_3$



Spectrum 3.27; ^{13}C (100MH_z) NMR spectrum (selected expansion) of KC-10 in $CDCl_3$



Spectrum 3.28; COSY correlations spectrum of KC-10 in $CDCl_3$

3.1.14 Characterisation of KC-11 as taraxerone

KC-11 was obtained as a white solid from the ethyl acetate extract of *Bruguiera gymnorrhiza* leaves.

The FTMS+p-ESIMS experiment showed a pseudo-molecular ion *m/z* at 425.7484 $[M+H]^+$ indicating for a molecular ion at *m/z* 424.7484 suggesting a molecular formula of $C_{30}H_{48}O$.

The IR spectrum displayed C-H stretching (2958, 2937 cm^{-1}), carbonyl (1708 cm^{-1}), 1449, 1463 and C-H bending (1373 cm^{-1}).

The ^1H NMR spectrum data (Table 3.17) showed signals typical for a taraxerene-type triterpenoid as aforementioned (page 115).

The ^{13}C NMR spectrum showed 30 carbon signals thus confirming the triterpenic structure. Both DEPT and HMQC experiments determined multiplicities as eight methyls, ten methylenes, four methines and eight quaternary carbons, including a signal at δ 217.6 accounting for a carbonyl (ketone) group.

The COSY spectrum data showed cross peaks between δ 1.87, δ 2.04 and δ 2.30, δ 2.54; δ 5.56 and δ 1.61, δ 1.64 attributed for the connection between H-1a,b and H-2a,b; H-15 and H-16a,b

The HMBC data (Table 3.17 and Figure 3.33), olefinic proton at δ5.56 showed ^3J correlation to the carbon at δ38.9 (C-8) and ^2J correlations to the carbons at δ157.7 (C-14) and δ36.7 (C-16) indicated the presence of a double bond at C-14 and C-15. The two geminal methyl at δ1.04 and δ1.07 showed ^3J correlation to the carbon at δ217.6 suggested carbonyl group situated at position 3 (Fig. 3.28).

The evidence given above and comparing this to previously published data in which this compound was isolated from the leaves of *Rhizophora stylosa* L. (Yang, X, *et al*, 2008) and found from the root barks of *Phyllanthus columnaris* (Jamal, A., K., *et al*,

2009), KC-11 was identified as taraxerone (Fig. 3.27) and this is the first report of its isolation from the leaves of *Bruguiera gymnorrhiza*.

Table 3.14: ^{1}H (400MH_Z) and ^{13}C (100MH_Z) NMR data of KC-11 in $CDCl_3$

Position	δ_H	δ_C	Selected HMBC correlations
1	2.04 (1H, *dd*, 3.04,13.09)/1.87(1H,*m*)	38.4	
2	2.30(1H, *m*)/2.54(1H, *m*)	34.2	C3, C1, C10
3	-	217.6	
4	-	47.6	
5	1.07(1H,*s*)	55.8	C3, C1
6	-	20.0	
7	-	35.1	
8	-	38.9	
9	-	48.8	
10	-	37.7	
11	-	17.5	
12	-	35.8	
13	-	37.6	
14	-	157.7	C17
15	5.56 (1H,*dd*, 7.92, 2.63)	117.3	
16	1.61(1H, *dd* 2.64,14.96)/1.64(1H, *dd*,2.64, 8.4)	36.7	C15
17	-	37.8	
18	-	48.7	
19	-	40.6	
20	-	28.8	
21	-	33.6	
22	-	33.4	
23	1.07(3H, *s*)	26.1	C14
24	1.04(3H, *s*)	21.5	
25	1.08(3H, *s*)	14.9	C4
26	0.88(3H, *s*)	30.0	C9
27	1.12(3H, *s*)	25.6	
28	0.81(3H, *s*)	29.9	
29	0.93(3H, *s*)	33.1	C20, C14
30	0.89(3H, *s*)	21.4	C20

Chemical shifts, (*ppm*)., Coupling constants (H_Z)

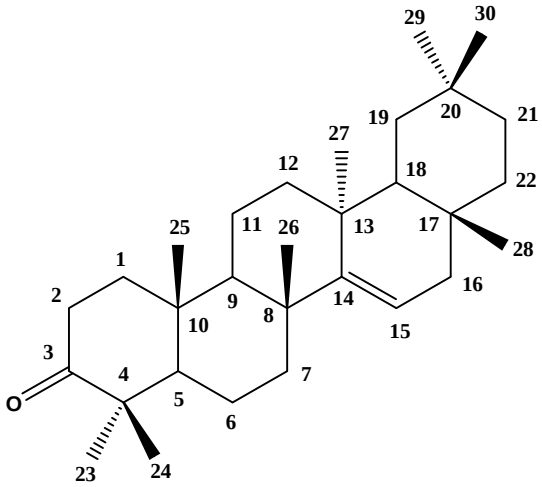


Fig. 3.27; Structure of KC-11

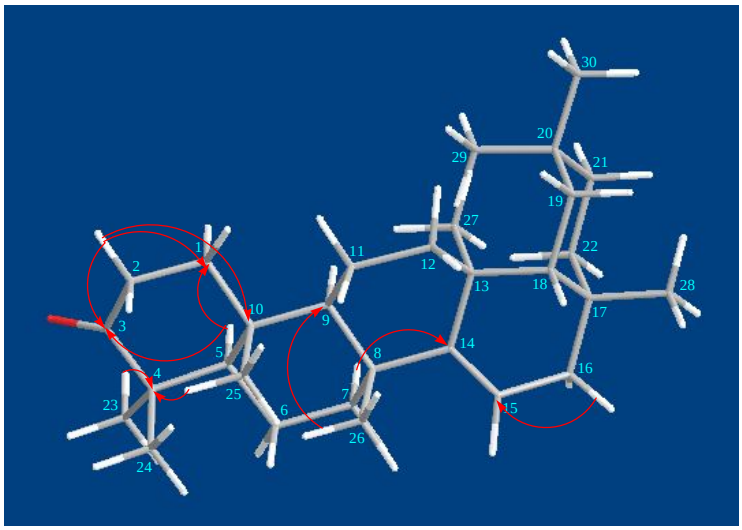
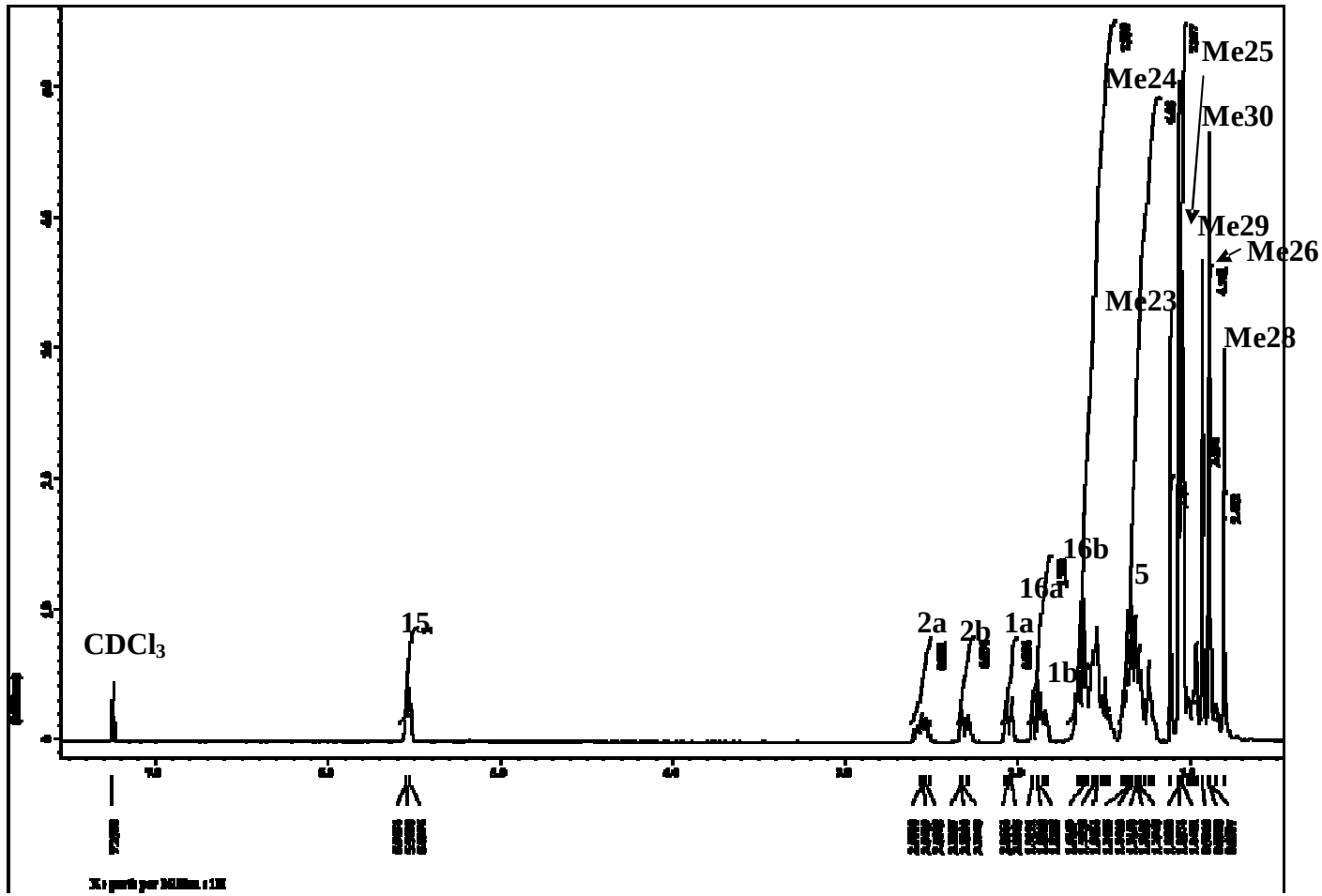
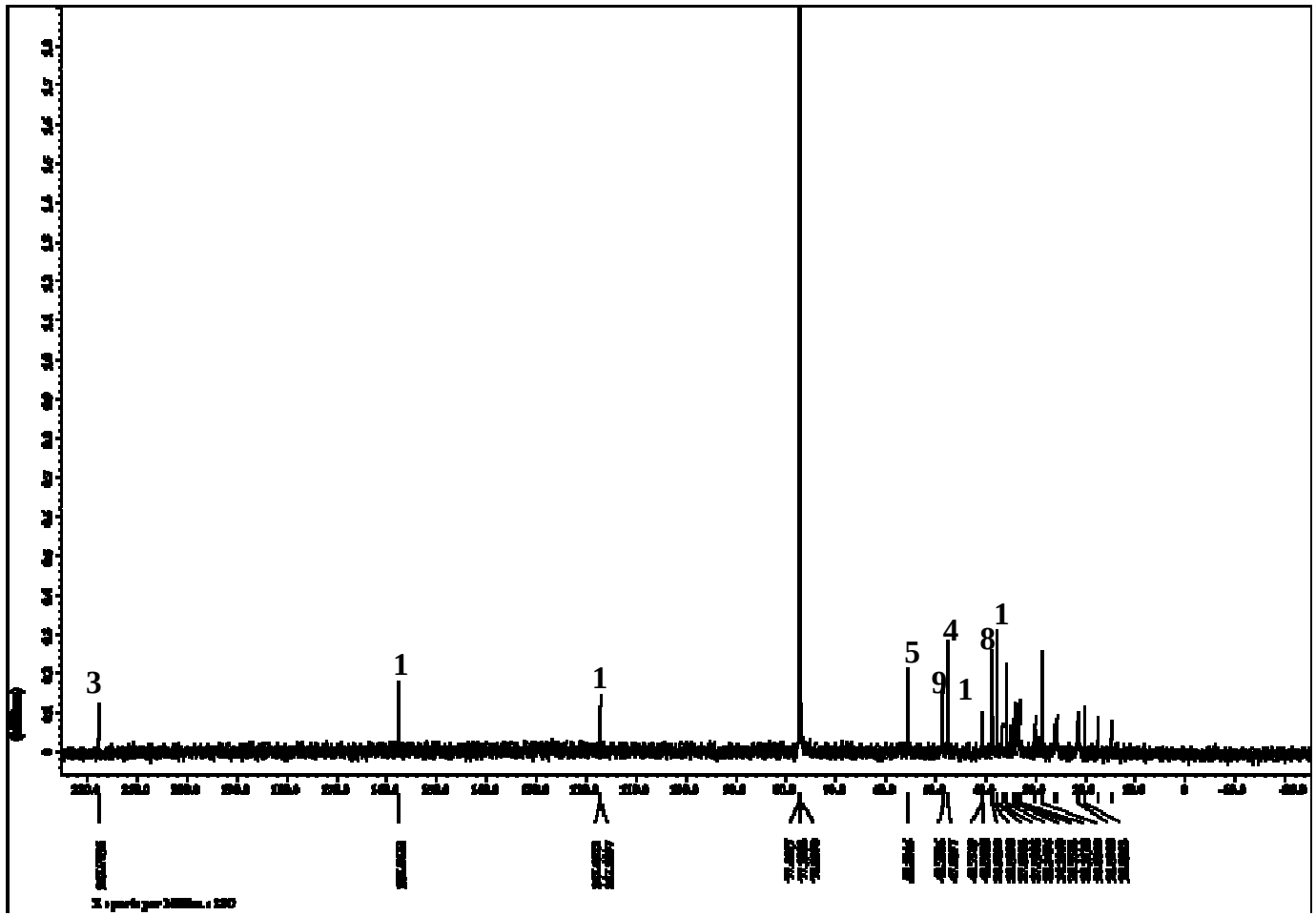


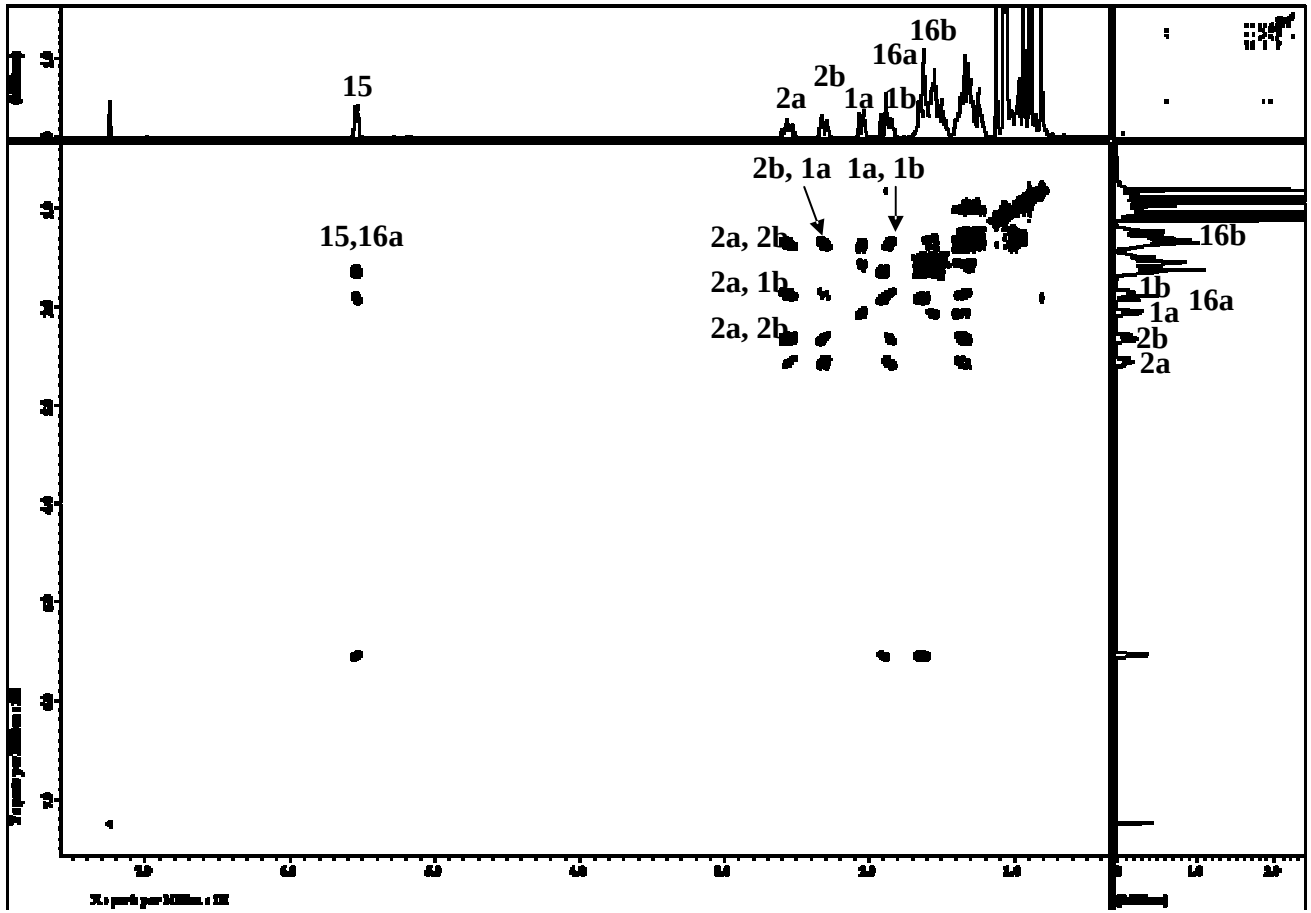
Fig.3.28; 3D Structure of KC-11 with representing for HMBC correlations



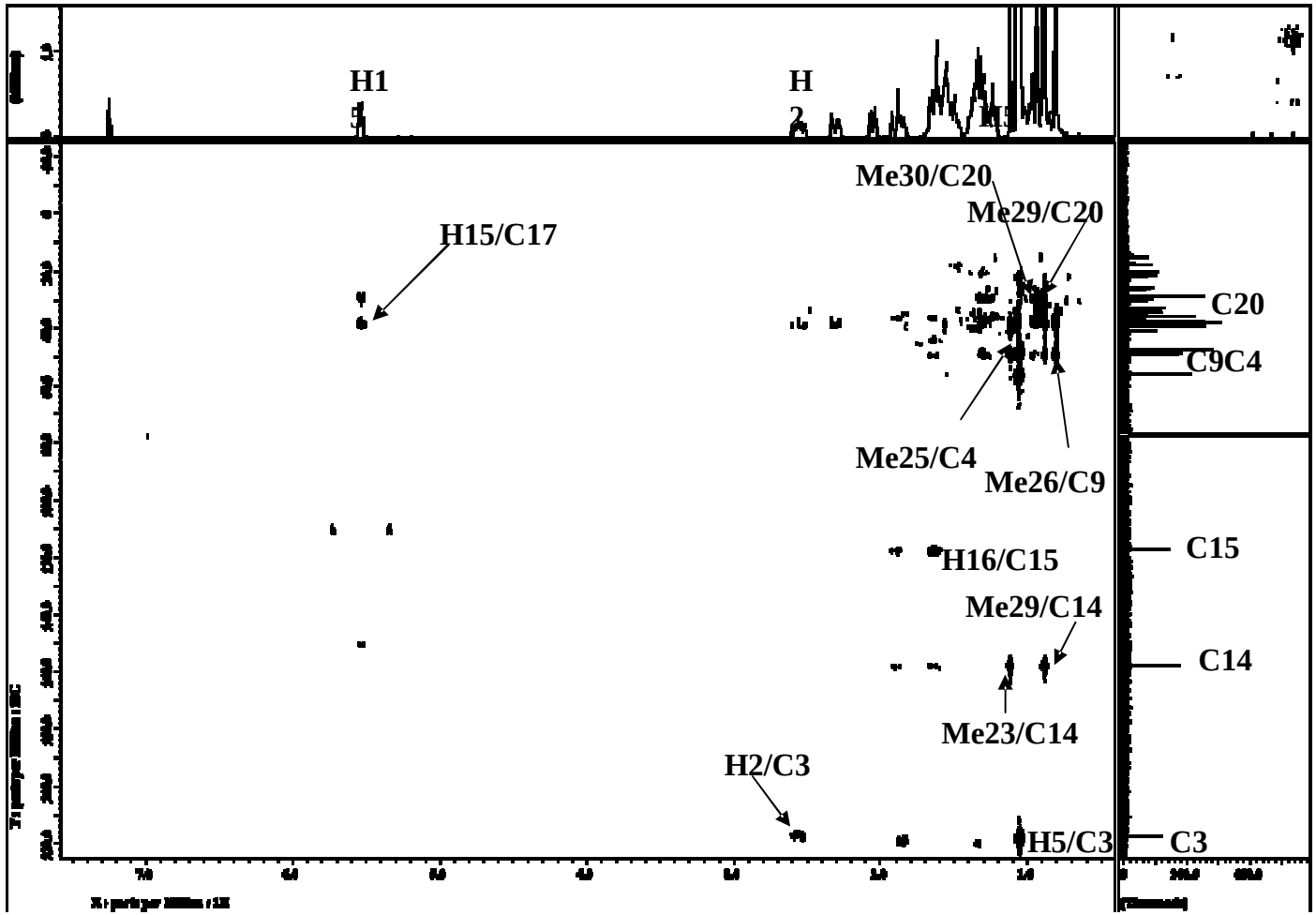
Spectrum 3.29; ^{1}H (400MH_z) NMR spectrum of KC-11 in $CDCl_3$



Spectrum 3.30; ^{13}C (100MH$_z$) NMR spectrum of KC-11 in $CDCl_3$



Spectrum 3.31; COSY correlations spectrum of KC- 11 in $CDCl_3$



Spect rum3.32; HMBC correlations spectrum of KC-11 in $CDCl_3$

3.1.15 Characterisation of KC-12 as betulinic acid

KC-12 was obtained as a white solid from the hexane extract of *Avicennia alba* dried stems. It was active under short wavelength UV light and it had a purple-pink colour on TLC after spraying with *p*-anisaldehyde-sulfuric acid followed by heating.

The positive mode ESI MS data showed a pseudomolecular ion $[M+H]^+$ at *m/z* 457 suggested for a molecular formula of $C_{30}H_{48}O_3$ with 7 double bond equivalents.

The IR spectrum (KBr) indicated the presence of hydroxyl (3461 cm^{-1}), C-H stretching (2945 cm^{-1}), carbonyl (1688 cm^{-1}), C=C stretching (1708 cm^{-1}) and C-O bending (1189 cm^{-1}) (Igoli, O., J., *et al*, 2008).

The FTMS-*n*-ESIMS experiment showed m/z 455.3525 $[M-H]^-$ indicating for a molecular ion 456 suggested a molecular formula of $C_{30}H_{48}O_3$.

The ^1H NMR spectrum data (Table 3.18) showed six methyl singlets at δ 0.74, δ 0.81, δ 0.92, δ 0.95, δ 0.96. The two singlet signals at δ 4.59 and δ 4.72 presented for exomethylene protons and a downfield signal of a vinylic methine presented at δ 1.68.

The ^{13}C NMR spectrum data obtained 30 carbon signals determined their multiplicity by DEPT and HMQC techniques presented six methyl groups, eleven methylene groups, six methine groups and seven quaternary carbons.

The downfield signal at δ 178.8 indicated for carboxylic carbon. The signal of an exomethylene carbon showed at δ 109.8, a tetrasubstituted of isopropyl group presented at δ 149.9 and an oxymethine signal showed at δ 77.99.

This compound was isolated from the stem bark of *Hymenocardia acida* (Hymenocardiaceae) (Igoli, J. and Gray, A. I., 2008). It was also found from a whole plant sample from *Ludwigia adscendens* (Shilpi, J., 2009). This is the first report of its isolation for the isolation from the stems of *Avicennia alba*. The data given confirmed that this compound was betulinic acid (Fig. 3.29 and 3.30).

Table 3.15: ^{1}H (400MH_Z) in $CDCl_3$ and ^{13}C (100MH_Z) in *d*-pyridine (C_5D_5N) NMR data of KC-12

Position	δ_H	δ_C
1	-	39.4
2	-	28.1
3	2.99(1H, *ddd*, 2.99, 6.44, 11.57)	77.9
4	-	37.4
5	-	55.8
6	-	18.6
7	-	34.7
8	-	40.9
9	-	50.8
10	-	39.1
11	-	21.0
12	-	25.9
13	2.25(1H,*d*,12.32)	38.5
14	-	42.7
15	-	30.1
16	2.18(2H,*dd*,3.18,12.32)	32.7
17	-	56.5
18	-	49.6
19	3.17(1H, *dd*, 5.06, 11.22)	47.6
20	-	149.9
21	1.95(1H,*d*,8)	31.0
22	-	37.4
23	0.96(3H,s)	28.3
24	0.95(3H, s)	16.2
25	0.74(3H,s)	16.3
26	0.81(3H,s)	16.3
27	0.92(3H,s)	14.7
28	-	178.7
29	4.59(1H,*s*)/4.72(1H,*s*)	109.8
30	1.68(3H,s)	19.3

Chemical shifts (*ppm*.), Coupling constants (H_Z)

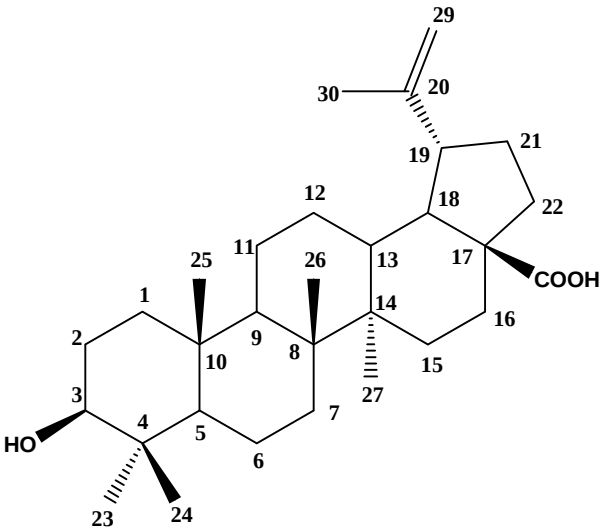


Fig. 3.29; Structure of KC-12

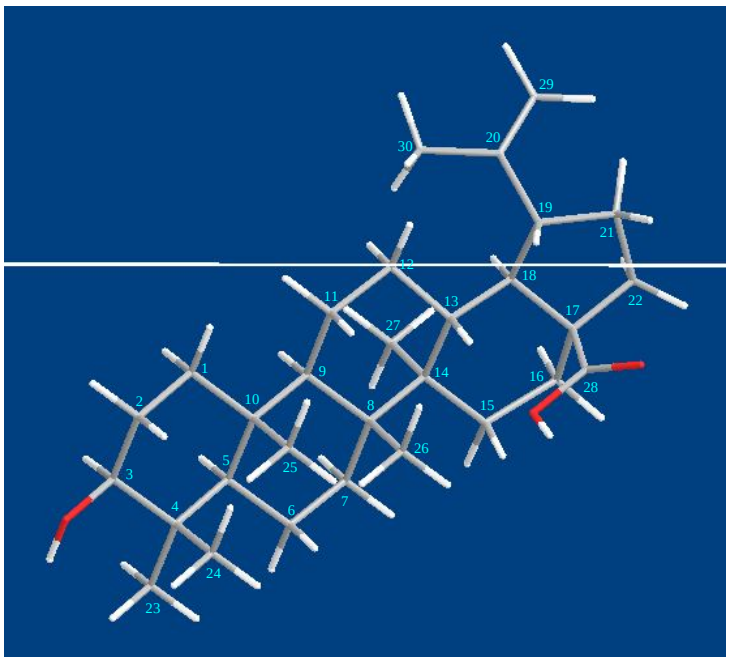
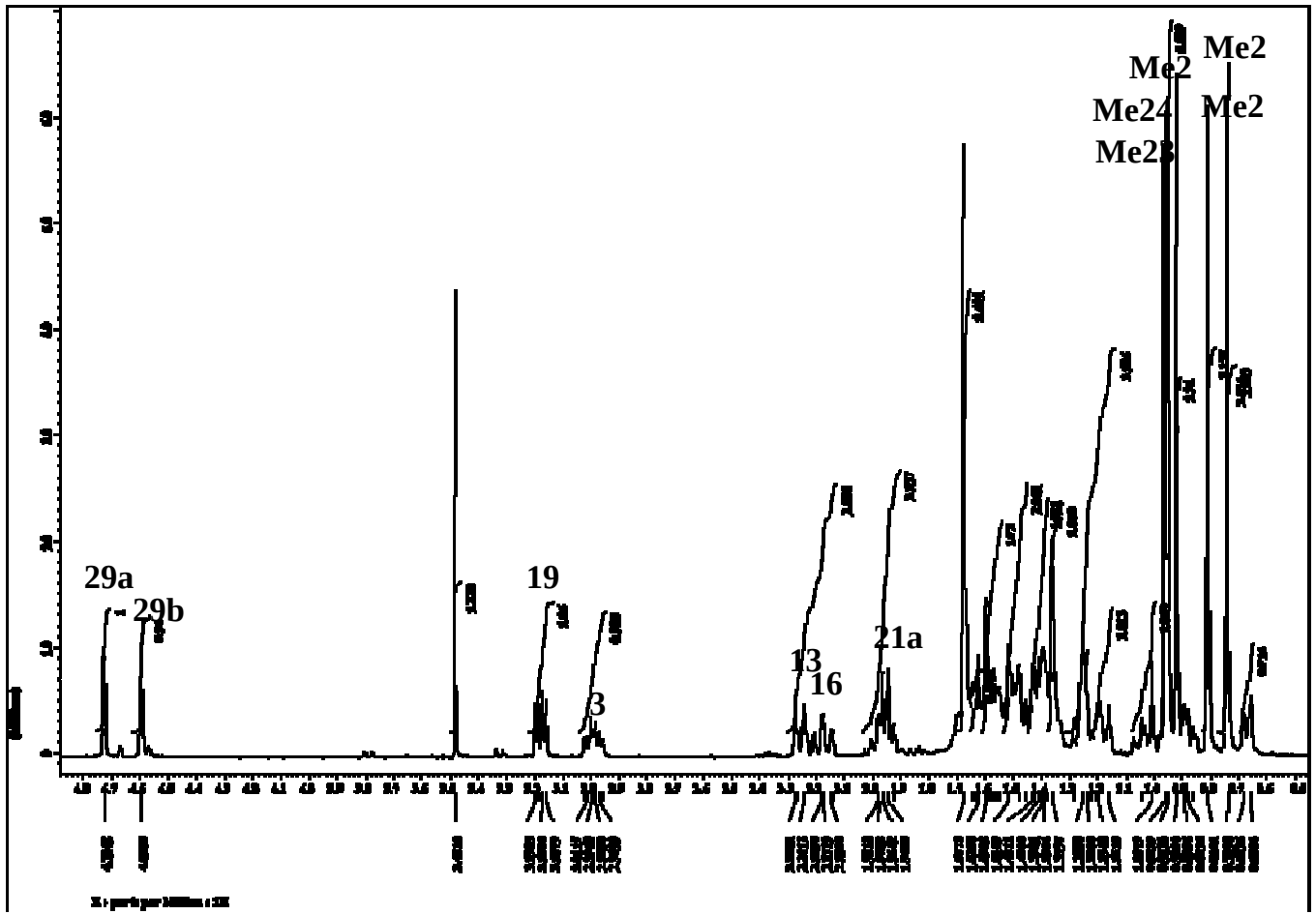
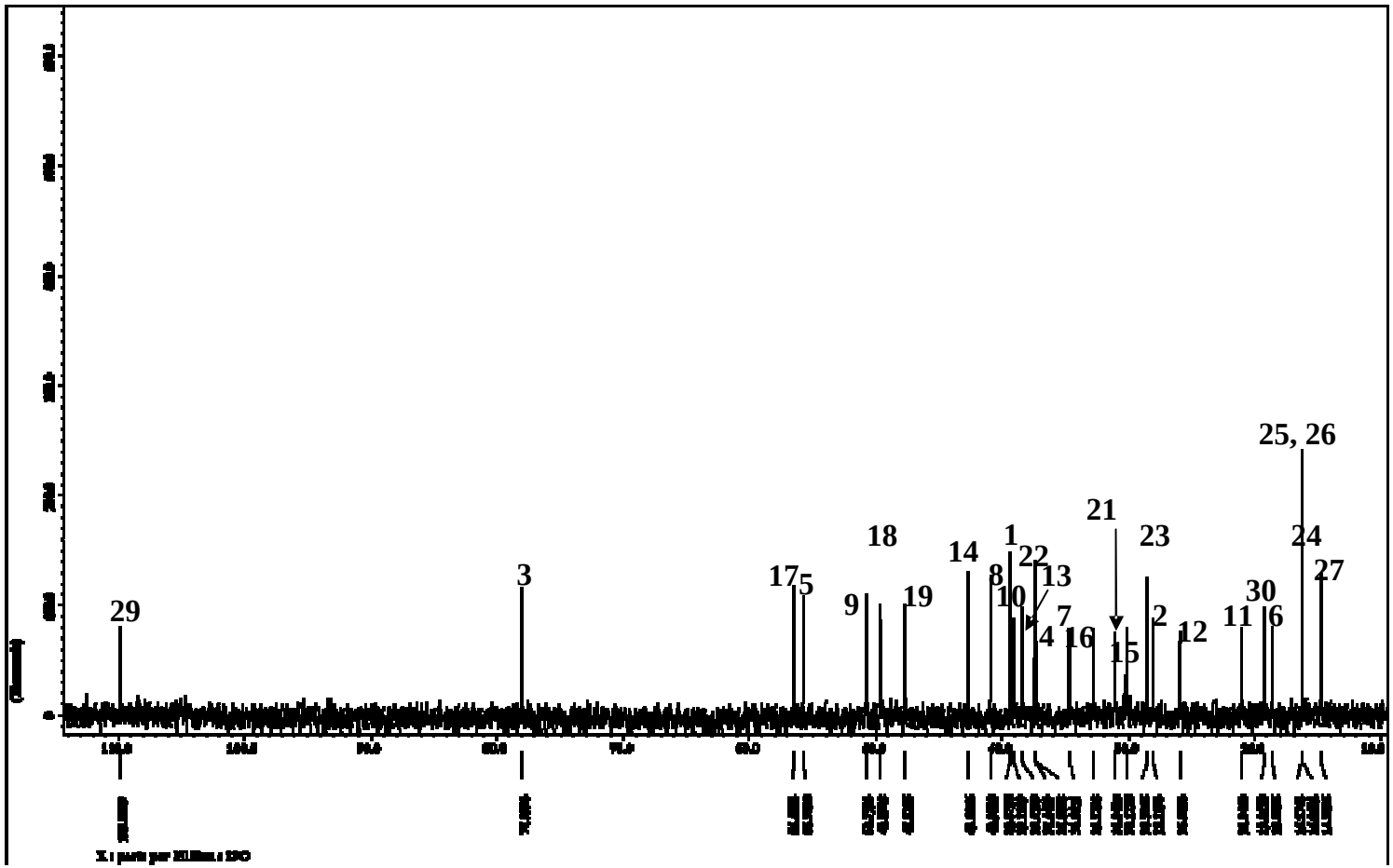


Fig.3.30; 3D Structure of KC-12



Spectrum 3.33; ^{1}H (400MH_z) NMR spectrum of KC-12 in C_5D_5N



Spectrum 3.34; ^{13}C (100MH_z) NMR spectrum of KC-12 (selected expansion) in $CDCl_3$

3.1.16 Characterisation of KC-13 as 3, 4-seco taraxerone

KC-13 was obtained as a white solid from the hexane extract of *Bruguiera gymnorrhiza* leaves. This compound was active under short wavelength, anisaldehyde-sulfuric acid spraying followed by heating.

The MS spectrum presented m/z 444 (Setzer, W., S., *et al*, 2000).

Distinctive features in the ^{1}H NMR spectrum data (Table 3.19) were an olefinic proton at δ5.54 (*dd, J*=8.34, 3.06 H_Z) assigned H-15 coupled with germinal protons of H-16 and singlets at δ 0.89, δ 1.09, δ 0.91, δ 0.81, δ 0.94 *and* 0.91 attributable to several methyl groups.The signals observed at δ 1.90 (2H, *dd, J*=12.31, 4.52 H_Z) and multiplets δ 2.11(1H, *m*), δ 2.18(1H, *m*) indicated for β methylene H-1 and α methylene H-2.

The ^{13}C NMR spectral data presented 30 carbon signals. The signal found at δ 180.2, indicated a carboxylic group. Two signals showed at δ 158.0 and δ 117.1 described for a double bond C-14 and C-15. A signal at δ180.2 indicated the presence of a carboxylic acid group.

No signals were observed at either δ79.2 or δ217.3 as in taraxerol (Mahato, S., B., *et al*, 1994) or taraxerone (Sakurai, N. *et al*, 1987.), respectively.

The COSY spectrum data showed cross peaks between δ5.54 and δ1.88, a signed for H-15 correlated to H-16a and H-16b supported for having a double bond group in this skeleton.

The DEPT experimental data presented the methyl signals at δ21.5, δ19.2, δ25.3, δ24.9, δ25.2, δ29.9, δ33.6 and δ18.0, the methylene signals at δ30.0, δ29.8, δ18.9, δ28.3, δ17.6, δ 35.8, δ36.7, δ40.6, δ33.2 and δ34.5, the methine groups at δ32.2, δ48.8, δ47.8 and δ40.5.

The HMBC spectral data (Table 3.19), the α methylene at δ2.11 and δ2.18 presented ^{2}J correlation to δ180.2 indicated the presence of a carbonyl group at C-3. The methyl protons at δ1.09 and δ0.91 showed ^{3}J correlation to carbon at δ158.0. The cross peaks displayed between methyl protons at δ0.82 to carbon at δ25.3 indicated the long range couple between Me-24 and C-25. The two methyls at δ0.89 and δ0.82 showed ^{2}J correlations to carbon signal at δ32.2. The COSY spectral data presented the couple of the methyls protons at δ0.89 and δ0.82 to the methane proton at δ1.87. These supported an isopropyl group situated at position 5 (Fig. 3.33). In addition, there was no linkage observed between positions 3 and 4, which suggested the presence of an open A ring (Setzer, W., N., *et al*, 2000). Furthermore, ^{13}C NMR characteristics of this compound were very similar to those in taraxerol (Mahato, S., B., *et al*, 1994) and taraxerone (Sakurai, N. *et al*, 1987), exceptted for the signals at δ 30.0, δ 29.8, δ 180.2, δ 32.2, δ 40.1 and δ 0.89.

From the evidence data given confirmed that this compound was 3, 4-seco taraxerone (Fig. 3.31). It was isolated from the leaf of *Alchornea latifolia* (Setzer, W., N., *et al*, 2000). This compound was the first report from the stems of *Bruguiera gymnorrhiza*.

Table3.16: ^{1}H (400MH_Z) and ^{13}C (100MH_Z) NMR data of KC-13 in $CDCl_3$

Position	δ_H	δ_C	Selected HMBC correlations
1	1.90 (2H, *dd*, 12.31, 4.52)	30.0	
2	2.11(1H, *m*)/2.18 (1H, *m*)	29.8	C-3
3		180.2	
4	1.87(1H, *d*, 5.8)	32.2	
5		40.5	
6		18.9	
7		28.3	
8		40.8	
9		48.8	
10		37.7	
11		17.6	
12		35.8	
13		38.9	
14		158.0	
15	5.54 (1H,*dd*, 8.34, 3.06)	117.1	
16	1.65(1H, *d* , 6.6)	36.7	
17		35.2	
18		47.8	
19		40.6	
20		28.9	
21		33.2	
22		34.5	
23	0.94(3H, *d*, 7.92)	21.5	C-4
24	0.82(3H, *d*, 7.04)	19.2	C-4
25	0.89(3H, *s*)	25.3	
26	1.09(3H, *s*)	24.9	Me-24, Me-27
27	0.91(3H, *s*)	25.2	
28	0.81(3H, *s*)	29.9	
29	0.94(3H, *s*)	33.6	
30	0.91(3H, *s*)	18.9	

Chemical shifts (*ppm*.), Coupling constants (H_Z)

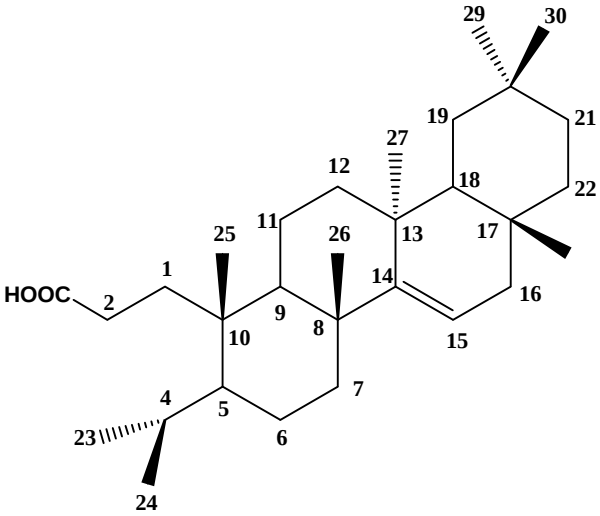


Fig. 3.31; Structure of KC-13

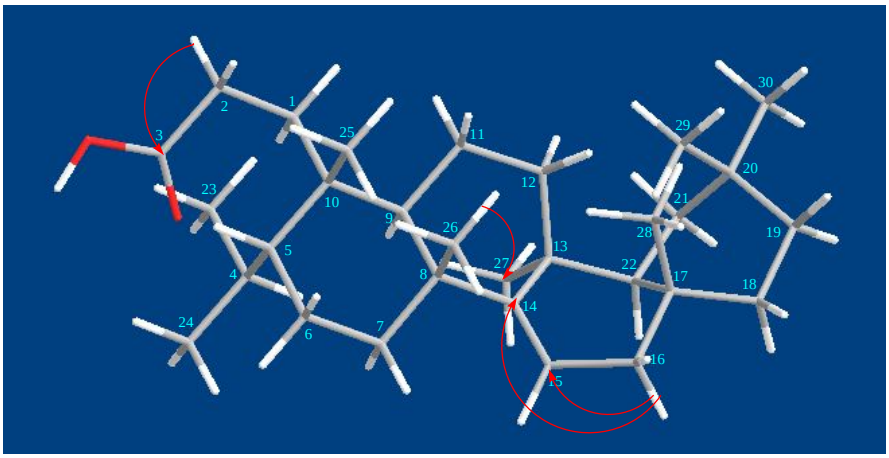
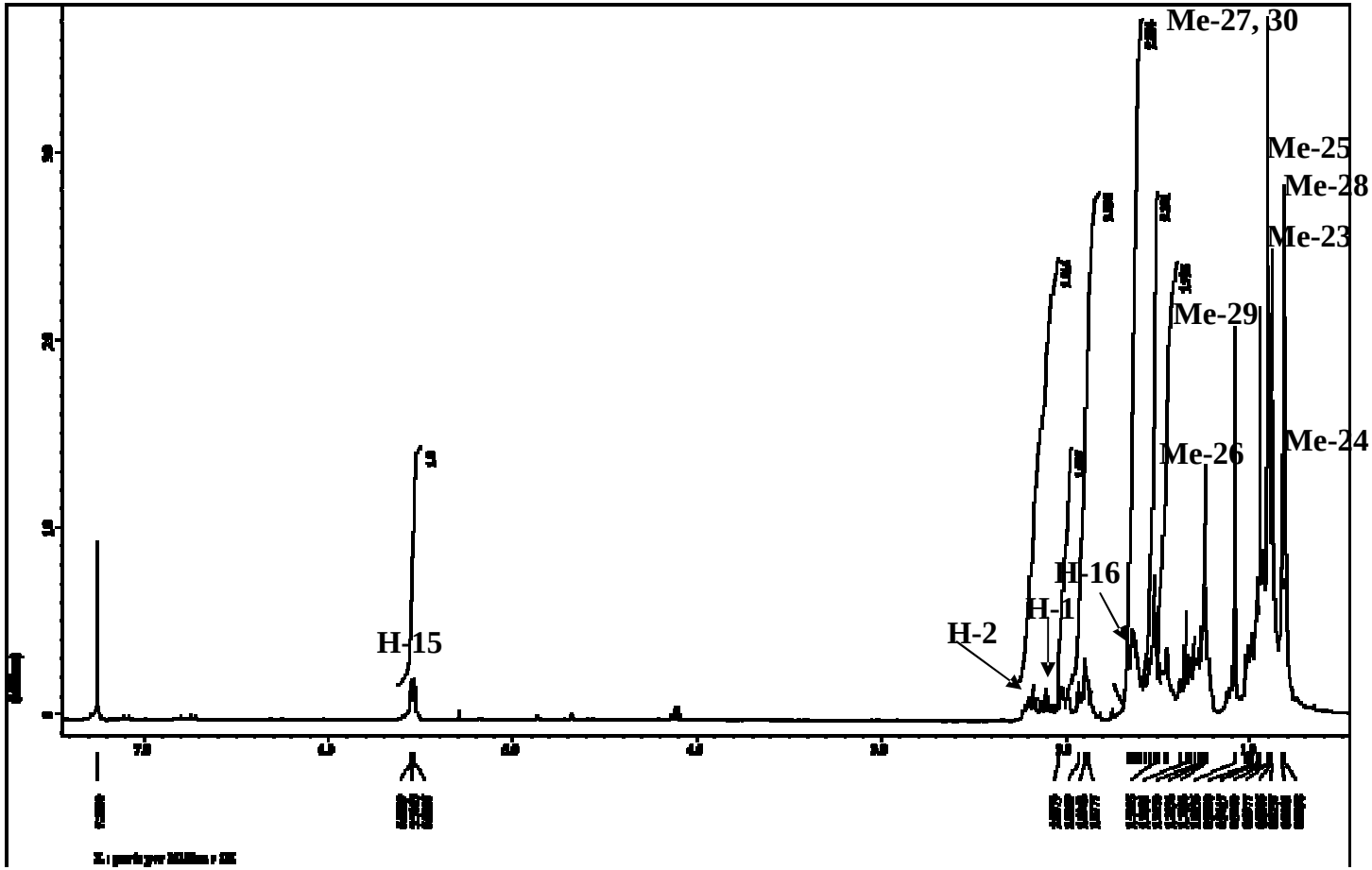
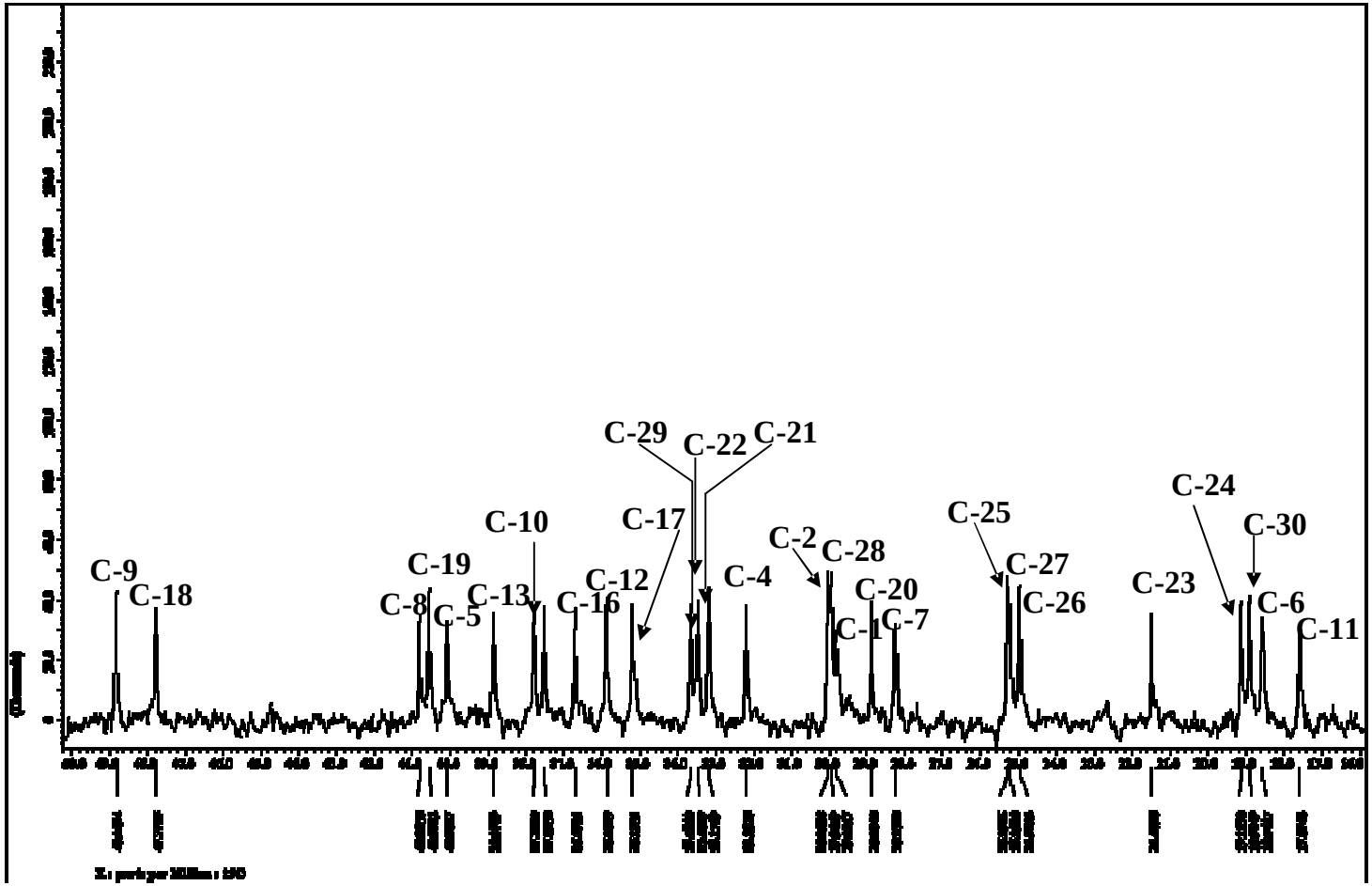


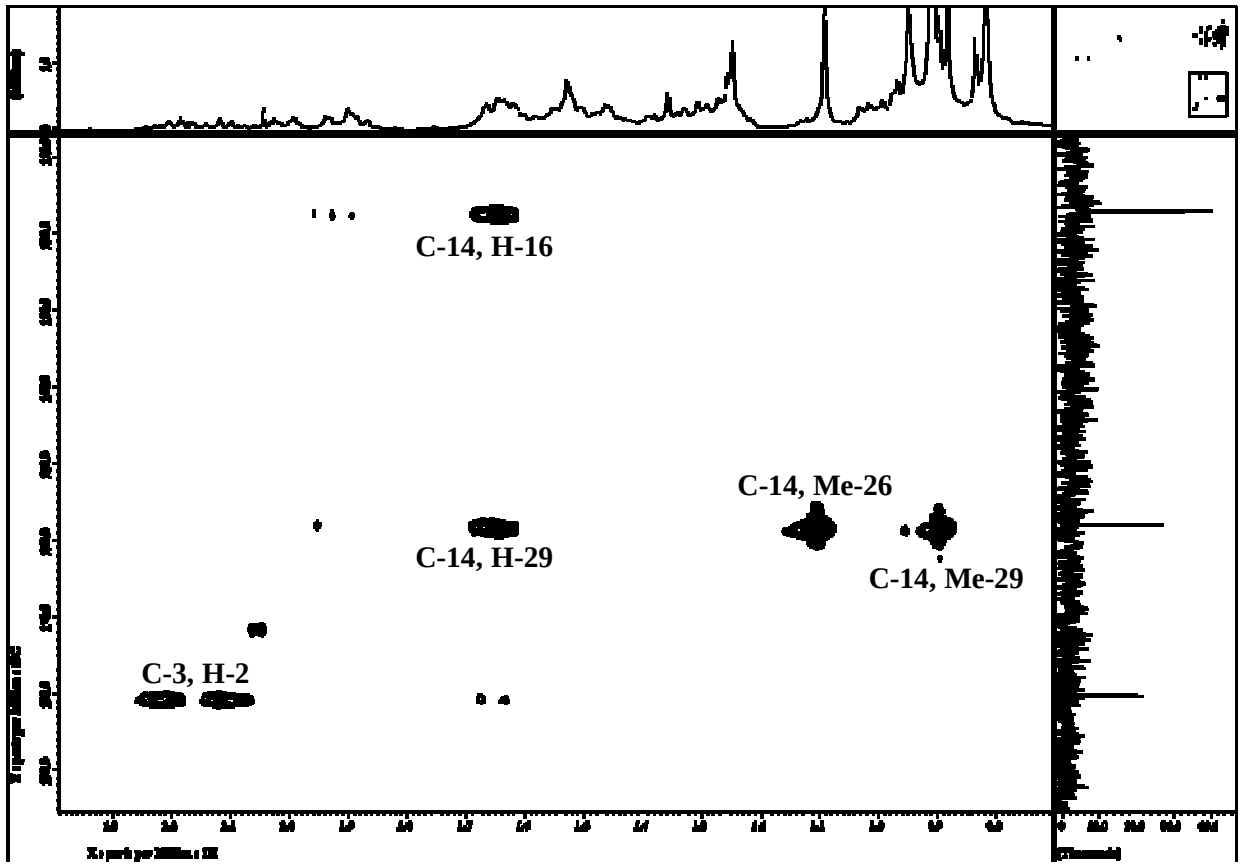
Fig. 3.32; 3D Structure of KC-13 with representing for HMBC correlations



Spectrum 3.35; ^{1}H (400M H_z) NMR spectrum of KC-13 in $CDCl_3$



Spectrum 3.36; ^{13}C (100M H_z) NMR spectrum (selected expansion) of KC-13 in $CDCl_3$



Spectrum 3.37; HMBC spectrum (selected) of KC-13 in $CDCl_3$

3.1.17 Characterisation of KC-14 as Stenocarpoquinone B

KC-14 was obtained as yellow oil from the ethyl acetate extract of *Avicenna alba* dried stems. This compound was active under short wavelength UV light and was colourless on TLC after spraying with *p*-anisaldehyde-sulfuric acid followed by heating.

The FTMS+c-ESIMS showed a quasi-molecular ion at 259 $[M+H]^+$ and indicated a molecular ion 258 suggesting a molecular formula of $C_{15}H_{14}O_4$.

The ^{1}H NMR spectrum data (Table 3.20) presented the double doublet signals at δ 8.06 (1H, *dd*, *J*=2, 2.7 H_Z), δ 8.09 (1H, *dd*, *J*=1.5, 8 H_Z), δ 7.68(1H, *ddd*, *J*=2.3, 6.5, 8 H_Z) and 7.75 (1H, *ddd*, *J*=1.4, 8, 8 HZ) for aromatic protons; a signal at δ3.18 (2H, *d*, *J*=12H_Z) coupled to a signal at δ 4.85 (1H, *t*, *J*=12 H_Z) suggested the presence of a methine proton attached to an oxygen bearing carbon. Two methyl signals at δ 1.27 (3H, *s*), δ 1.41 (3H, *s*) attributed to a side chain of two methyl protons attached to a quaternary carbon connected to a hydroxyl group.

The ^{13}C NMR spectrum data showed 15 carbon signals. The multiplicity determined by DEPT and HMQC techniques revealed two methyl, one methylene, five methine and seven quaternary signals. The carbon signals presented at δ177.83 and δ181.0 were assigned as two carbonyl groups C-1 and C-4. The set of signals found at δ126.09, δ 134.24, δ133.12 and δ126.65 of an aromatic structure. Deshielded at δ 92.19 and δ 71.73 indicated the presence of two carbons bearing oxygen groups.

The HMBC spectrum data (Table 3.20 and spectrum 3.40-3.43)) the vicinal methylene proton at δ3.18 showed ^3J correlation to quaternary carbon at δ160.14, one of carbonyl groups at δ82.2 and the quaternary carbon side chain at δ71.7. It was also displayed ^2J correlation to oxymethine carbon at δ92.1, quaternary carbon at δ125.1 and the long range correlation to another carbonyl group at δ177.8. These correlations indicated the presence of furan ring fused to the naphthoquinone nucleus at sp^2 carbons. The aromatic proton at δ8.06 revealed ^3J correlations to carbons at δ133.1 and δ131.6. The other two aromatic protons at δ7.75 showed ^3J correlations to

carbons at δ126.1, δ131.6 and the proton at δ8.09 showed ^{3}J correlations to carbons at δ134.2, δ132.9. These relationships suggested the presence of an aromatic ring. The methyl protons at 25.8 and 24.0 exhibited ^{2}J correlations to the quaternary carbon side chain at 71.7, ^{3}J correlations to the methane carbon at 92.1 and they had ^{3}J correlations to each other carbons, indicating the presence of the furan ring side chain.

Stenocarpoquinone has previously been isolated from the roots and stems of *Mendoncia cowanii* (S. Moore) Benoist (Acanthaceae) (Willium, R., B., 2005) and was reported from the trunkwood of *Tabebuia heptaphylla* (Schmeda-Hirschmann, G., *et al*, 2003).

The evidence data given suggesting this compound as stenocarpoquinone B (Fig. 3.33) and this is the first report of stenocarpoquinone B from *Avicennia alba*.

Table 3.17: ^{1}H (400MH_Z) and ^{13}C (100MH_Z) NMR data of KC-14 in $CDCl_3$

Position	δ_H	δ_C	Selected HMBC correlations
1		177.8	
2		160.1	
3		125.1	
4		182.2	
4a		132.9	
5	8.06 (1H, *dd*, 2.3, 8)	126.1	C-7, C-8a
6	7.68 (1H, *ddd*, 2.3, 6.5, 8)	134.2	C-8, C-4a
7	7.75 (1H, *ddd*, 1.4, 8, 8)	133.1	C-5, C-8a
8	8.09 (1H, *dd*, 1.5, 8)	126.3	C-6, C-4a
8a		131.6	
1’	3.18 (2H, *d*, 12)	28.4	C-2’, C-3’, C-2, C-1, C-3, C-4
2’	4.85 (1H, *t*, 12)	92.1	
3’	-	71.7	
4’-Me	1.41 (3H, *s*)	25.8	C-5’, C-3’, C-2’
5’-Me	1.27 (3H, *s*)	24.0	C-4’, C-3’, C-2’

Chemical shifts, *ppm*, coupling constants (H_Z)

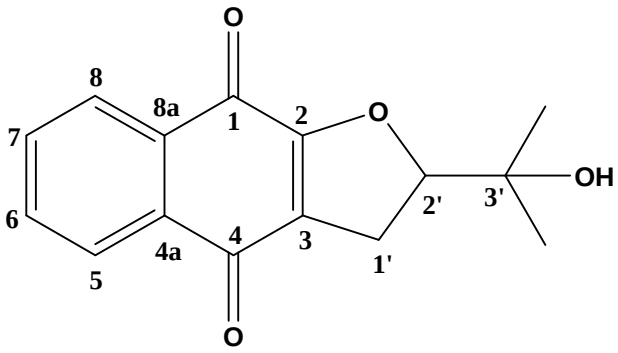


Fig. 3.33; Structure of KC-14

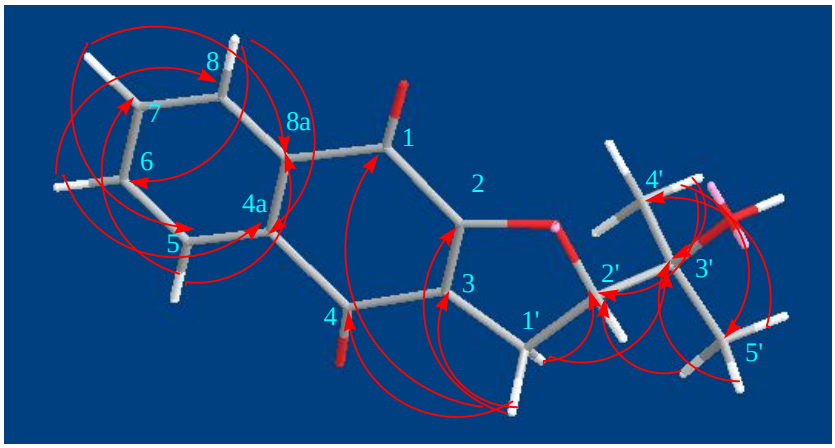
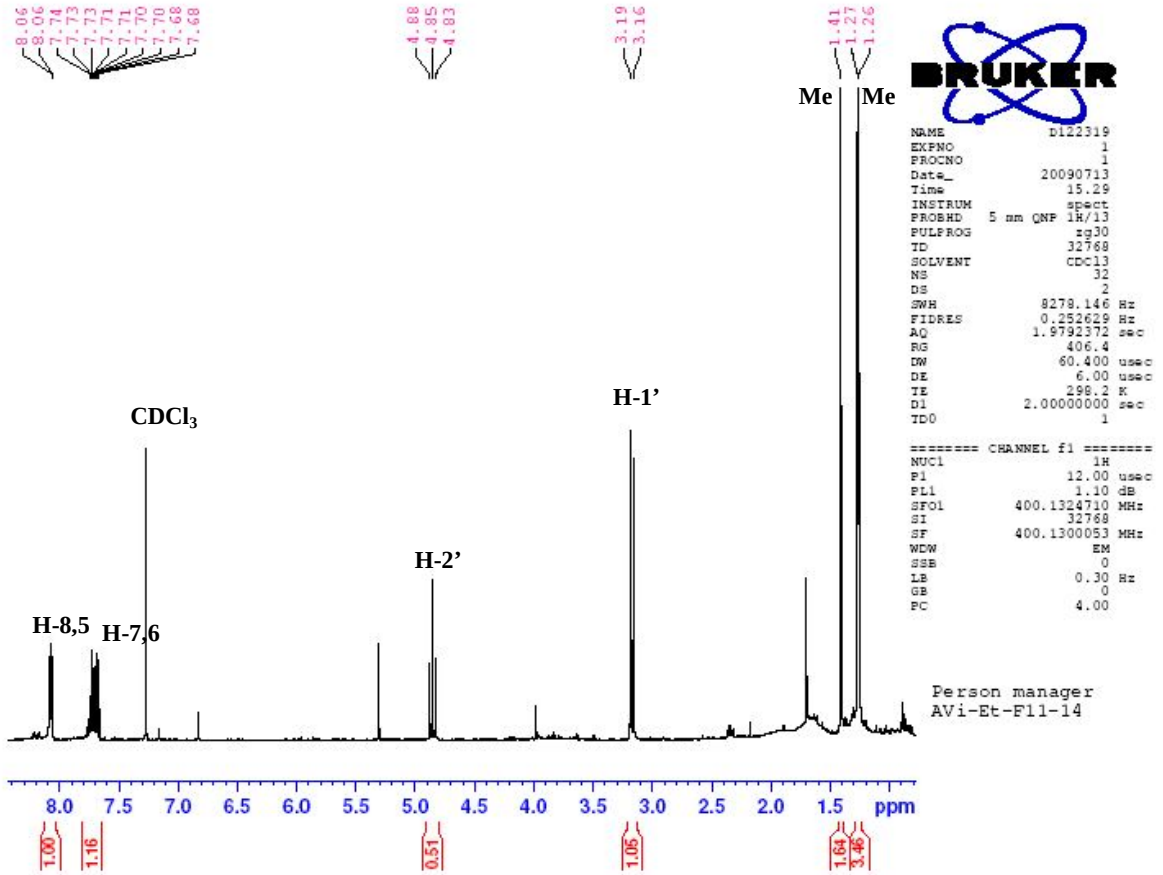
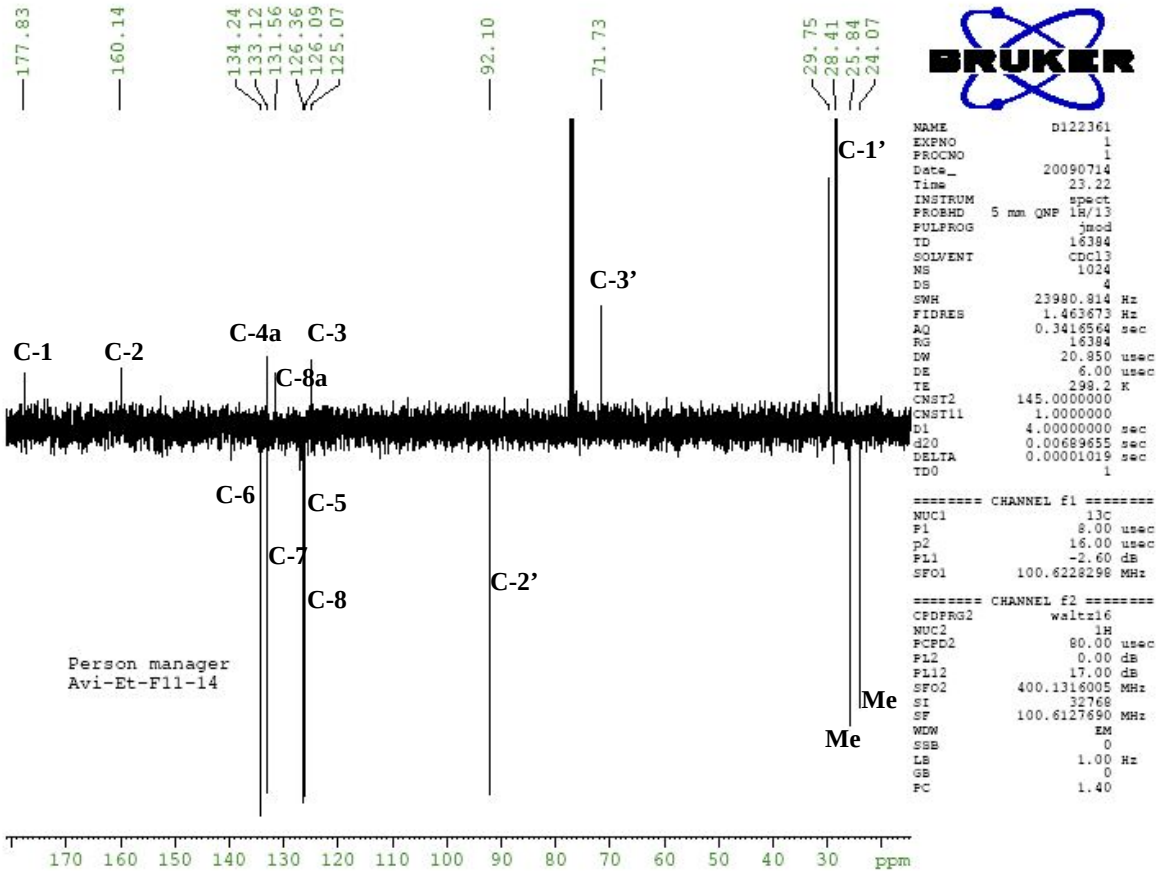


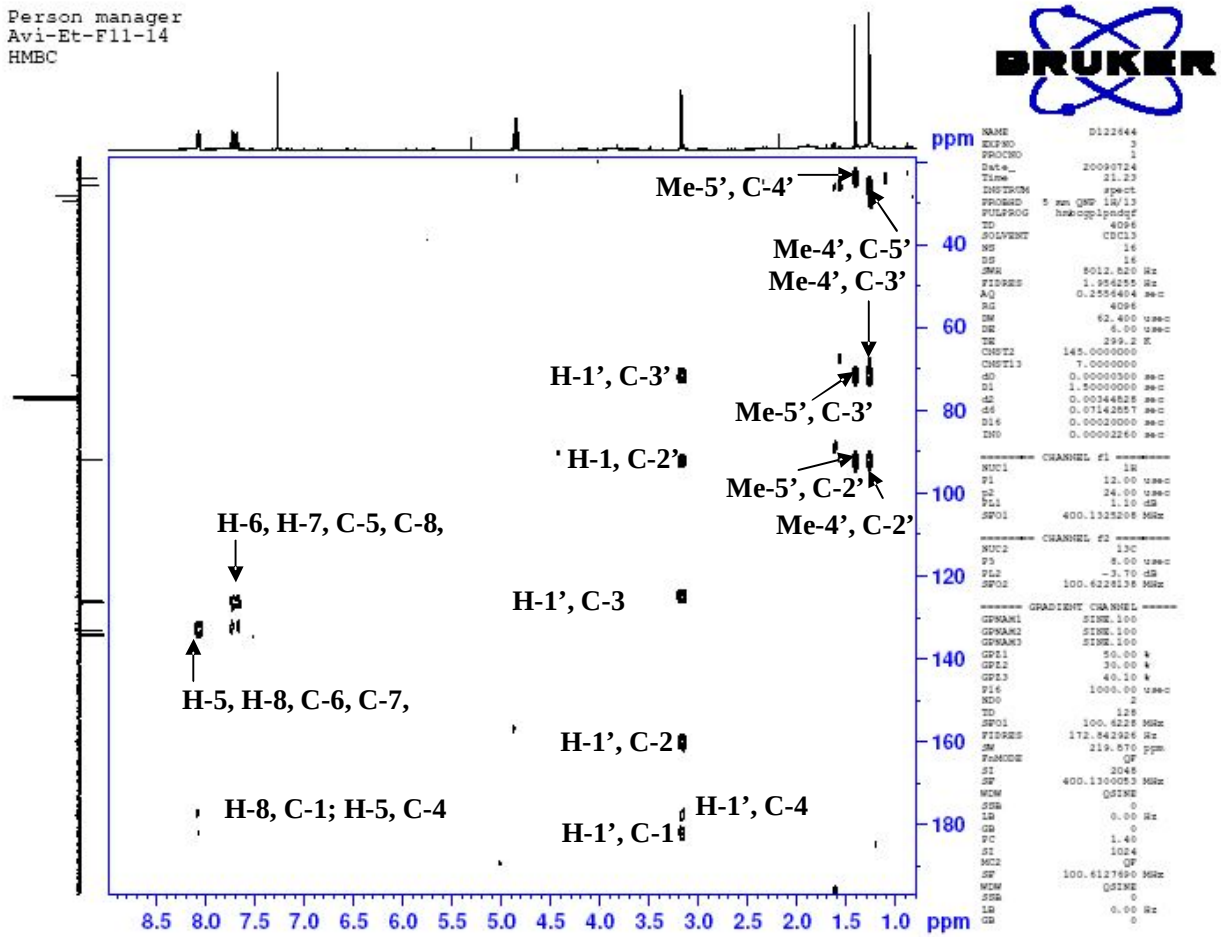
Fig. 3.34; 3D Structure of KC-14 with representing for HMBC correlations



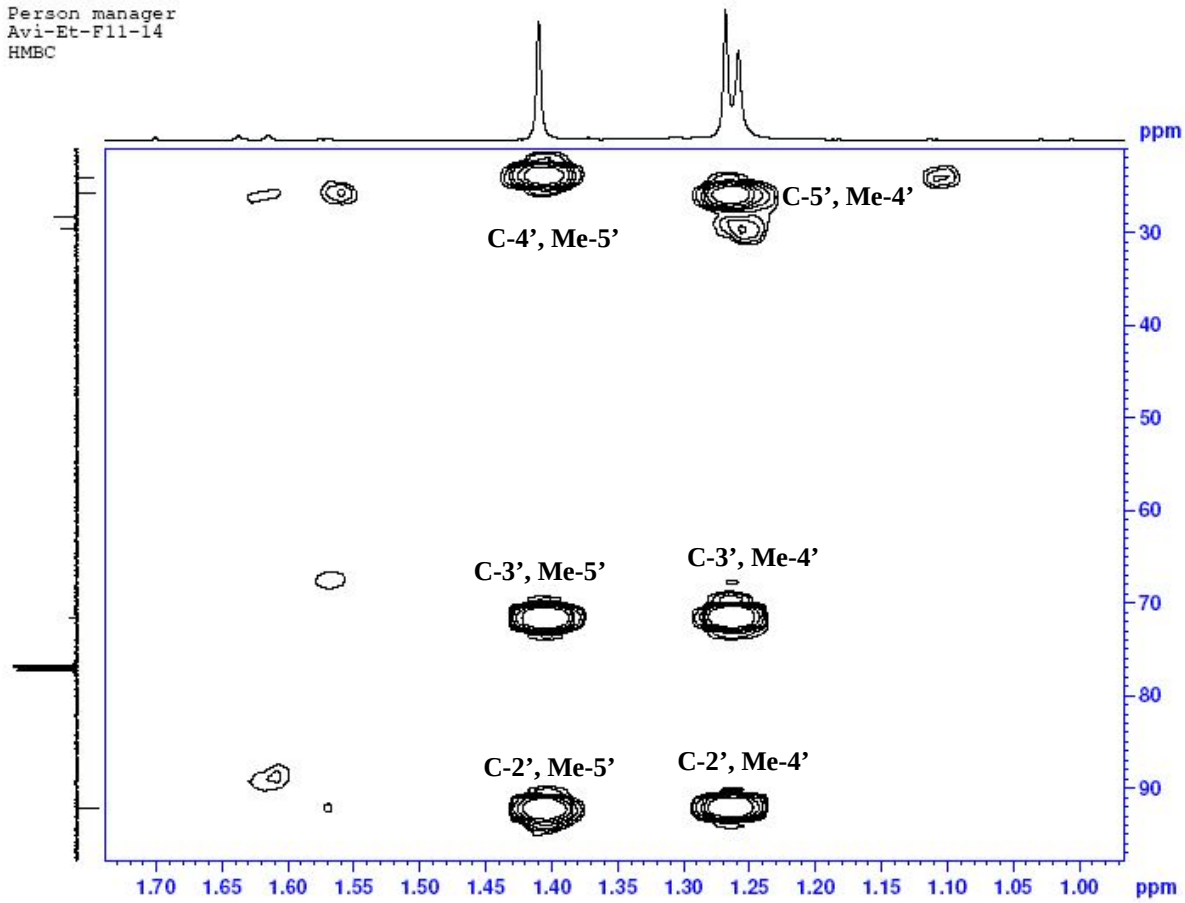
Spectrum 3.38; ^{1}H (400MH_z) NMR spectrum of KC-14 in $CDCl_3$



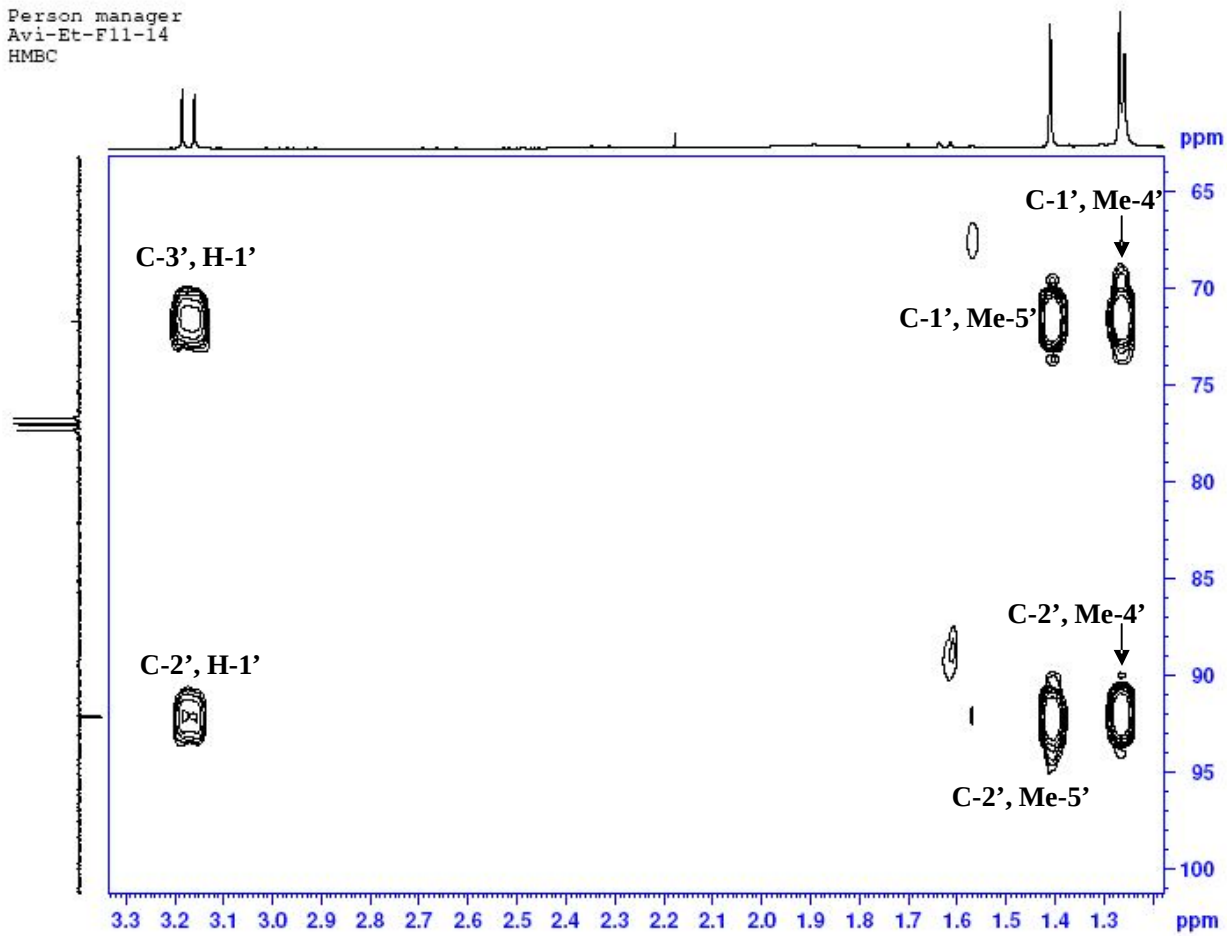
Spectrum 3.39; DEPT NMR spectrum of KC-14 in $CDCl_3$



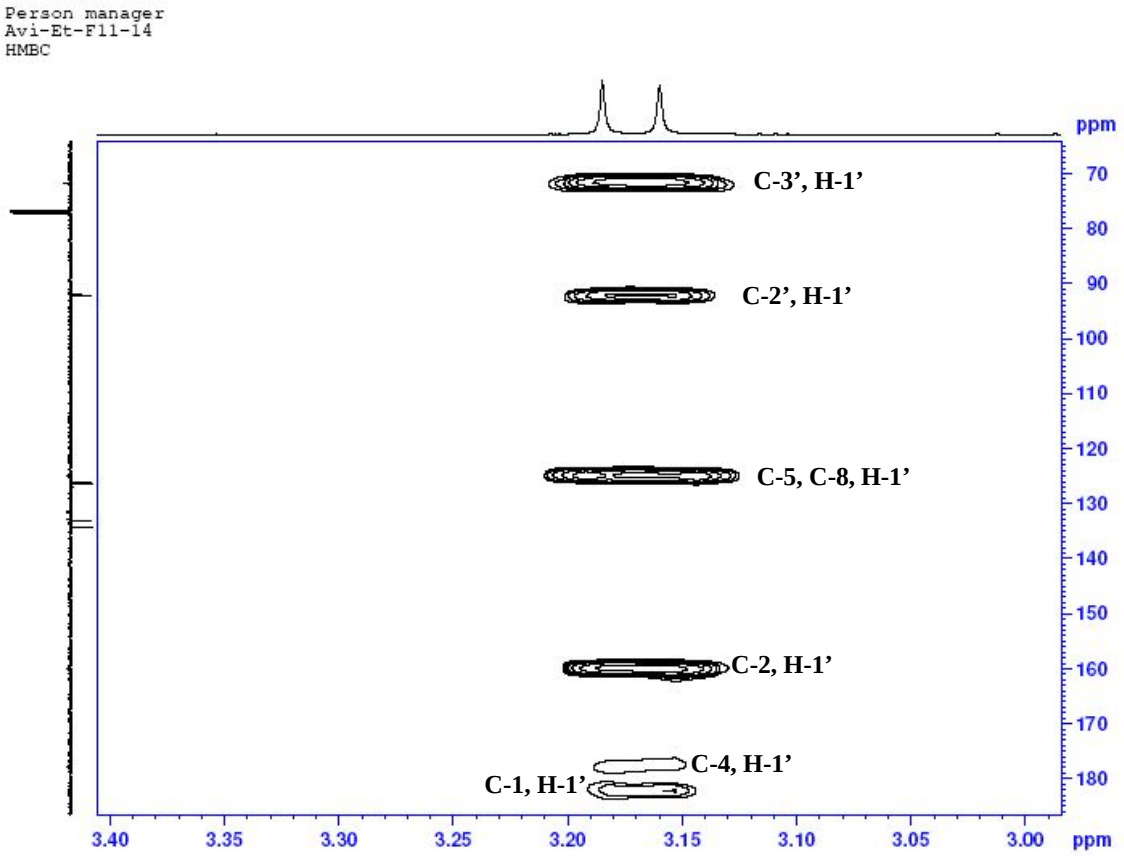
Spectrum 3.40; HMBC correlations spectrum of KC-14 in $CDCl_3$



Spectrum 3.41; HMBC correlations spectrum (selected expansion) of KC-14 in $CDCl_3$



Spectrum 3.42; HMBC correlations spectrum (selected expansion) of KC-14 in $CDCl_3$



Spectrum 3.43; HMBC correlations spectrum (selected expansion) of KC-14 in $CDCl_3$

3.2 Antimicrobial screening

3.2.1 Antimicrobial activity

In this study, eugenol and 4-allyl pyrocatechol, compounds isolated from *Piper betel* were against fifteen isolated (clinical isolates) strains of MRSA. The two compounds exhibited significant anti-MRSA activities against the test organisms with MIC of 64 and 128 µg/L (see Table 3.17).

Table 3.18 Anti-MRSA activity *in vitro* of selected antibiotics, eugenol and 4-allyl pyrocatechol isolated from *Piper betel* leaf

	MIC (µg /L)									
Isolates	**TET**	**VAN**	**GEN**	**AMP**	**OXN**	**CTX**	**CEF**	**CIP**	**EUG**	**4-AP**
LF78	0.25	2	1	32	64	128	>128	64	64	64
LF80	0.50	2	0.50	128	>128	>128	>128	128	64	128
LF81	0.50	2	32	64	>128	>128	>128	128	64	65
LF82	0.25	2	0.25	16	8	64	32	64	128	128
LF85	0.25	1	16	32	>128	>128	>128	128	64	128
LF87	0.25	2	0.50	64	>128	>128	>128	128	128	128
LF89	0.50	1	0.25	64	>128	>128	>128	128	64	64
LF91	0.25	1	32	64	>128	>128	>128	128	64	64
LF97	0.25	2	0.25	32	32	64	64	16	128	64
LF98	0.25	2	0.25	32	128	>128	>128	16	128	128
LF100	0.12	0.5	0.25	32	128	>128	128	128	128	128
LF102	0.25	1	0.25	32	128	>128	128	>128	128	128
LF105	0.12	0.5	0.50	4	32	64	32	32	128	128
LF109	0.25	1	0.50	64	>128	>128	>128	128	128	128
LF120	0.25	1	0.50	32	32	128	128	128	64	64
PA									256	256
SA									64	64

TET=tettracycline, VAN=vancomycin, GEN=gentamicin, AMP=amplicillin, OXA=oxacillin, CTX=cefotaxime, CEF=cefuroxime, CIP=ciprofloxacin, EUG=eugenol, 4-AP=4-allyl pyrocatechol, PA= *Pseudomonas aeruginosa* (Gram negative bacteria), SA=*Staphylococcus aureus* (Gram positive bacteria)

3.3 Discussion

3.3.1 Phytochemistry

Several different types of natural products have been isolated in this research including anthraquinones and steroids from *Cassia tora*, allyl benzenes and sesquiterpenes from *Piper betel* and triterpenoids from *Brugueira gymnorrhiza* and quinones from *Avecennia alba*. The chemical constituents in the plants are various depending on their environment conditions, season, part used, genetic variation, fertilization in soil, temperature, moisture and harvesting time and process (Awang, D.V.C., 2009).

3.3.1.1 Phenolic compounds

Eugenol is an active principle of Rhizoma acrori graminei used to treat Alzheimer's disease in Asia. Previous studies showed that eugenol has antidepressant activity inhibiting monoamine oxidase A (Tao, G., *et a*l., 2005). It is anti-inflammatory, inhibitingprostaglandin synthesis and reducing the tone of isolated gut muscle and myometrium on rat in vivo (Bennett, A., *et al*., 1988).

The structure-activity relationship (SAR) study, the position of double bond from the end of chain changed to the next position as in isoeugenol showed its activity more than eugenol. In addition, the phenolic group in eugenol molecule is also an important active site as shown in Mizutani study of hepatotoxicicity of eugenol and related compounds, 4-allyl pyrocatechol, the dimethoxy and a lactone ring with phenolic ring. Its allyl group showed toxic potential assuming that vinyl-quinone methide was formed by metabolic of eugenol. It also played a key role to induce hepatotoxicity in mice (Mizutani, T, *et al*, 1991).

The geometric chemical structure such as plane (defined by three or more points at the end of bond), centroid (the centre of uniform density mass, defined by point) and exclusion sphere (indicated the interatomic distance and using to mark steric requirements of binding, defined by point and distance) providing important information for computering drug designs.

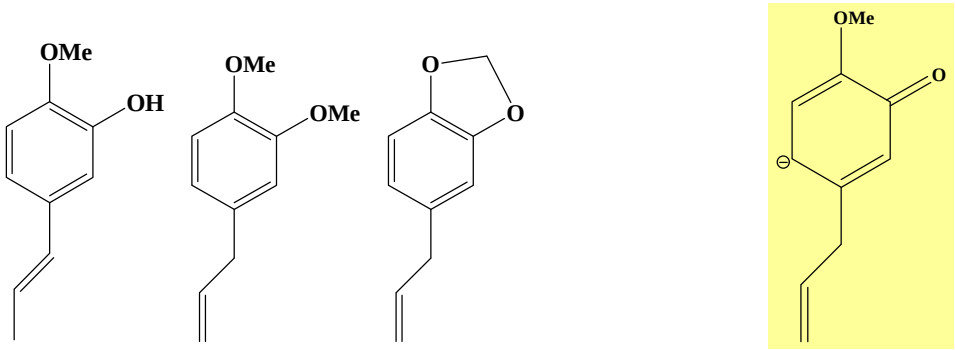


Fig. 3.35; Structure of eugenol, related compounds and vinyl-quinone methide intermediate

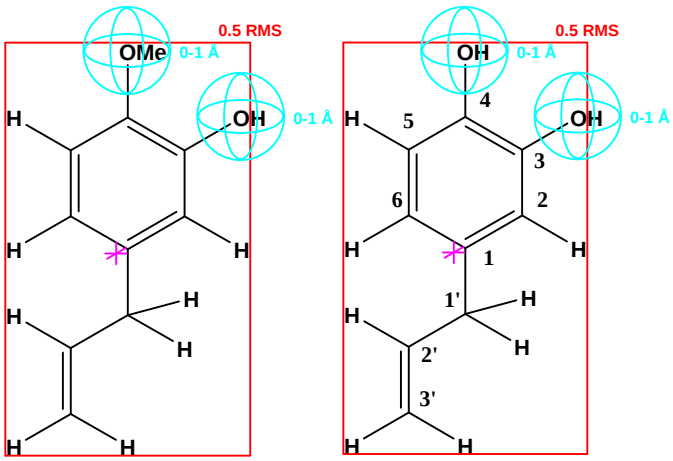


Fig. 3.36; Exclude sphere, plane and centroid of eugenol and 4-allyl pyrocatechol

Previous studies have showed that **4-allylpyrocatechol** showed protective activity against the photosentitization-induce damage to lipids and proteins of rat liver mitochondria (Bhattacharya, S., *et al*, 2007). It was found from *Piper betel* inflorescenes exhibited mutagenic potential in *Salmonella typhimurium* TA 102 without metabolic activation (Lee-Chen, S.-F., *et al*, 1996). This compound was tested for its antiplatelet effect for cardiovascular diseases and found that it inhibited arachidonic acid and collagen aggregation and TXB_2 production suggesting that it could be a potential therapeutic agent for prevention and treatment of atherosclorosis and other cardiovascular diseases though its anti-inflammatory and antiplatelet effects without effect on haemostatic functions (Chang, M., C., *et al*, 2007). Moreover, 4-allylpyrocatechol presented more potent than eugenol on dose-

dependent suppression of dimethylbenzanthracene-induced mutagenesis in *Samonella typhimurium* strain TA98 with metabolism activation (Amonka, A., J., *et al*, 1986).

3.3.1.2 Triterpenoids

Triterpenoids were the major compounds isolated from the leaf of *Brugueira gymnorrhiza*; namely, 3B-*E*-p-coumaroyltaraxerol, careaborin, taraxerol, seco-3, 4-taraxerone, taraxerone, 3β-lauryl-3-β-amyrin. All of these phytochemicals isolated from this plant in the present study, are the first report not only from the plant species but also from the Rhizophoraceae family. Only betulinic acid was a triterpene isolated and it is the first report from the stems of *Avicenna alba*.

Betulinic acid, was originally called gratiolone (Flekhter, O., B., *et al*, 2002), is an interesting and important compound owing to the variety of biological activities refered for example, as a cytotoxic agents against melanoma tumour cells (Pisha, E., *et al*, 1995) and solid tumours of childhood (Fulda, S., *et al*, 1997). A considerable amount of this compound was isolated from the outer bark of white-barked birch trees (*Betula alba*). The bark has been used by the Native American as a folk medicine and other trees species exhibited cytotoxic agents against a variety of cancer types. The anticancer mechanism of action of betulinic acid was a trigger for the mitochondrial pathway of apoptosis (instrinsic programmed cell death) in cancer cells (Fulda, S., *et al*, 2008). Betulinic acid also has antimalarial activity against chloroquine-resistant *Plasmodium falciparum* parasites *in vitro*, with IC_{50} values of 9.89, 10.01, 51.58 and 45.79 µM and *in vivo* in *P. berghi*-infected mice which this can be developed to be new antimalarial medicines (Santos de Sa, M, *et al*, 2009).Betulinic acidwas also isolated from the dried roots of *Saussurea lappa* C.B. Clarke (Compositive) traditionally used as an ethnomedicine to treat gastric pain, lack of appetite and vomiting, has potential lead moieties for development to new PTP1B (Protein tyrosine phosphatases 1B) inhibitors as a negative regulator of insulin signal transduction using for treatment of type B diabetes and obesity (Choi, J., Y., *et al*, 2009).

Mukherjee studied the structure-activity relationship of betulinic acid & found that the antiangiogenic activity was increased to six times that when a carbonyl substituted at position-3 & nine times that for amine derivatives, in comparison to the free hydroxyl (Mukherjee, R., *et al*, 2004).

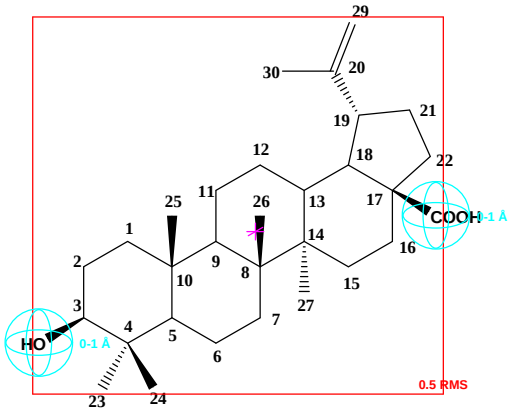


Fig. 3.37; Exclude sphere, plane and centroid of betulinic acid

The quantity of betulinic acid isolated from plant materials is generally low. A more effective method to obtain larger yieldshas been used synthesis from betulin with two steps such as oxidation by Jones reagent resulting in betulonic acid, followed by selective reduction with $NaBH_4$ to obtain betulinic acid in 60% yield, as shown below. Application of Jones reagent to the extract which treated birch bark with aqueous isopropanol in an autoclave at 80^0C resulted in betulin up to 90% and lupeol 5%. The percentage of potassium betulinoate was increased to 82% by the oxidation process. (Flekhter, O.B., *et al*, 2002).

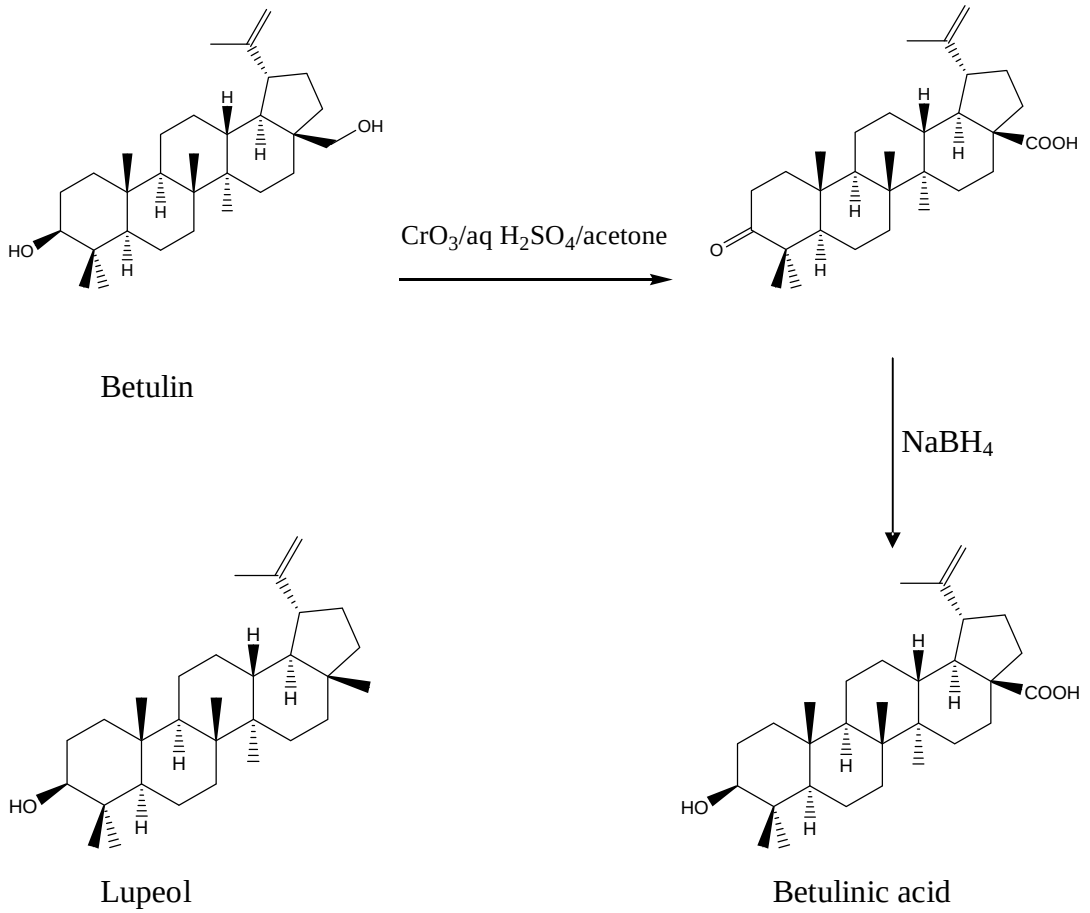


Fig. 3.38; Synthesis of Betulinic acid

3*β*-*E*-p-coumaroyltaraxerol and careaborin were a new oleanane-type triterpenoid *p*-coumaroyl ester, isolated from the stems and twigs of the mangrove plant *Rhizophora stylosa* (Li, D.-L., *et al*, 2008). Both compounds were isolated from a new plant species *Barringtonia maunwongyathiae* W. Chuakul (Lecythidaceae), recently discovered in Thailand. The *trans* compound, 3*β*-*E*-p-coumaroyltaraxerol, that demonstrated promise in cancer chemoprevention based on inhibition of TPA-induced ornithine decarboxylase expression, COX-1, COX-2 activities and phorbol ester-induced NF-kB luciferase expression as well as activation of the antioxidant response element-mediated luciferase expression (Jutiviboonsuk, A., *et al*, 2007). The *trans* configuration of the cinnamate moiety situated on other position in another triterpene also assessed with good biological activity. For example, 11α-O-trans-p-

coumaroyltaraxerol isolated from the roots of *Crabiodendron henryi* W. W. Smith exhibited antioxidant activity and vasodilator effects (Huang, X-Z., *et al*, 2008).

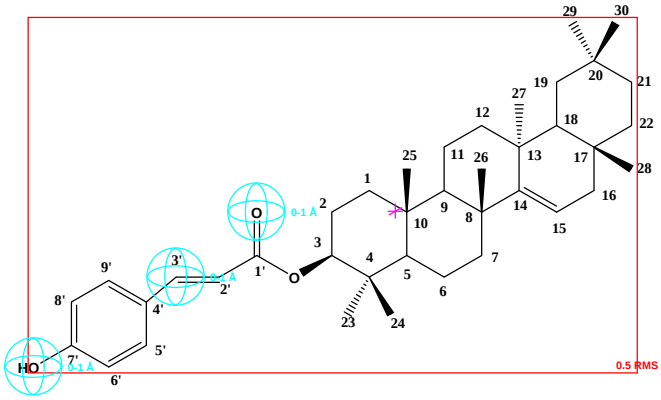


Fig. 3.39; Exclude sphere, plane and centroid of 3β-*E*-p-coumaroyltaraxerol

Seco-3,4- taraxerone was isolated from the leaf extract of *Alchornea latifolia* and showed *in vitro* cytotoxic activity against Hep-G2 and A-431 human cancer cell lines and possessed potent inhibitors of topoisomerase II (Setzer, S., *et al*, 2000).

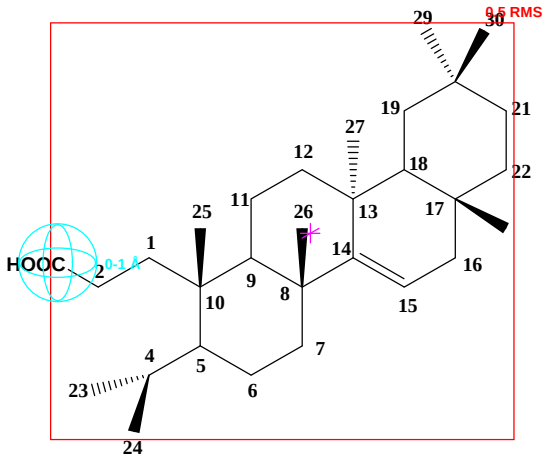


Fig. 3.40; Exclude sphere, plane and centroid of 3, 4-seco taraxerol

Taraxerone was isolated from the plant *Euphorbia hirta* and was tested for antibacterial and antifungal activities against fourteen bacterial and six fungal pathogens. The compound was active, but not significantly so, against most of the tested fungi. Its effective MIC was found to be between 64-128 µg/mL (Abu-Sayeed, M., *et al*, 2005). The compound was also isolated from the stems of *Diospyros*

maritime and evaluated for *in vitro* cytotoxicity in four cancer cell lines (Kuo, Y., H., *et al*, 1997). Taraxerone was isolated from the fruits of *Dregea volubilis* Benth (Asclepiadaceae) and demonstrated *in vitro* anti-leishmanial against promastigotes of *Leishmania donovani* (strain AG 83) and had anti-tumour activities (Moulisha, B., *et al*, 2009). Taraxerone, isolated from the aerial parts of *Laggera pterodonta*, exhibited weak antiviral activity against herpes simplex virus (type I and II) (Kuljanabhagavad, T., *et al*, 2009).

This compound was also isolated from hexane extracts of the flowers and leaves of *Acacia cochliacantha* and was tested for antibacterial activity. It showed potent activity against *S. aureus, B. subtilis, L. plantarum, E. coli, S. typhimurium, K. pneumoniae, Ps. Aeruginosa* with MIC. 0.4, 1.4, 1.4, 0.2, 2.8, 1.4, 2.8 µg/mL, respectively (Manriquez-Torres, J., J., *et al*, 2007).

The activity of related compounds, such as taraxeryl-3-acetate, showed three times higher antiviral activity against herpes simplex virus type II compared to taraxerone itself, but it was inactive against the type I virus. They exhibited equal cytotoxicity with ED_{50} > 50 µg/mL (Kuljanabhagavad, T., *et al*, 2009).
Taraxerone can be converted to acetyl taraxerone by acetylation after reduction of the carbonyl group and the reverse is also possible. The reduced product, taraxerol, displayed potent NO-reducing activity in microglial cells (Tsao, C.-C., *et al*, 2008). Taraxerol also inhibits the growth of Hela and BGG-823, with IC50 of 73.3µmol/L-1 and 116.0µmol/L-1, whilst taraxerone was inactive (Xuhong, Y., *et al*, 2008).

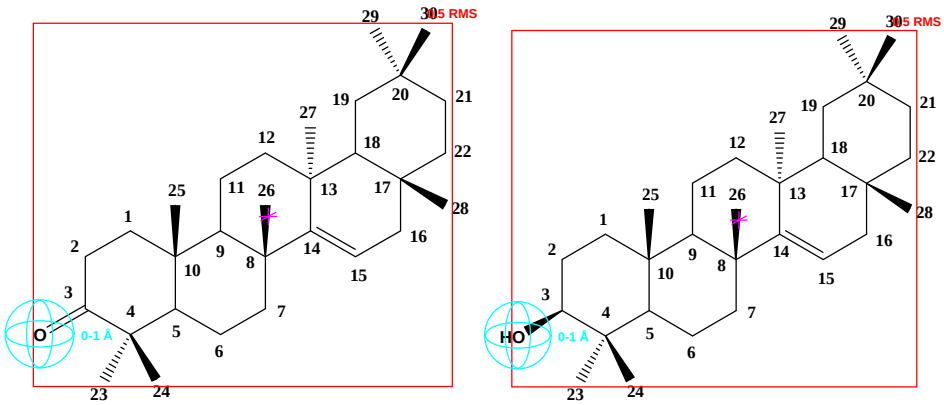


Fig. 3.41; Exclude sphere, plane and centroid of taraxerone and taraxerol

3.3.1.3 Sesquiterpenes

Sesquiterpenes were isolated and are the first report from the dried leaves of *Piper betel* including B-elemene, trans-cadamenene and Gamma-muurolene.

β-elemene, is a natural sesquiterpene, extracted from the ginger plant which possessed novel anticancer activity (Wang, G., *et al*, 2005), against leukemia and solid tumors and in a recent study it was part of a regimen for lung carcinoma and other cisplatin-resistant tumors (Li, Q., Q., *et al*, 2009). It effectively crosses the blood-brain barrier and shows promise for malignat brain tumor treatment (Wang, K., *et al*, 2005). This compound was able to inhibit the proliferation of cisplatin-resistant human ovarian cancer cells (Li, X., *et al*, 2005). This compound was synthesized from 2-allylpropane-1, 3-diol (Lee, J., *et al*, 2002) and geranylacetone (McMurry, J., E., *et al*, 1985).

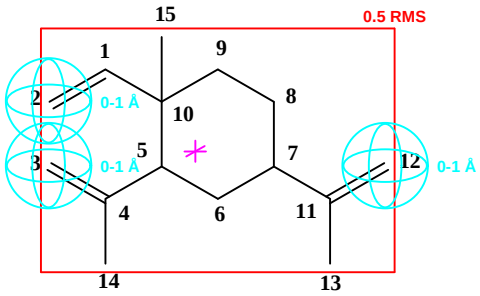


Fig. 3.42; Exclude sphere, plane and centroid of *β*-elemene

***Gamma*-muurolene** has been reported mostly as a constituent of essential oils. It has been found in 0.5% yield in *Artemisia campestris a*nd this plant exerted anti-freeradical activity by DPPH method (Akrout, A., *et al*, 2010). This compound, in 19% yield, was in the essential oils from the leaves of *Hippomarathrum microcarpum* (M.B.) B. Fedsch.; 19.2% from its flowers (Sefidkun, F., *et al*, 2003). It was also found in the essential oil from the aerial parts of *Ajuga chamaepitys* (L.) Schrebes, spp. in 40.3% yield (Arturo, V.-N., *et al*, 2004).

This compound was the first isolated as a single compound from the dried leaves of *Piper betel*.

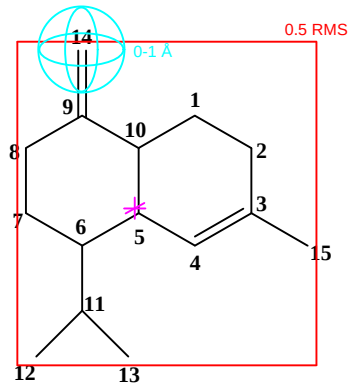


Fig. 3.43; Exclude sphere, plane and centroid of γ-muurolene

***Trans*-calamenene** was isolated from the soft corals of *Nephtha erecta* and *Nephthea chabroli* (Wang, S.-K., *et al*, 2009). This compound was found in essential oil of *Chrysanthemum balsamita* (L.) Baill., and has been used as a carminative and cardiotonic in traditional and folk medicine in Iran (Hassanpouraghdam, M.-B., *et al*, 2008). It was synthesized from menthone by the benzannulation approach while *cis*-calamenene was prepared using isomenthone as a starting material (Serra, S., *et al*, 2005).

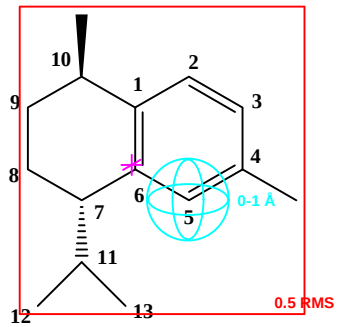


Fig. 3.44; Exclude sphere, plane and centroid of *trans*-calamenene

3.3.1.4 Quinones

Only one quinine, stenocarpoquinone, was isolated from the stems of *Avicennia alba*. It is also the first report from this study.

Stenocarpoquinone was isolated from the trunkwood of *Tabebuia heptaphylla*is used to treat cancer, for wound healing and inflammation (Schmeda-Hirschmann, G., *et al*, 2003). This compound, showed strong antiproliferative and moderate cytotoxic

activities as well as antibacterial effects, after isolation from twigs of *Avicennia marina* (Han, L., et al, 2007). It was a pigment isolated from wood of *Stenocarpus* R.Br. (Mock, J., *et al*, 1973).

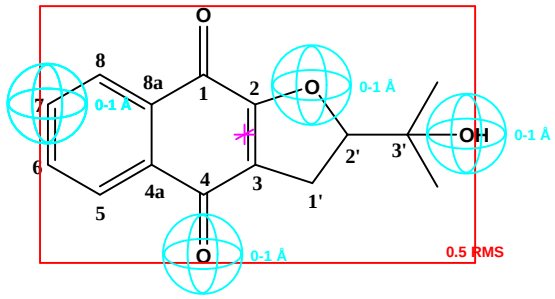


Fig. 3.45; Exclude sphere, plane and centroid of stenocarpoquinone B

In addition, the partial synthesis of stenocarpoquinone by dehydrogenation of the position 1'and 2' giving avicequinone C and then hydroxylation at position-8 of aromatic ring produces avicequinone D. Only hydroxylation of aromatic ring at position-8 yielded avicequinone E. These compounds gave better cytotoxicities against A2780 human ovarian cancer cell lines with avicequinone C , D and E having IC_{50} values 7.4, 8.8 and 9.8 µM, respectively, while that of stenocarpoquinone was 50 µM (Russell, B., 2005).

3.3.1.5 Steroids

Sitosterol was isolated from *Cassia tora* and *Bruguiera gumnorrhiza* while stigmasterol was isolated from *Cassia tora*.

Sitosterol is a plant sterol, known to prevent cardiovascular disease because it enhances glucose uptake and reduces triglyceride and cholesterol levels (Hwang, S.-L., *et al*, 2008). It exhibited inhibitory effects on the pathophysical process in ovalbumin-induced asthmatic mice which indicates its potential therapeutic molecule in asthma (Yuk, J., E., *et al*, 2007).This compound may have the potential to prevent/treat human cancer. From Choi's study of the effect of β-sitosterol on the growth of HT1 16 human cancer cells showed it was a possible active (Choi, Y., H., *et al*, 2003). It significantly increased the albumin content of plasma, but did not

affect the plasma total protein level in ovariectomized mice. These effects on plasma protein are similar to those of other oestrogens (Hassanein, R., R., *et al*, 1972).

β-sitosterol inhibited rhythmic contractions of pregnant and non-pregnant mouse uteri and mouse intestine. It relaxed the pregnant uterus and intestine and also decreased arterial blood pressure but did not change respiratory frequency as well as heart rate or the amplitude of contraction (Elghamry, M., I., *et al*, 1966). β-sitosterol, found in lipid content, exhibited strong antioxidant activity while ethyl acetate and butanol extracts showed moderate activity (Radwan, H., M., *et al*, 2006).

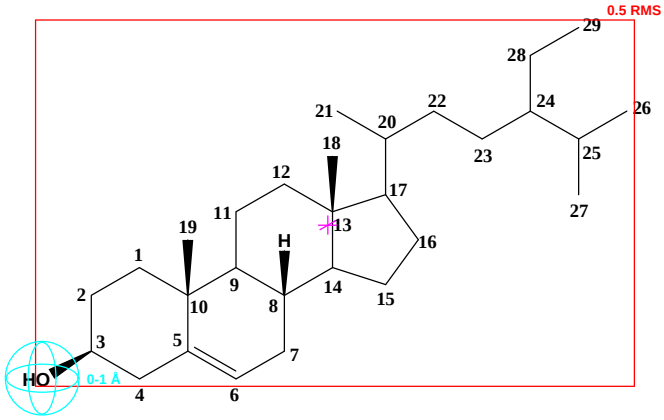


Fig. 3.46; Exclude sphere, plane and centroid of *β*-sitosterol

Stigmasterol is a main phytosterol in various plants, isolated from the bark of *Butea monosperma* and evaluated for its thyroid hormone and glucose regulatory efficacy in mice indicating for its thyroid inhibitory and hypoglycemic properties. It decreased the hepatic lipid peroxidation and increased the activities of catalase, superoxide dismutase and glutathione suggesting for its antioxidative potential (Panda, S., *et al*, 2009).

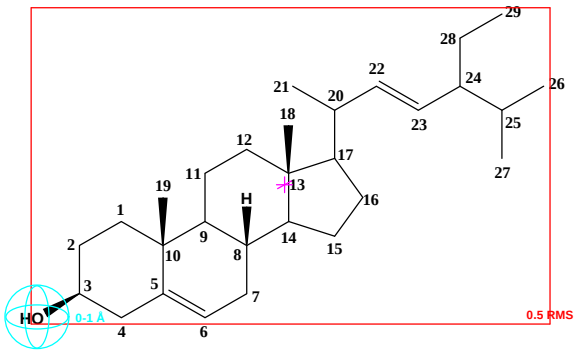


Fig. 3.47; Exclude sphere, plane and centroid of stigmasterol

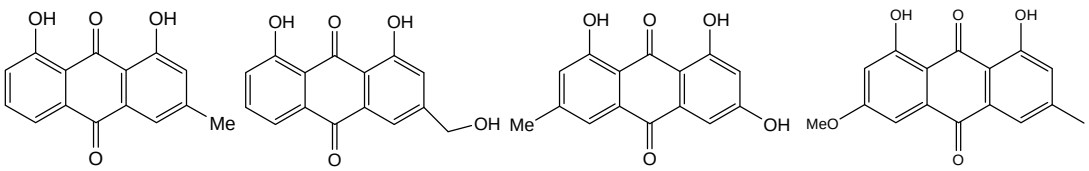
The mixure of β-sitosterol and stigmasterol, fernenol and arundoin were highly inhibited spore germination and germtube elongation. From the study of Yenjit on mango fruits they were significantly more effective than benomyl to control postharvest anthracnose disease of 100 and 200 mg/L using (Yenjit, P., *et al*, 2009).

3.3.1.6 Anthraquinones

Chrysophanol and physcion were isolated and are the first report from the leaves of *Cassia tora.*

Chrysophanol was isolated from the aerial parts of *Emex spinosa* (L.) growing in Egypt (Abd EI-Kader, A., M., *et al*, 2006), the roots of *Rheum palmatum* (Rhubarb) it showed relatively cytotoxic activities against tumor and normal cells (Shi, Y.-Q., *et al*, 2001). It inhibited noncompetitively phenylephrine-induced contraction of thoracic aorta in dose-dependent manner (Sun, X., *et al*, 1999). Chrysophanol with DNA-intercalating capability had antitumor activity *in vitro* (Kong, X., *et al*, 1992). It enhanced transformation of C3H/M2 mouse fibroblasts initiated by 3-methylcholanthrene suggesting that it may have tumor-promoting activities *in vitro* as it is one of hydroxyanthraquinones possess two hydroxy groups in the 1, 8-positions (Woelfle, D., *et al*, 1990). Yen studied antioxidant activity of anthraquinone and anthrone revealed that chrysophanol has accelerated the peroxidation of linoleic acid and accelerated the production of hydroxyl radicals supported for its pro-oxidant activity (Yen, G., C., *et al*, 2000).

Chrysophanol possesses dihydroxy groups at 1, 8-positions as its active site, leading its biological active as the study of Woelfle (Woelfle, D., *et al*, 1990). By comparing the activities of 1, 8 dihydroxyanthraquinones compounds such as aloe emodin, emodin, physcion and chrysophanol on toxicity against brine shrimp and R. scutatus resulted the toxicity in order: chrysophanol>aloe emodin>emodin>physcion with LC_{50} 0.00, 0.01, 0.05, 0.15 µg/mL, respectively (Sun, X., *et al*, 1999).



Chysophanol Aloe emodin Emodin Physcion

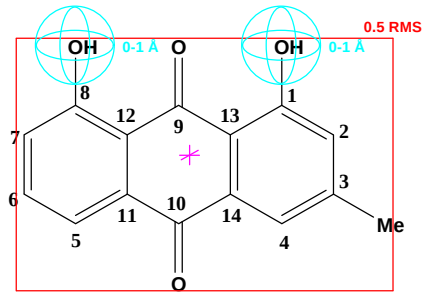


Fig. 3.48; Exclude sphere, plane and centroid of chrysophanol

The hydroxy at position-3 of emodin is involved in the inhibition activity of KCl-induced maximal contraction of thoracic aorta for 38.4% which was stronger than chrysophanol by 19.6% (Sun, X., *et al*, 1999). Chrysophanol is the first report isolated from *Cassia tora* leaf.

Physcion was isolated from *Eurotium repens mycelium*, was the highest cytotoxicity towards *Hella celles* caused 50% inhibition at concentration 0.1 ẁg/ml (Podojil, M., *et al*, 2008). It was also isolated from rhizomes of *Rheum emodi* and exhibited antifungal activity against *Candida albicans, Crytococcus neoformans, Trichophyton mentagophytes* and *Aspergillus fomigatus* at MIC 25-250 µg/mL (Agarwal, S., K., *et al*, 2000).

This compound found in the stem-barks of *Ventilago madraspatana* Gaerth. Showed activity inhibited *P. aeruginosa* (Subhalakshmi, B., *et al*, 2005). Physcion was isolated from Polygonum hypoleucum ohwi., have been used for a long time as a Chinese medicine, it presented inhibitory effects on Raj and K562 tumor cells proliferation of MIC10µg/mL at 22±3.2% and 24±3.6%, respectively (Kuo, Y.-C., *et al*, 1997). Physcion was the first report isolated from *Cassia tora* leaf.

The structure of physcion related to its biological activity, it has dihydroxy, dicarbonyl, a methoxy groups and aromatic systems as active sites. It showed much more bioactive than chrysophanol against plant powdery mildew (*Sphaerotheca fuliginea* (Schlecht.) Poll.) and a wheat powery mildew (*Blumeriagraminis* (DC) *speer* f. sp. tritic. Marchal.) (Yang, X., *et al*, 2007).

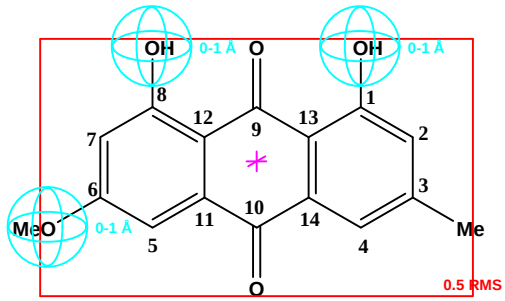


Fig. 3.49; Exclude sphere, plane and centroid of physcion

The synergistic interaction is very interesting to study; there was a significant activity of physcion and chrysophanol which acted together on decreasing of *Sphaerotheca fuliginea* (Schlecht.) Poll. when the ratios of physcion:chrysophanol ranged from 1:9 to 5:5. The synergistic degree increased with increasing chrysophanol proportion in the combination (Yang, X., *et al*, 2007).

3.3.2 Antimicrobial activity

In this study, only compounds isolated with sufficient amount were investigated for their biological activities, although other compounds detected in might exhibit the potential as novel medicines. However, structure modifications by using total, partial and biosynthesis strategies including computering drug designs have been used to increase the numerous potential of active compounds offering a high degree of new medicinal agents.

3.3.2.1 *Staphylococcus aureus* and antibiotics

Staphylococcus aureus is an important bacteria causing invasive infections, often resulting in fatality in patients without antibiotic treatment. Penicillins were later introduced to improve the prognosis of patients infected with *S. aureus*. However, resistance to the penicillins has appeared due to β-lactamase produced by *S. aureus* to hydrolyse penicillin. Methicillin was developed to treat Staphylococcal infections caused by β-lactamase-producing strains. In recent years, resistance of *S. aureus* has emerged, called Methicillin-Resistant *Staphylococcus aureus* (MRSA), but now resistant to all β-lactam antibiotics.

There are three key mechanisms of resistance; namely,

a) Hyperproduction of β-lactamases
b) Modification of normal-binding proteins (PBPs)
c) The presence of an acquired penicillin-binding protein (PBP2a).

S. aureus strains have four normal PBPs participating in the crosslinkage of peptidoglycan in the bacteria cell wall with high affitinity for β-lactam agents which cause bacterial death. On the other hand, PBP2a which is encoded by the *mec*A gene carried on a mobile genetic element known as SCCmec (type I-V), is a unique protein produced only by methicillin-resistant staphylococci with low affitinity for β-lactam antibiotics. This mutation development substituted the biosynthesis of PBPs in the presence of β-lactams and preventing cellysis. The resistance has emerged to all available β-lactams including penicillins, cephalosporins, β-lactam/β-lactamase inhibitor combinations, monobactams and carbapenems.

MRSA has become a major pathogen predominantly a nosocomial pathogen and has had widespread in hospitals worldwide (Hospital-Associated MRSA, HA-MRSA) causing bacterimia, pneumonia and surgical site infections. These infections represent a significant problem in healthcare systems causing increasing costs of hospitalization and high mortality and fatality in patients. The number of HA-MRSA infections was 59.5 % in USA and has increased from 22% (1995) to 57% (2001) in a nationwide surveillance study. The infection rate has recently increased throughout the world (Ji, Y., 2007).

MRSA has also appeared in the community (Community-Acquired MRSA, CA-MRSA) causing infections in patients, mostly skin abscesses and funculosis, severe necrotizing pneumonia and has often resulted in death. CA-MRSA strains resistant to β-lactams, susceptible to other antimicrobial classes and carries the staphylococcal

cassette chromosome *mec* (SCC*mec*) type IV. It also possesses unique combination of virolence factors and seems to be different from that in HA-MRSA. Virulence factors that *S. aureus* has produced, such as centerotoxins and toxic shock syndrome toxin-1 (TSST-1) cause toxic shock, severe soft-tissue infections and mortality in many cases. TSST-1 can be produced by HA-MRSA and methicillin-susceptible *S. aureus* (MSSA) strains. A family of synergohymenotropic toxins, PVL, is an important virulence factor in *S. aureus* causing tissue necrosis, necrotizing pneumonia and necrotizing cutaneous infections. Vancomycin has been the drug of choice used against all Staphylococci and for treatment of infections caused by MRSA. However, three vancomycin-resistant *S. aureus* (VRSA) strains have been documented in the USA (Michigan, New York and Pennsylvania) recently. VRSA has a mechanism for trapping and clogging the outer layer of peptidoglycan by binding vancomycin molecules. VRSA carries the VanA genes including Michigan isolates (MI-VRSA) and Pennsylvania isolates (PA-VRSA) which *mec*A was used for this resistance. All VRSA found are also resistant to oxacillin to dates using the essential protein PBP2a. β-lactam antibiotics with high binding affitinity to PBP2a are under development, ceftobiprole, one of these agents, is in clinical development (Ji, Y., 2007). There is, however, a need to investigate, discover and develop new antibiotic agents against all types of MRSA both β-lactams, non β-lactam and in particular phytochemical from natural products are extremely vital.

3.3.2.2 Antimicrobial activity of *Piper betel*

Piper betel has long been traditionally used to preserve teeth (Watt *et al*, 1962), as a mouth freshener and with wound healing properties from the leaf (Bhattacharya *et*

al., 2007). Previous biological investigations of the leaves showed antibacterial activity against Gram-positive and Gram-negative bacteria (Duke, 1929), antifungal activity (Evan, *et al.,* 1984; Krishna, *et al* 2005; Mohamed *et al*, 1996; Svaspan *et al*., 2003). Leaf extracts have shown potent antibacterial activity including against *S. aureus* and other *Staphylococcus spp*., (Svaspan *et al*., 2003; Chakraborty, D. and Shah, B., 2011), The leaf extract showed inhibition of HIV-1 intergrase (Tewtrakul *et al.,* 2006), antimicrobial activity against ten Gram-positive, twelve Gram-negative bacteria and *Candida tropicalis* (Nair *et al*., 2008; Chakraborty, D. and Shah, B., 2011). Other interested biological activities of *Piper betel* have previous discussed in the introduction chapter in this thesis.

3.3.2.3 Active compounds isolated from *Piper betel*

In previous investigations, 4-allyl pyrocatechol or hydroxchavicol were isolated from *Piper betel* against the fungus *Trichophyton mentagrophytes*, with Inhibition index value of 6.03, and also against bacteria *Staphylococcus aureus* and *Streptococcus sp.* with Inhibition index values of 4.00 and 3.33, respectively. Both *Piper betel* leaf extract and the essential oil from the leaves have already showed activity against several Gram-positive and Gram-negative species, including *Staphylococcus aureus* and *Streptococcus sp.* (Savaspun, K., *et al*, 2003). The leaf extract had antifungal activity against *P. personata, P. arachidis, in vitro,* (Krishma *et al*, 2005) and *Trichophyton mentagrophytes* (Savaspun, K., *et al*, 2003). Eugenol is an active principle of *Rhizoma acrori graminei* used to treat Alzheimer's disease in Asia and showed antidepressant activity inhibiting monoamine oxidase A (Tao, G., *et al*, 2005), anti-inflammatory, inhibiting prostaglandin synthesis and reducing the tone of isolated gut muscle and myometrium of rat *in vivo* (Bennett, A., *et al*, 1988) and antioxidant activity (Ogata, M., 2004). Eugenol, isolated from cloves, inhibited the growth of *Aspergillus flavus, A. parasiticus, A. niger, A. ochraceus* with the

inhibition zones ranging from 30 to 55 mm. at concentrations of 4.8 mg/disc (Reddy, C.S. *et al*., 2008).

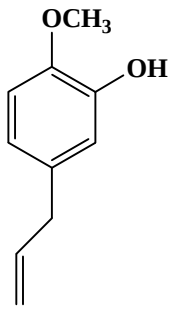
3.3.2.4 Anti-MRSA activity

In this thesis, 4-allyl pyrocatechol and eugenol not only exhibited anti-MRSA activity, but also they were active against epidemic FMRSA-15 and EMRSA-16 bacteria strains with MIC valves in the ranges of 64 and 128 μg/L compared to antibiotics such as cefotaxime (CEF) (16, 32, 64, 128, >128 μg/L), cefuroxime (CTX) (64, 128, >128μg/L), ciprofloxacin (CIP) (16, 32, 64, 128, >128 μg/L), oxacillin (OXN) (8, 32, 64, 128, >128 μg/L) and ampicillin (AMP) (4, 16, 32, 64, 128 μg/L) shown in the results chapter in this thesis. In general, the anti-MRSA effects of the two active compounds were similar mostly to CTX and slightly different to CEF, CIP, oxacillin (OXN) in the LF105 strain (MIC 32 μg/L); CTX and OXN in the strain LF82 (MICs 8, and 32 μg/L); CIP and OXN (MICs 16, and 32 μg/L) and OXN with only LF120 strain (MIC 32 μg/L).

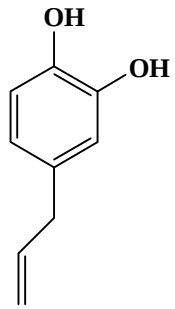
From the data (Table 3.17) both eugenol and 4-allyl pyrocatechol exhibited not only similar anti-MRSA activity to antibiotics OXN (LF78, 98,102, 100), AMP (LF81, 89, 91), CIP (LF109, 100, 87), CEF (LF102, 100) but also better activity on occasion than CTX (LF81, 78, 89, 91, 120), CEF (LF81, 78, 89, 91, 120), OXN (LF81, 91, 89), CIP (LF81, 89, 91, 120). The activity of eugenol was better than CIP, CEF, CTX, OXN (LF80, 85), AMP (LF80). While the anti-MRSA of eugenol and 4-allyl pyrocatechol were similar in most strains, in a few cases eugenol was better than 4-allyl pyrocatechol (in LF80 and 85 strains); on the other hand, only in LF97 strains, 4-allyl pyrocatechol performed better.

The structures of 4-allyl pyrocatechol and eugenol, compared to some antibiotics as shown in the Figure below, are simple, small compounds and interestingly far different from β-lactams antibiotics, for example, amplicillin, methicillin, oxacillin and cefotaxime and non-β-lactams antibiotics such as fluoroquinoline (ciprofloxacin), aminoglycoside (gentamicin), polyketide (tettracyclin), and glycopeptide (vancomycin). In addition, both 4-allyl pyrocatechol and eugenol were

uniquely discovered from natural product resources. These could give them advantages over not only some of antibiotics discussed but also antibiotics currently in clinical use at present because it might take a longer time for bacteria to mutate and become resistant, especially in mixtures/extracts. Moreover, these two active compounds could have fewer limitations, side effects as they were produced from living plants. This will be useful for their development/production, by both partial and total synthesis as new medicines and their structure-activity relationship (SAR) studies including their mode of actions.

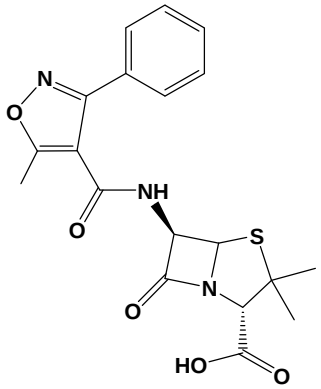


eugenol

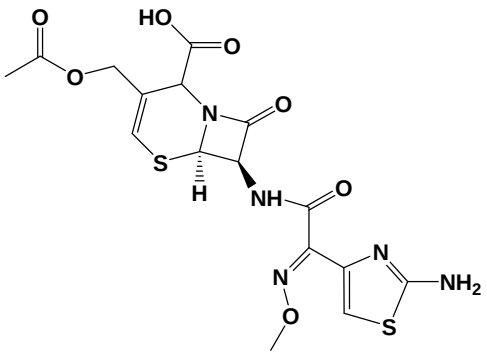


4-allyl pyrocatechol

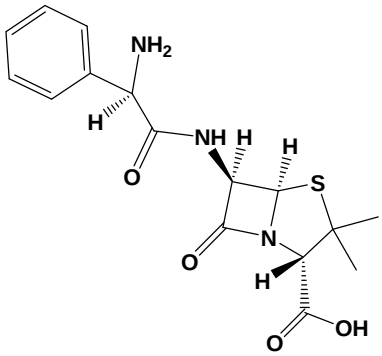
Fig. 3.50; Structure of active compounds against MRSA



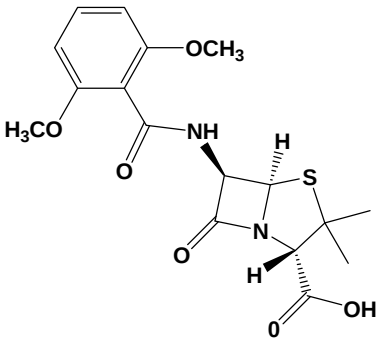
Oxacillin



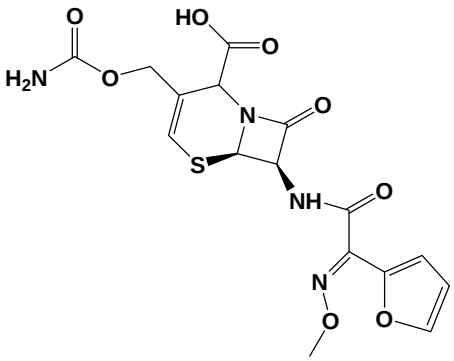
Cefotaxime



Amplicillin

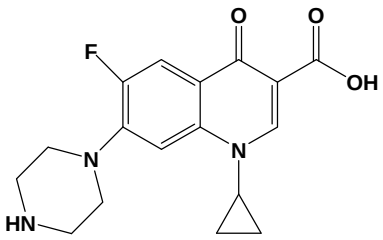


Methicillin

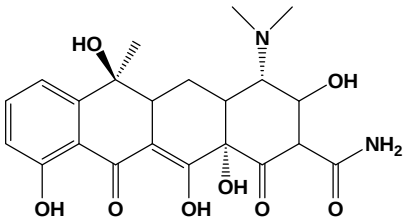


Cefuroxime

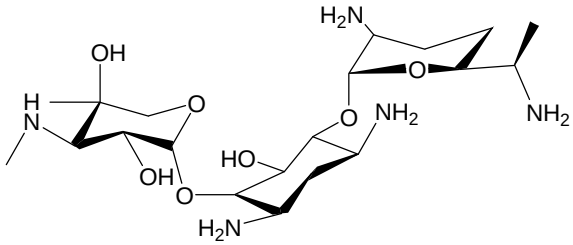
Fig. 3.51; Structure of antibiotics



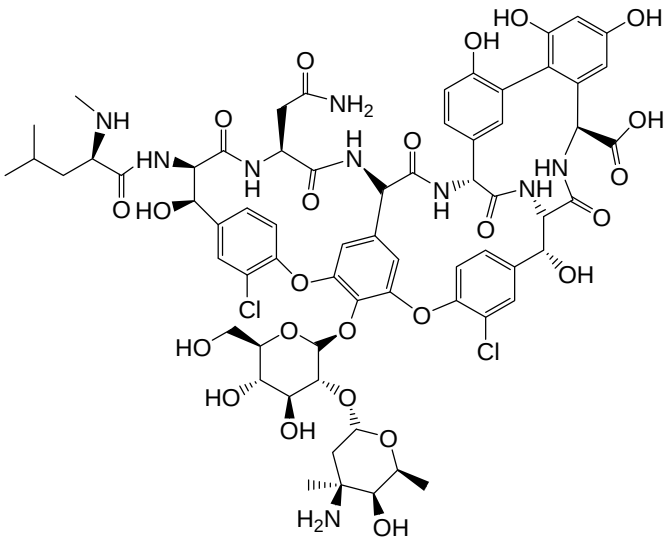
Ciprofloxacin



Tettracycline



Gentamicin



Vancomycin

Fig. 3.52; Structure of antibiotics

Furthermore, in drug modeling and discovery, pharmacophoric hypotheses are important tools providing excellent information of ligand - macromolecule recognition. Qualitative structure-activity relationships (QSAR) and pharmacophore analysis are basic concepts to predict the bioactivities of compounds. The 3D-QSAR models is somewhat limited by steric clashes or shielding and bioactivity-enhancing or reducing auxiliary groups, exclude spheres are added to the structure to resemble the steric constraints of the binding, providing pharmacophoric features of the binding model and identity of active sites (Al-Nadaf, A.H. and Taha, M.O., 2011; Al-masri, I.M., et al., 2008). Plane, centroid and other factors also provide important information used for calculations in drug modelling. 4-allyl pyrocatechol and eugenol could be interesting modeling compounds to be used in drug design to reduce cost of synthesis, varying bioavailability and lack of appropriate animal models (Al-Nadaf, A.H. and Taha, M.O., 2011) and for those against animals being used in pharmaceutical investigations for drug development.

Finally, there may be benefits to export-import industries and trading of medicinal plants worldwide because 4-allyl pyrocatechol and eugenol provide two key bioactive ingredient markers of a good product. The customers will have more confidence to consume standardised herbal and medicinal plants products. The better trading will promote more activity in the agricultural sectors and raise the economy of the country, Thailand. 4-allyl pyrocatechol and eugenol will be essential as the standardization agents in pre-harvest, post-harvest and for both quality and quantity control of medicinal plant products.

This biological investigation also substantiates some of the traditional uses as antimicrobial agents and showed the strong support for the traditional uses of *Piper betel* leaves as an antimicrobial agent due to the presence of the compounds, 4-allyl pyrocatechol and eugenol. *Piper betel* has stimulated a multitude of interest in biological investigations from pharmacists and chemists and researchers worldwide.

3.4 Future work

In Thailand from the past to present people use medicinal plants for their basic healthcare and this has declined in recent years because of imported pharmaceutical products. Medicinal plants are still used nowadays for illness treatments in various parts of Thailand. In some cases, the medicinal plants are used to treat the particular ailment after no improvement while using modern medicines in hospital.

Medicinal plants are very important, and this research will definitely be beneficial to Thailand, South East Asian countries and the world by the discovery of new medicinal agents. To begin with in Thailand, many researchers from various universities and research institutes study medicinal plants and their biological activities. Thailand Institute of Scientific and Technological Research (TISTR) by Pharmaceatical and Natural Products Department (PNPD) have worked extensively and uniquely developed medicinal plants to the pharmaceutical products for more than forty years. The results of this research as mentioned previously will be investigated, evaluated and developed to modern medicines in various pharmaceutical medicinal products, especially the isolated compounds from *Piper betel* leaves, particularly 4-allyl pyrocatechol. This compound possesses important structural features (e.g. dihydroxy groups) that can be synthetically converted to various target compounds as shown in Fig. 3.50 and some mentioned in the discussion. This future work will be corporated to universities in Thailand and possibly in the UK, depending upon the funding. The synthesized products can be evaluated and developed to medicinal products in the future. 4-allyl pyrocatechol and eugenol had already anti-MRSA activity and this opportunity can also be developed. Both compounds will be investigated further, for anti-fungal activity, to confirm their activities as they were found in this study to be active against *Candida albicans*. Replicates of the initial tests will have to be done to confirm the activity. The biological activity potential of 4-allyl pyrocatechol isolated from *Piper betel* leaves will be campared to that from the hydrolysis process from eugenol (Fig. 3.49). The result will be evaluated for anti-fungal dermatology treatment creams as single and a combination of two compounds and can be developed by PNPD. The developed pharmacological products will hopefully be introduced to use in Thailand,

transferring this knowledge to large-scale production by industry. This proposal can improve the well-being of Thai people including whom in particular the agricultural section may realize an increase of the economy of Thailand as an alternative to importing medicinal products from overseas.

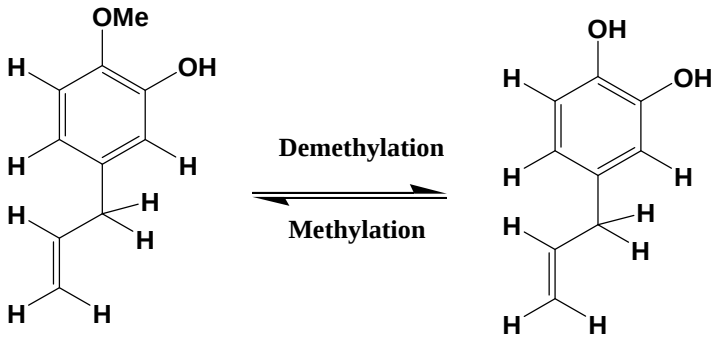
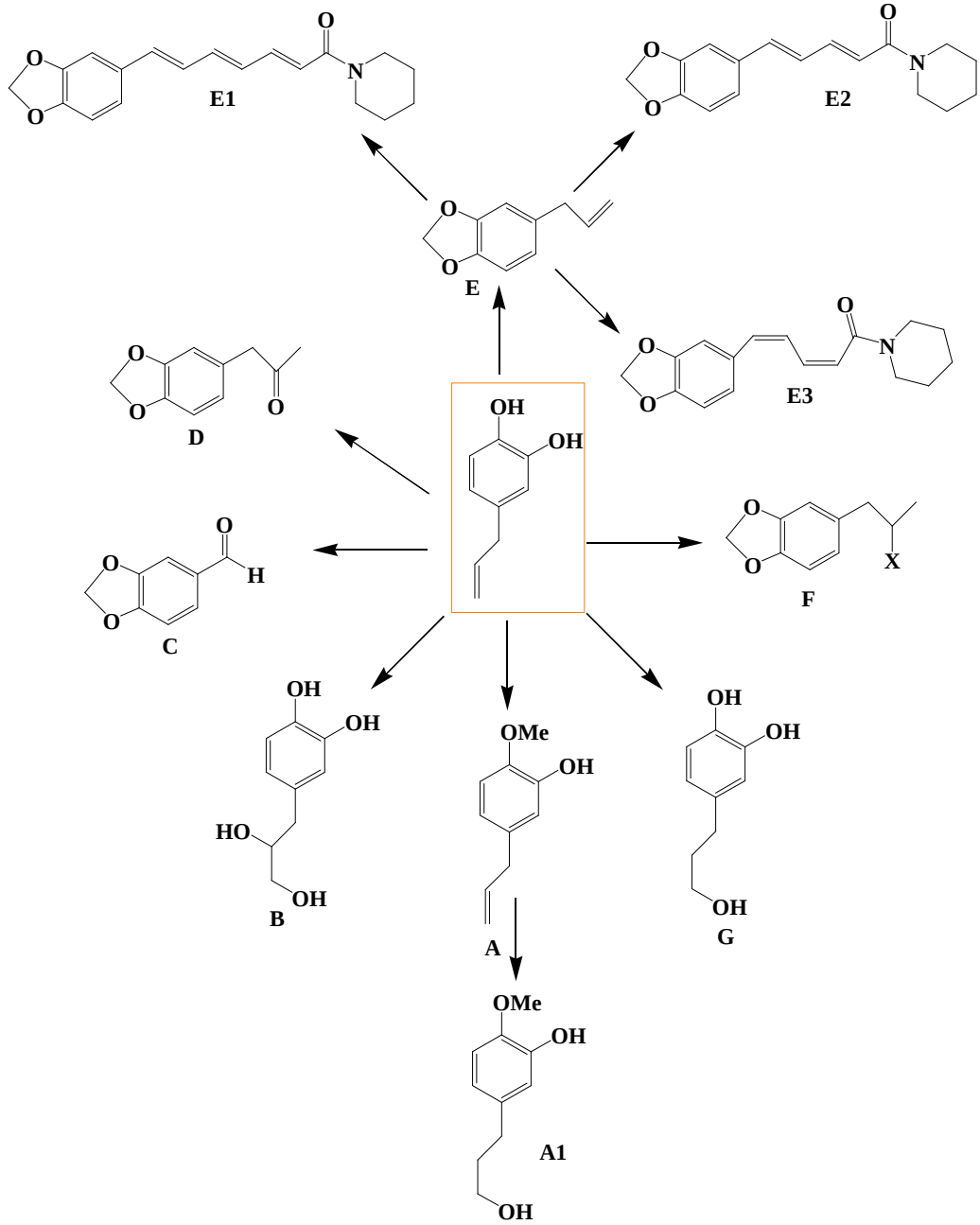


Fig. 3.53; Demethylation and methylation of eugenol and 4-ally pyrocatechol



A=eugenol, C=piperonal, D=piperonylacetone, E=safrole, F=halosafrole, E1=piperettine, E2=piperine, E3=chavacine

Fig.3.54; Partial synthesis of 4-allyl pyrocatechol

Appendix I

Physicochemical properties of isolated compounds

Physcion (KC-1)

^{1}H NMR in $CDCl_3$ solvent: 12.12 (1-OH), 7.08(H-2), 2.45(3-Me), 7.63(H-4), 7.36(H-5), 3.93(6-OMe), 6.69(H-7), 12.32(8-OH).

Chrysophanol(KC-2)

^{1}H NMR in $CDCl_3$ solvent: 12.02(1-OH), 12.13(8-OH), 7.10(H-2), 2.46(3-Me), 8.66(H-4), 7.81(H-5), 7.8(H-6), 7.28(H-7).

β-sitosterol (KC-3a) and stigmasterol (KC-3b)

^{1}H NMR in $CDCl_3$ solvent: 3.5(H-3), 5.3(H-6), 4.97(H-22), 5.1(H-23), 0.91-2.31(-CH_2-), 0.66(CH_3), 0.68(CH_3), 0.77(CH_3), 0.81(CH_3), 0.82(CH_3), 1.0 (CH_3).

Eugenol (KC-4)

IR spectrum : ν_{max} (KBr disc) cm^{-1}; 3386(O-H), 1652(C=C), 1436(C=C), 1406 (C=C) and 1012(C-O) , 905 (C-O). MS: 164 (100), 163(54), 133(100)[M^+], 132(38), 105(17), 75(10), 41(6) and 29(12), m/z 164.12 $C_{10}H_{12}O_2$.

^{1}H NMR in $CDCl_3$ solvent: 3.87(OH), 6.83(H-2), 6.81(H-5), 6.70(H-6), 3.32(H-1'), 5.98(H-2'), 5.07(H-3'), 5.10(H-3')

^{13}C NMR in $CDCl_3$ solvent: 133.55(C-1), 110.86(C-2), 145.12(C-3), 145.67(C-4), 115.08(C-5), 119.98(C-6), 39.74(C-1'), 137.82(C-2'), 115.64(C-3').

4-allyl pyrocatechol (KC-5)

IR spectrum : ν_{max} (KBr disc) cm^{-1}; 3241.8(O-H), 1604.0, 1528.3 (C=C), 1443.0(C=C), 1345.6(C=C), 1253.5(C=C), 1185.5(C=C) 1109.4 (C-O), cm^{-1} , 812.8 (C-O) and 787.8(C-O). MS m/z 150.1 (Cadogan, J., I., G., *et al*, 1996), $C_9H_{10}O_2$.

^{1}H NMR in $CDCl_3$ solvent: 6.76(H-2), 6.68(H-5), 6.57(H-6), 3.20(H-1'), 5.85(H-2'), 5.02(H-3'), 5.05(H-3').

^{13}C NMR in $CDCl_3$ solvent: 133.39(C-1), 115.46(C-2), 141.74(C-3), 143.35(C-4), 115.71(C-5), 121.16(C-6), 39.59(C-1'), 137.69(C-2'), 115.82(C-3').

Trans-calamenene (KC-6a)

MS; m/z 202[M^+](9), 202, 159, 144, 129, 115, 105, 91; HR-EIMS found m/z 202.1696[M^+] $C_{15}H_{22}$ (202.1722).(Nakashima, K., *et al*, 2002).

^{1}H NMR in $CDCl_3$ solvent: 6.94(H-2), 7.11(H-3), 7.01(H-5), 2.67(H-7), 1.59(H-8), 1.82(H-8), 1.93(H-9), 1.33(H-9), 2.71(H-10), 0.70(H-12), 0.99(H-13), 1.25(H-140, 0.99(H-15).

^{13}C NMR in $CDCl_3$ solvent: 140.1(C-1), 126.8(C-2), 126.2(C-3), 134.5(C-4), 128.8(C-5), 140.0(C-6), 43.8(C-7), 21.1(C-8), 30.9(C-9), 32.5(C-10), 32.0(C-11), 17.4(C-12), 21.5(C-13), 22.4(C-14), 21.3(C-15).

β-elemene (KC-6b)

^{1}H NMR in $CDCl_3$ solvent: 5.82(H-1), 4.9(H-2), 5.72(H-2), 4.58(H-3), 4.81(H-3), 2.00(H-5), 1.59(H-6), 1.93(H-7), 1.56(H-8), 1.43(H-9), 4.84(H-12), 1.74(H-13), 1.70(H-14).

^{13}C NMR in $CDCl_3$ solvent: 150.4(C-1), 109.9(C-2), 112.1(C-3), 147.8(C-4), 52.7(C-5), 32.9(C-6), 45.7(C-70, 26.9(C-8), 45.7(C-7), 26.9(C-8), 39.9(C-9), 39.9(C-10), 150.5(C-11), 108.3(C-12), 21.1(C-13), 24.8(C-14), 16.7(C-15)., 0.98(H-15).

Gamma-muurolene (KC-7)

MS m/z 204, 41, 55, 67, 81, 91, 105, 119, 133, 147, 161 and 204, $C_{15}H_{24}$ (Leitậo, S., G., et al, 2008).

^{1}H NMR in $CDCl_3$ solvent: 2.00(H-1), 2.09(H-2), 5.56(H-4), 2.55(H-5), 1.95(H-5), 1.42(H-6), 0.99(H-7), 2.70(H-8), 2.38(H-10), 1.67(H-11), 0.92(H-12), 0.79(H-13), 4.60(H-14), 4.66(H-14), 1.69(H-15).

^{13}C NMR in $CDCl_3$ solvent: 25.4(C-1), 31.68(C-2), 133.68(C-3), 124.62(C-4), 39.78(C-5), 44.8(C-6), 25.9(C-7), 30.91(C-8), 154.34(C-9), 43.58(C-10), 23.9(C-11), 21.7(C12), 15.5(C-13), 106.61(C-14), 26.7(C-15).

Taraxerol (KC-8a)

^{1}H NMR in $CDCl_3$ solvent: 3.19 (H-3), 5.52(H-15), 0.80-1.09 (Me).

Cis-careaborin (KC-8b)

IR spectrum : ν_{max} (KBr disc) cm^{-1}; 3360(OH), 2935, 2855 (C-H), 1604 (C=C), 1513 (C=C), 1454 (C=C), 1375 (C-H), 1167 (C-O), 1704(carbonyl).

LRESIMS, *m/z* at 571 $[M-H]^-$, *m/z* 572 $C_{39}H_{56}O_3$ (Jutiviboonsuk, A., 2006).

^{1}H NMR in $CDCl_3$ solvent: 3.19(H-3), 5.53(H-15), 1.91(H-16), 0.90(Me-23), 0.94(Me-24), 0.97(Me-25), 1.09(Me-26), 0.80(Me-27), 0.89(Me-28), 0.94(Me-29), 0.89(Me-30), 5.80(H-2'), 6.80 (H-3'), 6.77(H-5',9'), 7.60(H-6',8').

^{13}C NMR in $CDCl_3$ solvent: 26.7(C-1), 23.8(C-2), 81.1(C-3), 36.7(C-4), 55.7(C-5), 18.8(C-6), 33.8(C-7), 39.8(C-8), 48.8(C-9), 37.6(C-10), 17.6(C-11), 35.1(C-12), 38.0(C-13), 158.1(C-14), 116.9(C-15), 37.5(C-16), 35.8(C-18), 41.1(C-19), 28.8(C-20), 33.1(C-21), 33.1(C-22), 28.1(C-23), 16.8 (C-24), 15.6(C-25), 26.0(C-26), 29.8(C-27), 29.9(C-28), 33.4(C-29), 21.4(C-30). 167.5(C-1'), 116.3(C-2'), 144.1(C-3'), 127.4(C-4'), 130.0(C-5',9'), 115.9(C-6',8'), 157.6(C-7').

3β-E-p-coumaroyl taraxerol (KC-9)

IR spectrum ν_{max} (CH_3Cl) cm^{-1}3360(OH), 2939 (C-H), 2863(C-H), 1604 (C=C), 1513(C=C), 1452(C=C), 1373 (C-H), 1169 (C-O), 1681(carbonyl). LRESIMS m/z 571 $[M-H]^-$ 572 $C_{39}H_{56}O_3$ (Jutiviboonsuk, A., 2006).

^{13}C NMR in $CDCl_3$ solvent: 3.19(H-3), 5.51(H-15), 1.61(H-16, (H-16), 2.01(H-19), 0.97(Me-23), 0.89(Me-24), 0.79(Me-25), 1.08(Me-26), 0.89(Me-27), 0.81(Me-28), 0.94(Me-29), 0.89(Me-30), 5.80(H-2'), 6.80(H-3'), 7.60(5',9'), 6.77(H-6',8'). ^{13}C NMR in $CDCl_3$ solvent: 36.7(C-1), 21.4(C-2), 79.2 (C-3), 35.8 (C-4), 49.3(C-5), 18.8(C-6), 33.7(C-7), 39.0(C-8), 48.8(C-9), 37.7(C-10), 17.5(C-11), 35.1(C-12), 38.0(C-13), 157.9(C-14), 116.9(C-15), 37.7(C-16), 35.1(C-17), 48.8(C-18), 41.3(C-19), 28.8(C-20), 30.0(C-21), 33.1(C-22), 28.0(C-23), 21.4(C-24), 15.5(C-15), 25.9(C-26), 29.7(C-27), 28.8(C-28), 33.4(C-29), 21.1(C-30), 171.7(C-1'), 117.7(C-2'), 143.0(C-3'), 127.5(C-4'), 132.4(C-5',9'), 115.0(C-6',8'), 158.1(C-7').

3β-laulyl-β-amyrin (KC-10)

ESI MS$[M+H]^+$ at *m/z* 611.3192(609) $C_{42}H_{72}O_2$

IR spectrum ν_{max} (CH_3Cl) cm^{-1} : 2914 (C-H), 2850(C-H), 1728 (C=C), 1472(C=O), (1468)(C=O), 1707(C=O), 1378 (C-H), 1173 (C-O).

^1H NMR in $CDCl_3$ solvent: 1.59(H-2), 4.49(H-3), 5.15(H-12), 0.85(H-23), 0.86(H-24), 0.95(H-25), 0.81(H-26), 1.09(H-27), 0.86(H-28), 0.94(H-29), 0.86(H-30), 2.27(-CH_2-CO), 1.24(-CH_2-), 0.88(-CH3).).

^{13}C NMR in $CDCl_3$ solvent: 38.3(C-1), 27.0(C-2), 80.6(C-3), 37.8(C-4), 55.3(C-5), 18.4(C-6), 32.7(C-7), 39.9(C-8), 47.6(C9), 36.9(C-10), 23.6(C-11), 121.7(C-12), 145.3(C-13), 41.8(C-14), 26.1(C-15), 26.2(C-16), 32.6(C-17), 47.3(C-18), 46.9(C-19), 31.2(C-20), 32.0(C-21), 37.2(C-22), 28.1(C-23), 15.6(C-24), 16.9(C-25), 22.8(C-26), 16.9(C-27), 28.5(C-28), 33.4(C-29), 23.8(C-30), 173.7(C=O), 34.93(-CH_2-C=O), 29.82-29.2(-CH_2-), 14.23(CH_3).

Taraxerone (KC-11)

FTMS+p-ESIMS *m/z* at 426.7484 $[M+H]^+$ *m/z* 425.7484 , $C_{30}H_{48}O$.

IR spectrum ν_{max} (KBr) cm^{-1} : 2958(C-H), 2937(C-H), 1708 (C=O), 1449(C-H), 1463(C-H), (1373 (C-H).

^1H NMR in $CDCl_3$ solvent: 2.04(H-1), 2.30(H-2), 1.07(H-5), 5.56(H-15), 1.61(H-16), 1.64(H-16), 1.07(Me-23), 1.04(Me-24), 1.08(Me-25), 0.88(Me-26), 1.12(Me-27), 0.81(Me-28), 0.93(Me-29), 0.89(Me-30).

^{13}C NMR in $CDCl_3$ solvent: 38.4(C-1), 34.2(C-2), 217.6(C-3), 47.6(C-4), 55.8(C-5), 20.0(C-6), 35.1(C-7), 38.9(C-8), 48.8(C-9), 37.7(C-10), 17.5(C-11), 35.8(C-12), 37.6(C-13), 157.7(C-14), 117.3(C-15), 36.7(C-16), 37.8(C-17), 48.7(C-18), 40.6(C-19), 28.8(C-20), 33.6(21), 33.4(C-22), 26.1(C-23), 21.1(C-24), 14.9(C-25), 30.0(C-26), 25.6(C-27), 29.9(C-28), 33.1(C-29), 21.4(C-30).

Betulinic acid (KC-12)

ESI MS $[M+H]^+$ at *m/z* 456 $C_{30}H_{48}O_3$. FTMS-p-ESIMS m/z 455.3525 $[M-H]^-$ 456 $C_{30}H_{48}O_3$.

IR spectrum ν_{max} (KBr) cm^{-1} : 3461(OH), 2945(C-H), 1688(C=O), 1708(C=C), 1189(C-O) (Igoli, O., J., *et al*, 2008).

^{1}H NMR in $CDCl_3$ solvent: 2.99(H-3), 2.25(H-13), 2.18(h-16), 3.17(H-19), 1.95(H-21), 0.96(Me-23), 0.95(Me-24), 0.74(Me-25), 0.81(Me-26), 0.92(Me-27), 4.59(Me-29), 4.72(Me-29), 1.68(Me-30).

^{13}C NMR in $CDCl_3$ solvent: 39.4(C-1), 28.1(C-2), 77.9(C-3), 37.4(C-4), 55.8(C-5), 18.6(C-6), 34.7(C-7), 40.9(C-8), 50.8(C-9), 39.1(C-10), 21.0(C-11), 25.9(C-12), 38.5(C-13), 42.7(C-14), 30.1(C-15), 32.7(C-16), 56.5(C-17), 49.6(C-18), 47.6(C-19), 149.9(C-20), 31.0(C-21), 37.4(C-22), 28.3(C-23), 16.2(C-24), 16.3(C-25), 16.3(C-26), 17.4(C-27), 178.6(C-28), 109.8(C-29), 19.3(C-30).

3, 4-seco taraxerone (KC-13)

MS m/z 444 (Setzer, W., S., *et al*, 2000).

^{1}H NMR in $CDCl_3$ solvent: 1.90(H-1), 2.11(H-2), 5.54(H-15), 1.65(H-16), 0.89(Me-23), 0.82(Me-24), 0.89(Me-25), 1.09(Me-26), 0.91(Me-27), 0.81(Me-28), 0.94(Me-29), 0.91(Me-30).

^{13}C NMR in $CDCl_3$ solvent: 30.0(C-1), 29.8(C-2), 180.2(C-3), 32.2(C-4), 40.1(C-5), 18.9(C-6), 28.3(C-7), 40.8(C-8), 48.8(C-9), 37.7(C-10), 17.6(C-11), 35.8(C-12), 38.9(C-13), 158.0(C-14), 117.1(C-15), 36.7(C-16), 35.2(C-17), 47.8(C-18), 40.6(C-19), 28.9(C-20), 33.2(C-21), 34.5(C-22), 21.5(C-23), 19.2(C-24), 25.3(C-25), 24.9(C-26), 25.2(C-27), 29.9(C-28), 33.6(C-29), 18.9(C-30).

Stenocarpoquinone B (KC-14)

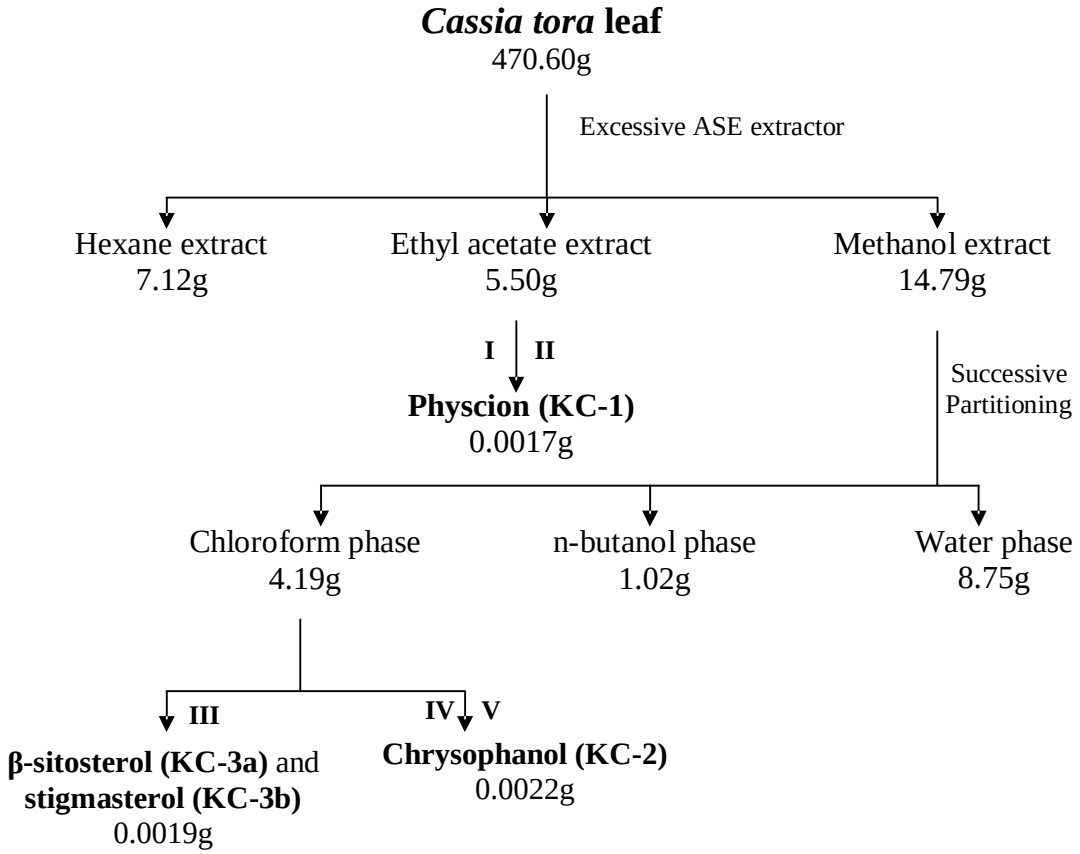
FTMS+c-ESIMS m/z 256, molecular formula of $C_{15}H_{14}O_4$.

^{1}H NMR in $CDCl_3$ solvent: 8.06(H-5), 7.68(H-6), 7.75(H-7), 8.09(H-8), 3.18(H-1'), 4.85(H-2'), 1.41(Me-4'), 1.27(Me-5').

^{13}C NMR in $CDCl_3$ solvent: 177.8(C-1), 160.1(C-2), 125.1(C-3), 182.2(C-4), 132.9(C-4a), 126.1(C-5), 134.2(C-6), 133.1(C-7), 126.3(C-8), 131.6(C-8a), 28.4(C-1'), 92.1(C-2'), 71.7(C-3'), 25.8(C-4'), 24.0(C-5').

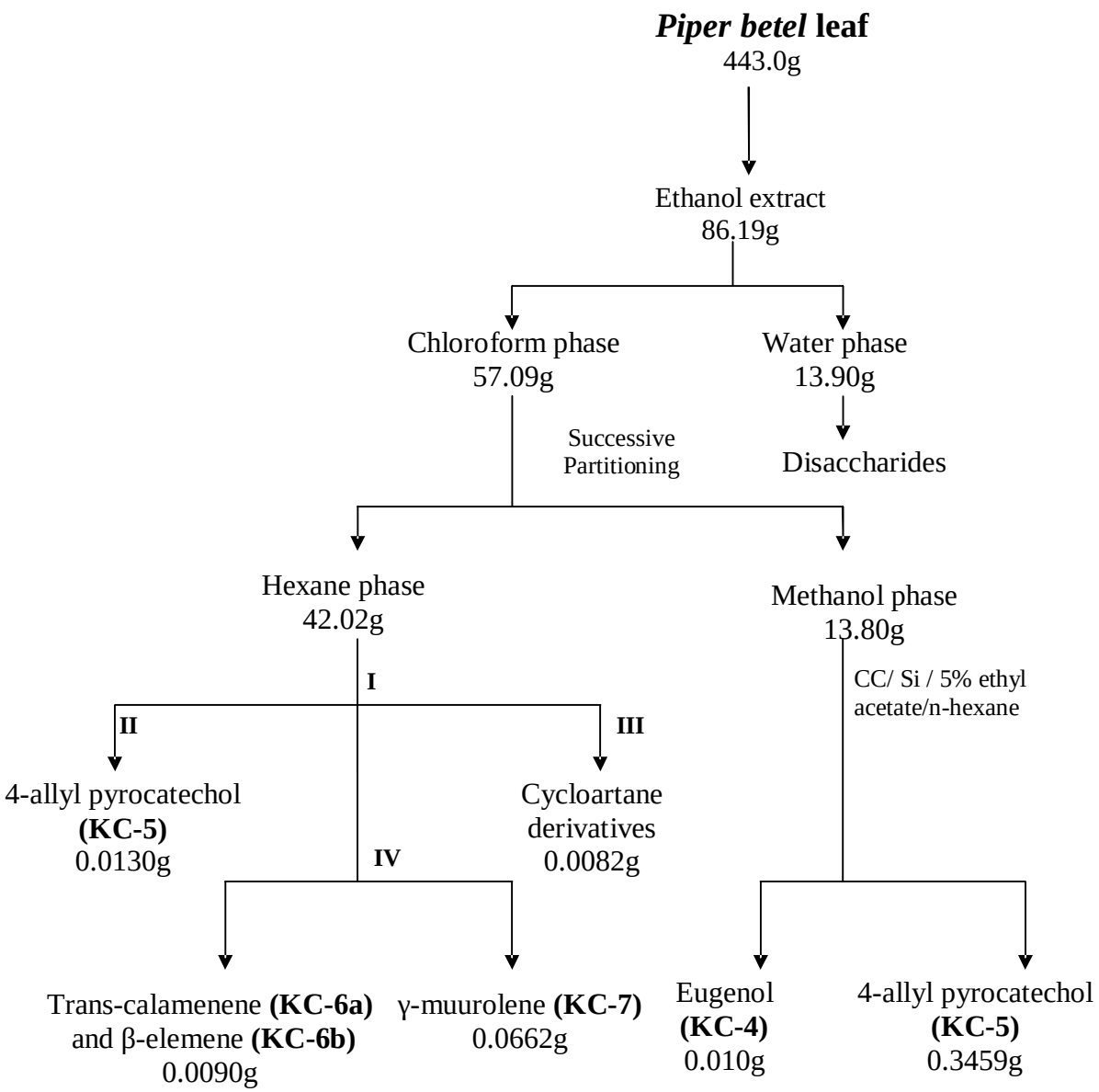
Appendix II

Isolation schemes



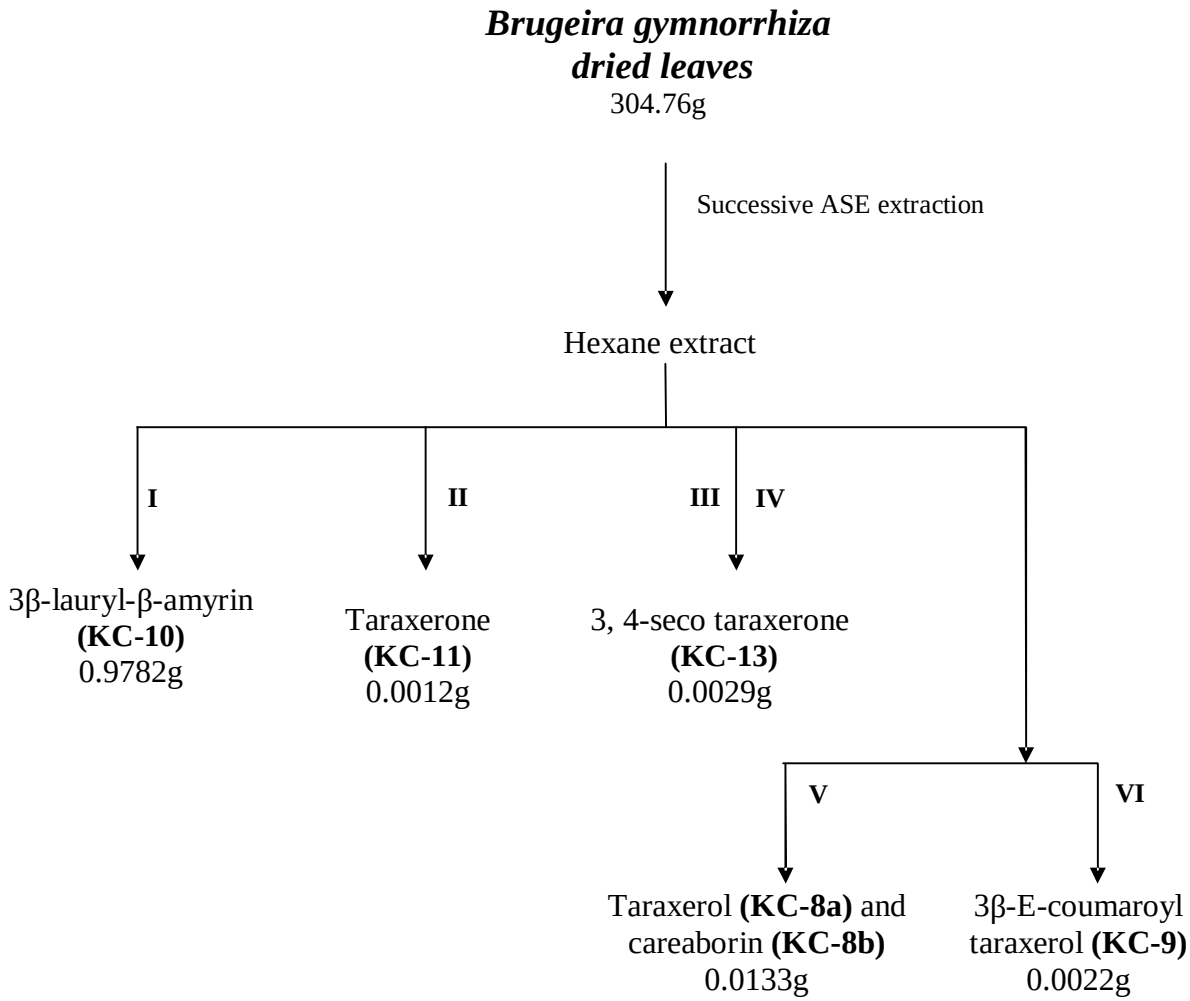
I= VLC/ 50, 60, 70, 80, 90% chloroform/heaxane, 100%chloroform, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90% ethyl acetate/chloroform, 100%ethyl acetate, **II**=CC/ Si /10%ethyl acetate/chloroform, **III**= VLC/50%ethyl acetate/chloroform, **IV**=VLC/ 10, 20, 30, 40, 50, 60, 70, 80, 90% chloroform/hexane, 100% chloroform, 10, 20, 30, 40, 50, 60, 70, 80, 90% chloroform/ethy acetate, 100% ethyl acetate, 5 and 10% ethyl acetate/methanol, **V**=CC/ Si / 20%ethyl acetate/hexane

Scheme 1; Isolation of compounds from *Cassia tora* leaf



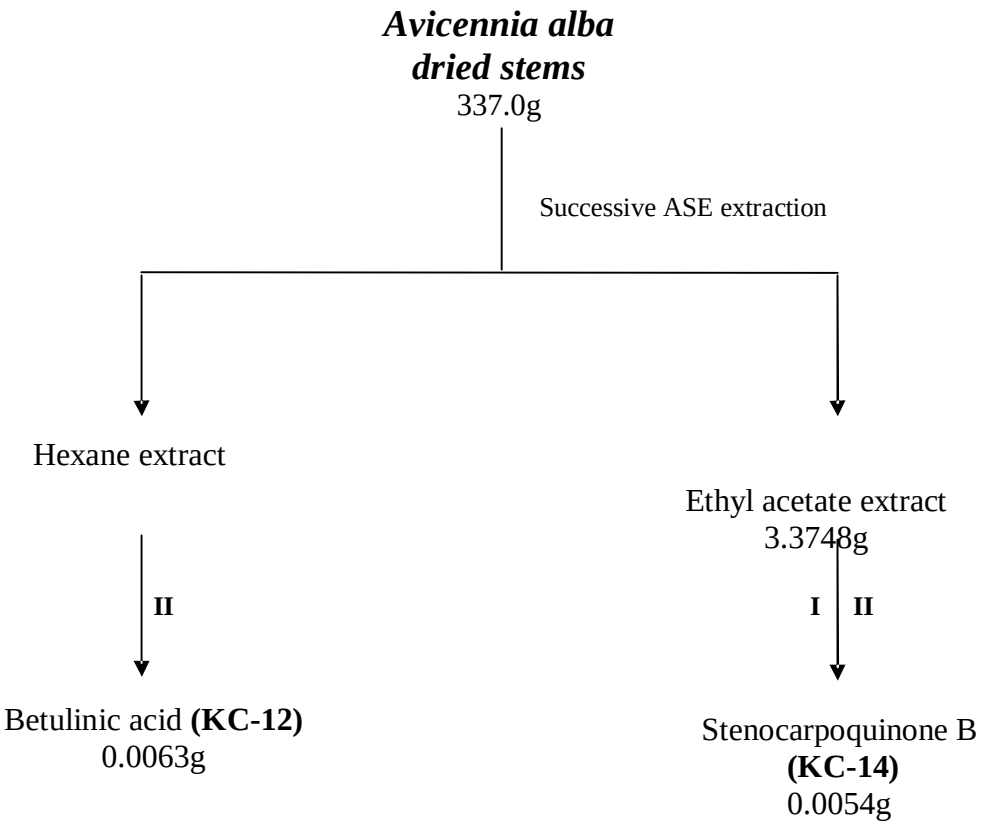
I=CC/ Florisil / 100% hexane, 10% ethyl acetate/n-hexane, **II**=PTLC/ 20% ethyl acetate, n-hexane,
III=CC/GF/ 95% chloroform/ n-hexane, **IV**=CC/Si / 100% n-hexane, 2, 2.5, 3% ethyl acetate/n-hexane

Scheme 2; Isolation of compounds from *Piper betel* leaf



I=VLC /100% n-hexane, **II**=VLC/ 4%ethyl acetate/n-hexane, **III**=VLC /15%ethyl acetate/n-hexane, **IV**=PTLC/30%ethyl acetate/n-hexane, **V**=VLC /10%ethyl acetate/n-hexane, **VI**=CC/ GF / 95%dichloromethane/n-hexane

Scheme 3; Isolation of compounds from *Brugeira gymnorrhiza* leaf



I=VLC /10%ethyl acetate/n-hexane, **II**=CC/ GF / 95%dichloromethane/n-hexane

Scheme 4; Isolation of compounds from *Avicennia alba* stems

References

Abd El-Kader, A.M., Abd El-Mawla, A.M.A., Mohamed, M.H., Ibraheim, Z.Z. (2006). Phytochemical and biological studies of *Emex spinosa* (L.) Campd. growing in Egypt. Bulltin of Pharmaceutical Sciences. 29:328-347.

Abu, R.S.M. (1995). Chemical investigation of *Bruguiera gymnorhiza*. Journal of the Bangladesh Chemical Society. 8:101-104.

Abu-Sayeed, M., Ali, M.A., Bhattacharjee, P.K., Anwarul, I., Astaq, G.R.M., Khan, M., Yeasmin, S. (2005). Biological evaluation of triterpenoids of *Euporbia hirta*. Pakistan J. of Scientific and Industial Research. 48:12-125.

Acharya, T.K., Chatterjee, I.B. (1975). Isolation of chrysophanic acid-9-anthrone, the major antifungal principle of *Cassia tora.* Lloydia. 38:218-20. [Chemical abstract]

Achmadi, S., Syahbirin, G., Choong, E.T., Hemingway, R.W. (1994). Catechin-3-O-rhamnoside chain extender units in polymeric procyanidins from mangrove bark. Phytochemistry. 35:217-19.

Agarwal, S.K., Singh, S.S., Verma, S., (2000). Antifungal activity of anthraquinone derivatives from *Rheum emodi*. J.of Ethnopharmacology. 72:43-46.

Akrout, A., Jani, H.E., Amouri, S., Neffati, M. (2010). Screening of antiradical and antibacterial activity of essential oils of *Artemisia campestris* L., *Artemisia Herba* Alba Asso, & *Thymus capitatus* Hoff. ET LINK. growing wild in the Southern of Tunesia. Recent research in Science and Technology. 2:29-39.

Al-Attar, A.M. (2011). Protective effect of *Avicennia alba* leaves extract on grastric mucosal damage induced by ethanol. Research Journal of Medicinal Plant. 5; 477-490.

Ali, I., Khan, F.G., Suri, K.A., B.D., Satti, N.K., Duitt, P., Afrin, F., Qazi, G., Khan, I, A. (2010). *In Vitro* antifungal activity of hydroxychavicol isolated from *Piper betel* L. Annals of Clinical Microbiology and Antimicrobials. 9:7.

Allan, J.A. and Duke, N.C. (2006). Species profiles for Pacific island agroforestry. www.traditionaltree.org. 22/07/10.

Allen, N.O., Allen, E.K. (1981). The Leguminosae, a source book of characteristics, uses, and nodulation. University of Wisconsin Press. U.S.A. 806 pages.

Al-masri, I.M., Mohammad, M.K., Taha, M.O. (2008). Discovery of DPP IV inhibitors by pharmacophore modeling and QSAR analysis followed by *in silico* screening. ChemMedChem. 3:1763-1779.

Al-Nadaf, A.H. and Taha, M.O. (2011). Discovery of new rennin inhibitory leads via sequential pharmacophore modeling, QSAR analysis, *in silico* screening and *in vitro* evaluation. J. of Molecular Graphics and Modelling. 29:843-864.

Amonka, A.J., Nagabhushan, M., Souza, A.U.D., Bhide, S.V. (1986). Hydroxychavicol: a new phenolic antimutagenic from betel leaf. Food and Chemical Toxicity. 24: 1321-1324.

Andrews, J.M. (2005) BSAC standardized disc susceptibility testing method (version 4). J. Antimicrob. Chemother. 56; 60-76.

Arambewela, L., Arawwawala, M., Rajapaksa, D. (2006). *Piper betel*: a potent natural antioxidant. International J. of Food Science and Technology. 41(Suppl.1):10-14.

Arturo, V.-N., Jose, P.-A., M., Jesus, P.-P., Ana, I., Jesus, S. (2004). Volatile constituents of the essential oil of *Ajuga chamaepitys* (L.) Schrebes, spp. J. of Essential Oil Research. July/August. [Chemical abstract]

Ashwini, P. and Krishnamoorthy, M. (2011). Antioxidant activity of ethanolic extract of *Cassia tora* L. International Journal of Research in Ayurveda& Pharmacy. 2; 250-252.

Atsuro, N., Seiji, H. (1994) Trachrysone as safe antioxidant. Jpn. Kokai Tokkyo Japan. [Chemical abstract]

Awang, D.V.C. (2009). Taylor's Herbs of Choice. The therapeutic use of phytomedicinals. CRC press. Taylor&Francis Group. New York. USA. 269 pages.

Azuma, H., Toyota, M., Asakawa, Y., Takaso, T., Tobe, H. (2002). Floral scent chemistry of mangrove plants. Journal of Plant Research. 115:47-53.

Baimai, V., Brockelman, Y.W. (1998). Biodiversity research and training programme in Thailand. Pure & Appl. Chem. 70: 2073-78.

Baker, J.G. (1877). Flora of Mauritius and the Seychelles; a description of the flowering plants and ferns of those islands. Taylor and Co., London.557 pages.

Bamroongrugsa, B. (1999). Bioactive substances from the mangrove resource. Songklanakarin J. Sci. Technol. 3; 377-386.

Bansyuni, M., Oku, H., Tsujimoto, E., Kinjo, K. (2007). Triterpene synthase from the Okinawan mangrove tribe, Rhizophoraceae. Federation of European Biochemical Societies Journal. 274:5028-5042.

Bateman, H., Klepac, A., Moody, M., Sweeney, B., Tomnay, S. (2004). The essential flowers gardening encyclopedia. Kyodo Printing Co. Pte. Ltd., Singapore. 608 pages.

Becconsall, J.K. (2005). Basic one- and two- dimensional NMR spectroscopy. Wiley-VCH Verlag GmbH &Co. KGaA. Germany. 406 pages.

Bennett, A., Stamford, I.F., Tavares, I.A., Jacobs, S., Capasso, F., Mascolo, N., Autore, G. (1988). The biological activity of eugenol, a major constituent of nutmeg (*Myristica fragrans*): Studies on prostaglandins, the intestine and other tissues. Phytotherapy Reasearch. 2:124-130.

Bharucha, F.R. and Rangnekar, P.V. (1957). Free amino acids and organic acids of halophytes of Bombay. Naturwissenschaften. 44:469.

Bhattacharya, S., Banejee, D., Bauri, A.K. (2007). Healing property of the *Piper betel* phenol, allylpyrocatechol against indomethacin-induced stomach ulceration and mechanism of action, World L. Gastroenterol.27:3705-13.

Bhattacharya, S., Chaudhuri, S.R., Chattopadhyay, S., Bandyopadhyay, S. (2007). Healing properties of some Indian medicinal plants against indomethacin-induced gastric ulceration of rats. J.of Clinical Biochemistry and Nutrition. 41:106-114.

Bhattacharya, S., Mula, S., Gamre, S., Kamat, J.P., Bandyopadhyay, S.K., Chattopadhyay, S., (2007). Inhibitory property of *Piper betel* extract against photosensitization-induced damages to lipids and proteins. Food Chemistry. 100: 1474-1480.

Bhattacharya, S., Subramanian, M. (2005). Radioprotective property of the ethanol extract of *Piper betel* leaf. JRR. 46**:**165-171.

Braithwaite, A., Smith, F.J. (1996). Chromatographic methods. Blackie Academic&Professional, Great Britain. 571 pages.

Castro, I.R. (2006). A guide to families of common flowering plants in the Philippines. Econofast Press. The Philipines. 199 pages.

Chakraborty, D. and Shan, B. (2011). Antimicrobial, anti-oxidative and anti-hemolytic activity of *Piper betel* leaf extracts. International Journal of Pharmacy and Pharmaceutical Sciences. 3; 192-199.

Chang, L.-Y., Lin, J.-C., Chang, C.-W., Ho, W.-H., Chan, Y.-T., Peng, J.-L., Hung, S.-L. (2009). Inhibitory effects of safrole on phagocytosis, intracellular reactive oxygen species, and the activity of myeloperoxidase released by human polymophonuclear leukocytes. Journal of Periodontology. 80:1330-1337.

Chang, M.C., Uang, B. J., Tsai, C.Y., Wu, H.L., Liu, B.R., Lee, C.S., Chen, Y.J. (2007). Hydroxychavicol, a novel betel leaf component, inhibites platelet aggregation by suppression of cyclooxygenase thromboxane production and calcium mobilization. British Journal of Pharmacology. 152:73-81.

Chan, S.J., Wu, B.N., Yeh, J.L. (1995). C-fiber-evoked autonomic cardiovascular effects after injection of *Piper betle* inflorescene extracts, J. Ethnopharmacology. 45:183-8.

Changtragoon, S. (2007). 22 Opportunities in using the conservation of biodiversity to alleviate poverty in Thailand.
http://www.fao.org/docrep/008/ae537e/ae537e0o.htm 30/05/07.

Chans, M.C., Uang, B.J., Tsai, C.Y. (2007). Hydroxychavicol, a novel betel leaf component inhibits platelet aggregation by suppression cyclooxygenase thromboxane production and calcium mobilization. Br.J.Pharmacol. 152:73-82.

Chantawanaskul, P. Puchanichanthranon, Wongsiri, S. (2005). Inhibitory effects of some medicinal plant extracts on the growth of *Ascosphaera apis*. Acta herticulturae. 678; 183-189.

Chatterjee, A. and Pakrashi, S.C. (1992). The treatise on Indian Medicinal Plants. CSIR. New Deli 2:44-45pp.

Chaveerach, A., Makkamul, P., Sudmoon, R., Tanee, T. (2006). Ethnobotany of the genus Piper (Piperaceae) in Thailand, Ethnobotany Research & Applications. 4:223-231.

Chen, G., Zhu, Y., Wang, H-Z., Wang, S-J., Zhang, R-Q. (2007). The metabolites of a mangrove endophytic fungus, *Penicillium thomi*. Journal of Asian Natural Products Research. 9:159-164.

Choi, J.E., Junh, J.H., Lee, H.J., Lee, J.H., Kang, S.S. (1995). A naphthalene glycoside from *Cassia tora*. Phytochemistry. 40:997-999.

Choi, J.S., Lee, H J., Kang, S.S. (1994). Alaternin, cassiaside and rubrofusarin gentiobioside, radical scavenging principles from the seeds of *Cassia tora* on 1,1-diphenyl-2-picrylhydrazyl(DPPH) radical. Arch.Pharm.Res. 17:462-6.

Choi, J.S., Lee, H J., Park, K.Y., Ha, J.O., Kang, S.S. (1997). *In vivo* antimutagenic effects of anthraquinone aglycones and naphthopyrone glycosides from *Cassia tora*. Planta Med. 63:11-4. [Chemical abstract]

Choi, J.Y., Na, M., Hwang, I.H., Lee, S.H., Bae, E.Y., Kim, B.Y., Ahn, J.S. (2009). Isolation of betulinic acid, its methyl ester and guaiane sesquiterpenes with protien tyrosine phosphatase 1B inhibitory activity from the roots of *Saussurea lappa* C.B.Clarke. Molecules. 14:266-272.

Choi, Y.H., Kong, K.R., Kim, Y.-A., Jung, K.-O., Kil, J.-H., Rhee, S.-H., Park, K.-Y. (2003). Induction of bax and activation of caspases during β-sitosterol-mediated apoptosis in human colon cancer cells. International J. of Oncology. 23:1657-1662.

Chukeatirote, E. and Saisavoey, T. (2009). Antimicrobial property and antioxidant composition of crude extracts of *Puerarin mirifica, Butea superba* and *Macana macrocarpa.*. Maejo International Journal of Science and Technology. 3; 212-221.

Claridge, T.D.W. (2006). High-Resolution NMR Techniques in organic chemistry. Volume 19. ELSEVIER . Netherlands, 382 pages.

Cunningham, G.E., Ghosh, D. (1963). Properties of some Indian mangroves. Leather Science (Madras). 10:127-37.

Dahot, M. (1999). Antibacterial and antifungal activities of small proteins of *Indigofera oblongifolia*. Journal of Ethnopharmacology. 64; 277-282.

Deore, S.L., Khadabadi, S.S., Kamdi, K.S., Ingle, V.P., Kawalkar, N.G., Sawarkar, P.S., Patil, U.A., Vyas, A.J. (2009). *In Vitro* anthelmintic activity of *Cassia tora*. International J. of ChemTech Research. 1:177-179.

Dhaked, P.S., Bairagi, G.B., Hatkar, A.D., Zaferuddin, S.D., Tembhurne, S.V., Sakarkar, D.M. (2010). Accelaratory effect of ethanol extract of *Piper betel* on gastrointestinal transit: involving calcium innervations in mice. International Journal of PharmTech Reasearch. 2:563-567.

Duke, A.J. (1929). Handbook of medicinal herbs. CRC press Inc. U.S.A. 870 pages.

Ebadi, M. (2007). Pharmacodynamic basis of herbal medicine 2 nd edition. CRC press, Taylor&Francis Group, New York, USA. 699 pages.

Elghamry, M.I., Shihata, J.M., (1966). Biological activity of phytoestrogens. III. Experimental studies on some biological properties of β-sitosterol. Planta Medica. 14:352-357.

Evans, P.H., Bowers, W.S., Funk, E.J. (1984). Identification of fungicidal and nematocidal components in the leaves of *Piper betle* (Piperaceae), J. of Agri. Food Chem., 32:1254-1256.

Fan, R.-Z., Song, W.-D., Gu, C.-S., Han, W.-D. (2009). Analysis of volatile components and fatty acids in hypocotyl of *Bruguiera gymnorhiza*. Zhiwu Yanjiu. 29: 500-504.

Fan, R.-Z., Song, W.-D., Han, W.-D., Hong, P.-Z., Xie, R.-Z. (2008). Analysis of leaves of *Bruguiera gymnorhiza* for its elementary chemical composition, Lihua Jianyan, Huaxue Fence. 44:754-756.

Fathilah, A.R. (2011). *Piper betle* L. and *Psidium guajava* L. in oral health maintenance. Journal of Medicinal Plants Research. 5; 156-163.

Farnsworth, N.R., Bunyaprapratsara, N. (1992). Thai medicinal plants recommended for primary health care system. Medicinal plants information center, Mahidol university. Bangkok. 402 pages.

Filipescu, G., Grigorescu, E., Streit, G. (1985). Pharmacognostic study of some *Cassia* L. and *Solenostema* L. I. Preliminary morphoanatomical and chemical study of *Cassia alata* L., *C. occidentalis* L., *C. mimosoides* L., *C. angustifolia* L. and *Solenostema argeria* L. An. Stiint.Univ. “Al. I. Cuza” Iasi, Sect. 2a. 31:53-6. [Chemical abstract]

Flekhter, O.B., Nigmatullina, L.R., Baltina, L.A., Karachurina, L.T., Galin, F.Z., Zarudii, F.S., Tolstikov, G.A. (2002). Synthesis of betulinic acid from betulin extract and study of the antiviral and antiulcer activity of some related terpenoids. Pharm. Chem. J. 36:484-487.

Fulda, S. (2008). Betulinic acid for cancer treatment and prevention Int. J. Mol. Sci. 9: 1096-1107.

Fulda, S., Friesen, C., Los, M., Scaffidi, C., Mier, W., Benedict, M., Nunez, G. (1997). Betulinic acid triggers CD95 (APO-1/FAS)-and p53-independent apoptosis

via activation of caspases in neuroectodermal tumors. Cancer Reasearch. 57:4956-4964.

García-Sosa, K. Villarreal-Alvarez, N., Lübben, P., Peňa-Rodríguez, L.M. (2006). Chrysophanol, an antimicrobial anthraquinone from the root extract of *Colubrina greggii*. J. Mex. Chem. Soc.50:76-78.

Ghosh, A., Misra, S., Dutta, A.K., Choudhury, A. (1985). Pentacyclic triterpenoids and sterols from seven species of mangrove. Phytochemistry. 24:1725-7.

Ghosh, K., Bhattacharya, T.K. (2005). Chemical constituents of *Piper betle* Linn. (Piperaceae) roots. Molecules. 10:798-802.

Ghosh, M.G. and Gouri, P. (1979). Chemical investigation on some mangrove species. part I. Genus Avicennia. J. of the Indian Chemicals Society. 56:111-113.

Ghosh, P., thakur, S. Itoh, T., Matsumoto, T. (1982). Sterols from flowers of *Cassia-siamea* Lam, *Cassia-sophera* Linn. and *Cassia-fistula* Linn. Indian J. Chem. Sec.B-Org.Chem. Inclu. Med Chem.21:796-797.

Gong, J-X, Shen, X., Yao, L.-G., Jiang, H. (2007). Total synthesis of Gymnorrhizol, an unprecedented 15-membered macrocyclic polydisulfide from the Chinese mangrove *Bruguiera gymnorhiza*. Organic Letters. 9:1715-1716.

Grabley, S. and Thiericke, R. (1999). Drug discovery from nature. Springer. Germany. 347 pages.

Grieve, M. (1974). A modern herbal. Jonathan, Cape Ltd., London. 915 pages.

Gritsanapan, W., Nualkaew, S. (2001). Variation of anthraquinone content in *Cassia* suttensis.Warasarn Phesatchasat. 28:28-34. [Chemical abstract]

Guo, Y-W., Huang, X-C., Zhang, W., Sun, Y-Q. (2006). Bioactive natural products from Chinese tropical marine plants and invertebrates. ACS Symposium Series. 925(Herbs): 73-91.

Hajare, R., Darvhekar, V.M., Shewale, A., Patil, V. (2011). Evaluation of antihistaminic activity of *Piper betel* leaf in guinea pig. African Journal of Pharmacy and Pharmacology. 5; 113-117.

Han, L., Huang, X., Duhse, H.-M., Moellman, U., Fo, H., Grably, S., Sattler, I., Lin, W. (2007). Unusual naphthoquinone derivatives from the twigs of *Avicennia marina*. J. of Natural Products. 70:923-927.

Han, L., Huang, X., Sattler, I., Dahse, H-M., Fu, H., Grabley, S., Lin, W. (2005). Three new pimarane diterpennoids from marine mangrove plant, *Bruguiera gymnorhiza*.Pharmazie. 60:705-707.

Han, L., Huang, X., Sattler, I.,Dahse, H.-M., Fu, H., Lin, W., Grabley, S. (2004). New diterpenoids from the marine mangrove *Bruguiera gymnorhiza*. Journal of Natural Products. 67:1620-1623.

Han, L., Huang, X-S, Sattler, I., Fu, H-Z, Grably, S, Lin, W-H, (2007). Two new constituents from mangrove *Bruguiera gymnorhiza*. Journal of Asian Natural Products Research, 9, 327-331.

Han, L., Huang, X., Sattler, I., Moellmann, U., Fu, H., Lin, W., Grabley, S. (2005). New aromatic compounds from the marine mangrove *Bruguiera gymnorhiza*. Planta Medica. 71:160-164.

Harborne, J.B., Boulter, D., Turner, B.L. (1971). Chemotaxonomy of the Leguminasae. Academic Press. London and New York. 612 pages.

Hassanpouraghdam, M.-B., Tabatabaie, S.-J., Nazeniyeh, H., Vojodi, L., Aazami, M.-A., Shoja, A.-M. (2008). *Chrysanthemum balsamita* (L.) Baill.: Forgotten medicinal plant. Medicine and Biology. 15:119-124.

Hassnein, R.R., Elmougy, S.A., Elghamry, M.I. (1972). Biological activity of phytoestrogens. VI. fractionation of plasma proteins associated with β-sitosterol treatment. Planta Medica. 22:412-417.

Hatano, T., Uebayashi, H., Ito, H., Shiota, S., Tsuchiya, T., Yoshida, T. (1999). Phenolic constituents of Cassia seeds and antibacterial effects of some naphthalenes and anthraquinones on methicillin-resistant *Staphylococcus aureus*. Chem.Pharm.Bull. 47:1121-7.

Hayata, M., Ikehara, N. (1984). Polyphenol oxidase in mangrove leaves, *Bruguiera gymnorhiza* (L.) Lamk. Galaxea. 3:71-80.

Hogg, R.W., Gillan, F.T. (1984). Fatty acids, sterols, and hydrocarbons in the leaves from eleven species of mangrove. Phytochemistry. 23:93-7.

Homhual, S., Bunyapraphatsara, N., Kondratyuk, T., Herunsalee, A., Chaukul, W. (2006). Bioactive dammarane triterpenes from the mangrove plant *Bruguiera gymnorhiza*. Journal of Natural Products. 69:421-424.

Homhual, S., Zhang, H-G., Bunyapraphatsara, N., Kondratyuk, T., Santarsiero, B.D. (2005). Bruguiesulfurol, a new sulfur compound from *Bruguiera gymnorhiza*. Planta Medica. 72:255-260.

Hu, Y., Boping, Y., Wang, D. (2007). Study on the bioactivity of 3',4',5'-trihydroxy-7-hydroxy-5-methoxy flavone extracted from *Bruguiera gymnorhiza* by *Caenorhabditis elegans*. Zhongguo Haiyang Yaowu. 26:20-26.

Huang, X.-Y., Wang, Q., Liu, H.L. (2009). Diastereoisomeric macrocyclic polydisulfides from the mangrove *Bruguiera gymnorhiza*, Phytochemistry. 70:2096-2100.

Huang, X-Z., Liu, Y., Yu, S.-S., Hu, Y.-C., Qu, J. (2007). Triterpenoids from the roots of *Crabiodendron henryi* W. W. Smith. J. of Integrative Plant Biology. 49:1615-1618.

Huang, X., Z., Yin, Y., Dai, J., H., Liang, H., Dai, Y., Bai, L. (2010). Two new ceramides from stems of *Piper betel* L. Chinese Chemical Letters. 21:433-436.

Huo, Y.-C., Sun, C.-M., Ou, J.-C., Tsai, W.-J. (1997). A tumor cell growth inhibitor from *Polygonum hypoleucum ohwi*. Life Sciences. 61:2335-2344.

Hwang, L.S., Wang, C.K., Sheu, M.J., Kao, I.S. (1992). Phenolic-compounds of *Piper betle* flower as flavoring and neuronal- activity modulating agents, ACS Symposium Series. 506:200-213.

Hwang, S.-L., Kim, H.-N., Jung, H.-H., Kim, J.-E., Choi, D.-K. (2008). Beneficial effect of β-sitosterol on glucose and lipid metabolism in L6 myotube cell are mediated by AMP-activated protein kinase. Biochemical and Biophysical Research Communication. 377:1253-1258.

Hyun, S.K., Lee, H., Kang, S.S., Chung, H.Y., Choi, J.S. (2009). Inhibitory activities of *Cassia tora* and its anthraquinone constituents on angiotensin-converting enzyme. Phytotherapy Research. 23:178-184.

Igoli, O.J., Gray, I.A. (2008). Friedelanone and Other Triterpenoids from *Hymenocardia acida*. International Journal of Physical Sciences. 3:156-158.

Islam, M.S., Lain, C.L., Kameyama, N., Wu, B., Hogetsu, T. (2006). Developmental and characterization of ten new microsatellites markets in a mangrove tree species *Bruguiera gymnorhiza* (L.). Molecular Ecology Notes. 6:30-32.

Ismail, N.H., Alyahya, S.A., Shaari, K., Yamin, B.M. (2005). 1-(1,8-Dihydroxy-6-methoxy-3-methylnaphthalen-2-yl)ethanone. Acta Crytallographica, Section E: Structure Reports Online. 61:67- 68.

Ito, C., Katsuno, S., Kondo, Y., Tan, H., T.-W., Furukawa, H. (2000). Chemical constituents of *Avicennia alba*. Isolation and structural elucidation of new naphthoquinones and their analogues. Chemical &Pharmaceutical Bulletin. 48:339-343.

Jang, D.S., Lee, G.Y., Kim, Y.S., Lee, Y.M., Kim, C.-S., Jeong, L., Kim, J.S. (2007). Anthraquinones from the seeds of *Cassia tora* with inhibitory activity on protein glycation and aldose redutase. Biological&Pharmaceutical Bulletin. 30:2207-2210.

Jeng, J.H., Wang, Y.J., Chang, W.H. (2004). Reactive oxygen species are crucial for hydroxychavicol toxicity toward KB epithelial cells. Cell Mol. Life Sci. 61:83-96.

Ji, Y. (2007). Methicillin-resistant *Staphylococcus aureus* (MRSA) protocols. Human Press. Totowa, New Jersey. USA.

Jia, Z.-B., Chen, W.-W., Jiang, J.-X., Ding, X.-L. (2009). Study on anthraquinone constituents in the seed of *Cassia tora* L. Linchan Huaxue Yu Gongye. 29:100-102.

Joy, P.P. (1998). Medicinal plants, Kerala Agricultural University. Aromatic and Medicinal Plants Research Station. India.
Joshi, S. (1994) Indian J. Pharm. 26:78.

Jung., H.J., Kang, S.S. (1996). The NMR assignments of antraquinones from *Cassia tora*. Arch.Pharm.Res. 19:302-306. [Chemical abstract]

Jutiviboonsuk, A. (2006). Phytochemistry and pharmacologycal studies of *Barringtonin mawongyathae* W. Chawkul and *Bursera tonkinensis* Guillaum. PhD.Thesis. Mahidol University. Thailand.

Jutiviboonsuk, A., Zhang, H.-J., Kondratyuk, T.P., Herunsalee, A., Chaukul, W, Pezzuto, J.M., Fong, H.H., S., Bunyapraphatsara, N. (2007). Isolation and characterization of cancer chemopreventive compounds from *Barringtonia maunwongyathiae*. Pharmaceutical Biology. 45:185-194.

Kaneda, M., Morisgita E., Shibata, S. (1969). Chemical studies on the oriental plant drugs.XXI. The constituents of *Cassia tora* L. 2. A glycoside of rubrofusarin. Chem.Pharm.Bull. 17:456-61. [Chemical abstract]

Kanjwani, D.G., Marathe, T.P., Chiplunkar, S.V., Sathaye, S.S. (2008). Evaluation of immunomodulatory activity of methanolic extract of *Piper betel*. Scandinavian J. of Immunology. 67:589-593.

Karimulla, S., Kumar, B.P. (2011). Anti diabetic and anti hyperlipidemic activity of bark of bark of *Bruguiera gymnorrhiza* on streptozotocin induced diabetic rats. 1; 4-7.

Kee, C.H. (1999). The Pharmacology of Chinese herbs. Second Edition. CRC publishers. U.S.A. 512 pages.

Keat, E.C., Razak, S.S., Fadil, N.M., Yusof, F.M., Chan, L.H., Chyi, F.K., Teoh, S.L. (2010). The Effect of *Piper betel* extract on the wound healing process in experimentally induced diabetic rats. La Clicnica terapeutica. 161:117-120.

Keng, H. and Keng, R.-S., L. (1978). Order and families of Malayan seed plants: synopsis of orders and families. Singapore University Press. Singapore. 437 pages.

Kim, Y.M., Lee, C.H., Kim, H.G., Lee, H.S. (2004). Anthraquinones isolated from *Cassia tora* (Leguminosae) seed show an antifungal property against phytopathogenic fungi. J.Agric.Food.Chem. 52:6096-6100.

Koocharoenpisal, N. (1997). Chemical constituents from the leaves of *Piper betle* Linn. and their biological activity, Thesis (M.Sc.). Chulalongkorn University Library. 211. Bangkok.

Kong, X., B., Rubin, L., Chen, L., I., Ciszewska, G., Watanabe, K., A.,Tong,W., P., Sirotnak, F., M., Chou, T.-C. (1992). Topoisomerase II-mediated DNA cleavage activity and irreversibility of cleavable complex formation induced by DNA intercalator with alkylating capability. Molecular Pharmacology. 41:237-244.

Krishna, K.G., Suresh, P. (2005). International Journal of Pest Management. 51:327-334. [Chemical abstract]

Kuljanabhagavad, T., Suttisri, R., Pengsuparp, T., Ruangrungsi, N. (2009). Chemical structure and antiviral activity of aerial parts from *Laggera pterodota*. J. Health Res. 23:175-177.

Kumar, R., Singh, S., Agarwal, S.C., Singla, S., Pundir, R.K., Varshney, S.C. (2009). Chemical composition and antimicrobial activity of essential oil of *Piper betel* (L.) Cv. Kapoori Leaves. Indian Perfumes. 53:38-40.

Kuo, Y.H., Chang, C., Li, S.Y., Chou, C.J., Chen, C.F., Kuo, Y.H., Lee, K.H. (1997). Cytotoxic constituents from the stems of *Diospyros maritime*. Planta Medica. 63:363-365.

Laphookhieo, S., Karalai, C., Ponglimanont, C. (2004). New sesquiterpenoid and triterpenoids from the fruits of *Rhizophora mucronata*. Chem. Pharm. Bull. 52:883-885.

Lavania, M., Chauhan, P.S., Chauhan, S.V. (2006). Induction of plant defense enzymes and phenolics by treatment with plant growth-promoting Rhizobacteria *Serratia marcescens* NBRI1213. Curr. Microbiol. 52:363-368.

Lee-Chen, S.-F., Chen, C.-L., Ho, L.-Y., Hsu, P., C., Chang, J.-T., Sun, C.-M., Chi, C.-W., Lin, T.-Y., (1996). Role of oxidative DNA damage in hydroxychavicol-induced genotoxicity. Mutagenesis. 11:519-523.

Lee, G.Y., Jang, D.S., Lee, Y.M., Kim, J.M., Kim, J.S. (2006). Naphthopyrone glucosodes from the seeds of *Cassia tora* with inhibitory activity on advanced glycation end products (AGEs) formation. Arch.Pharm.Res. 29:587-590. [Chemical abstract]

Lee, H.J., Choi, J.S., Jung, J.H., Kang, S.S. (1998). Alaternin glucoside isomer from *Cassia tora*. Phytochemistry. 49:1403-1404.

Lee, H.J., Jung, J.H., Kang, S.S., Choi, J.S. (1997). A rubrofusarin gentiobioside isomer from roasted *Cassia tora*. Arch.Pharm.Res. 20:513-515. [Chemical abstract]

Lee, H.S. (2003). Inhibitory effects of quinizarin isolated from *Cassia tora* seeds against human intestinal bacteria and aflatoxin B-1 biotransformation. J. of Microbiology and Biotechnology. 13:529-536. [Chemical abstract]

Lee, J., Kim, S., Kim, D., Chang, J. (2002). Stereoselective synthesis of β-elemene by a doubly diastereo differentiating internal alkylation : A remarkable difference in the rate of enolization between syn and anti ester. Tetrahedron Letters. 57:7.

Lewis, J. (1956). Flora of tropical East Africa. 1.Balkema. London. 20 pages.

Li, H.L., Liu, T.S. (1976). Flora of Taiwan. Epoch Publishing Co. Ltd.. Taiwan.

Li, D.-L., Li, X.-M., Wang, B.-G. (2008). Pentacyclic triterpenoids from the mangrove Plant *Rhizophora stylosa*. Natural Products Research, Part A: Structure and Synthesis. 22:808-813.

Li, Q.Q., Wang, G., Zhang, M., Cuff, C.F., Huang, L., Reed, E. (2009). Beta-elemene, a novel plant-drived antineoplastic agent, increases cisplatin chemosensitivity of lung tumors cells by triggering apoptosis. Oncol. Rep. 1:161-70.

Li, X., Wang, G., Zhao, J., (2005). Antiproliferative effect of beta-elemene in chemoresistant ovarian carcinoma cells is mediated thought arrest of the cell cycle at the G2-M Phase. Cell Mol Life Sci. 62: 894-904.

Liao, H. and Zhou, J. (2008). Molecular cloning and analysis of a chalcone synthase gene of *Cassia tora*. Xibei Zhiwu Xuebao. 28:1728-1733.

Lin., H.-L., Shen, X., Jiang, H.-L., Guo, Y.-W. (2008). Structural studies on an unusual novel macrocyclic polydisulfide from the Chinese mangrove *Bruguiera gymnorhiza*. Youji Huaxue. 28:246-251.

Liu, M., Seidel, V., Katerene, D.R., Gray, A.I. (2007). Colourimetric broth microdilution method for the antifungal screening of plant extracts against yeasts. Method. 42:325-329.

Mahato, S.B., Kundu, A.P. (1994). ^{13}C NMR Spectra of pentacyclic triterpenoids-a compilation and some salient features. Phytochemistry. 37:1517-1575.

Maity, T.K., Mandal, S.C., Bhakta, T., Pal, M., Saha, B.P. (2001). Metabolism of 1,8-dihydroxy 3-hydroxy methyl anthraquinone(aloe-emodin) isolated from the leaves of *Cassia tora* in albino rats. Phytother.Res. 15:459-60. [Chemical abstract]

Maji, M.D., Chattopadhyay, S.Kumar, P., Saratchandra, B. (2005). *In vitro* screening of some plant extracts against fungal pathogens of mulberry (Morus spp.). Archives of Phytopathology and Plant Protection. 38:157-164.

Manjusree, P., Roy, D.K., Pal, P.R. (1997). Emodin from the leaves of *Cassia tora* Linn. Indian J. Pharm. 39:116-7. [Chemical abstract]

Manriquez-Torres, J.J., Zuniga-Estrada, A., Gonzalez-Ledesma, M., Torres-Valencia, J., M. (2007). The antibacterial metabolites and proacacipetalin from *Acacia cochliacantha*. J. Mex. Chem. Soc. 51:228-231.

McMurry, J.E., Ovasley, P.K. (1985). Synthesis of helminthogermancrene and β-elemene. Tetrahedron Letters. 26: 2171-2172.

Meselhy, M.R. (2003). Constituents from moghat, the Roots of *Glossostemon bruguieri* (Desf.). Molecules. 8:614-621.

Mfilinge, P.L., Meziane, T., Bachok, Z., Tsuchiya, M. (2005). Total lipid and fatty acid classes in decomposing mangrove leaves of *Bruguiera gymnorhiza* and Kandelia candel: Significance with respect to lipid input. Journal of Oceanography. 61:613-622.

Mira, P., Kumar, A., Khare, P., Gupta, S., Kumar, N., Dube, A. (2009). Pro-apoptotic Effect of the landrance *Bangla Mahoba* of *Piper betel* on *leishmania donovani* may be due to the high content of eugenol. Journal of Medicinal Microbiology. 58:1058-1066.

Miroyoshi, M., Iizuka, T., Nagai, M., Hoshi, K. (2003). Adenine, an inhibitor of platelet aggregation, from the leaves of *Cassia alata*. Biol.Pharm.Bull. 26:1361-4.

Misra, S., Datta, A.K., Chattopadhyay, A., Choudhury, S., Ghosg, A. (1987). Hydrocarbons and wax esters from seven species of mangrove leaves. Phytochemistry. 26:3265-8.

Mizutani, T., Satoh, K., Nomura, H. (1991). Hepatotoxicity of eugenol and related compounds in mice depleted of glutathione: structureal requirements for toxic potency. Research Communications in Chemical Pathology and Pharmacology. 73:87-95.

Mock, J., Murphy, S., T., Ritchie, E., Taylor, W.C. (1973). Chemical studies of the proteaceae. VI two naphthoquinones from *Stenocarpus salignus*. Astralia J. of Chem. 26:1121-1130. doi 10.107/CH9731121.

Mohamed, S., Saka, S., El-Sharkawy, S.H., Ali, A.M. (1996). Antimycotic screening of 58 Malaysian Plants against Plant Pathogents. 47:259-267. [Chemical abstract]

Morimoto, S., Nonaka, G., Chen, R.F.M. Nishioka, I. (1988). Tannins and related-compounds.61. Isolation and structures of novel bi-flavanoids and triflavanoids from the leaves of *cassia-fistula* L. Chem.Pharm.Bull. 36:39-47.

Motoyoshiya, J. (2007). Synthesis of hypericin via emodin anthrone derived from a two-fold diels-alder reaction of 1, 4-benzoquinone. Natural Product Communications. 2:2007.

Moulisha, B., Bikash, M.N., Kumar, G.A., Sukdeb, B., Kanti, H.P. (2009). *In vitro* anti-leishmanial and anti-tumor activities of a pentacyclic triterpenoids compound isolated from *Dregea volubilis* Bebth *Asclepiadaceae*. Tropical J. of Pharm. Research. 8:127-131.

Msaji, K., Choji, I., Mayumi, N., Yasuko, I., Yoshio, T. (1978). Studies on the evaluation of crude drug.VII. Separation and quantitative estimation of

anthraquinones in *Cassia* seeds on column of Sephadex LH-20. Shoyakugaku Zassh. 32:267-72. [Chemical abstract]

Mukherjee, P.K., Saha, K., Saha, B.P., Pal, M., Das, J. (1996). Antifungal activities of the leaf extracts of *Cassia tora* Linn.(Fam.Leguminasae). Phytother.Res. 10:521-522.

Mula, S., Banerjee, D., Patro, B., Bhattacharya, S., Barik, A., Bandyopadhyay, S.K., Chattopadhyay, S. (2008). Inhibitory property of the *Piper betel* phenolics against photosensitization-induced biological damages. Bioorganic&Medicinal Chemistry. 16:2932-2938.

Murata, K., Nakao, K., Hirata, N., Namba, K., Nomi, T., Kitamura, Y. (2009). Hydroxychavicol: a potent xanthine oxidase inhibitor obtained from the leaves of betel, *Piper betel*. J.of Natural Medicines. 63:355-359.

Mukherjee, R., Jaggi, M., Rajendran, P., Siddiqui, M.J., a., Srivastava, S.K., Vardham, A., Burman, A., C. (2004). Betulinic acis and its derivatives as anti-angiogenic agents. Bioorganic& Medicinal Chemistry Letters. 14:2181-2184.

Nair, R. and Chanda, S. (2008). Antimicrobial activity of *Terminalia catappa, Manilkara zapota* and *Piper betel* leaf extract. Indian J. of Pharmaceutical Sciences. 70:390-343.

Nakagawa, Y., Suzuki, T., Nakajima, K., Ishii, H., Ogata, A. (2009). Biotransformation and cytotoxic effects of hydroxychavicol, an intermediate of safrole metabolism, in isolated rat hepatocytes. Chemico-Biological Interactions. 180:89-97.

Nam, J. and Choi, H. (2008). Effect of butanol fraction from *Cassia tora* L. seeds on glycemic control and insulin secretion in diabetic rats. Nutrition Research and Practice. 2:240-246.

Nelson, G. (1996). The shrubs and woody vines of Florida: A Reference and Field. Press Inc. USA. 391 pages.

Nisar, M., Ghafoor, A., Ahmad, H., Khau, M.R., Qureshi, A.S., Ali, H. and Islam, M. (2008). Evaluation of genertic diversity of pea germplasm through phenotypic trait analysis. Pak. J. Bot. 40; 2081-2086.

Nishikawa, M. and Fukui, Y. (2008). *Cassia tora* seed extracts, their manufacture, method for reduction of diarrhea-inducing activity of the extracts and foods and beverages containing them. Japan Kokai Tokkyo Koho. 12pp.

Obiajunwa, E.I., Adebajo, A.C., Omobuwajo, O.R. (2002). Essential and trace element contents of some Nigerian medicinal plants. J.Radioanalytical and Nuclear Chemistry. 252:473-476. [Chemical abstract]

Ogata, M. (2004). Biological activity of eugenol. Japan Aroma Research. 5:259-262.

Ogata, M., Hoshi, M., Urano, S., Eud, T. (2000). Antioxidant activity of eugenol and related monomeric and dimeric compounds. Chem. Pharm. Bull. 48:1467-1469.

Panda, S., Jafri, M., Kar, A., Msheta, B.K. (2009). Thyroid inhibitory, antoperoxidative and hypoglycemic effects of stigmasterol isolated from *Butea monosperma*. Fitoterapia, 80:123-0126.

Pandey, A., Bani, S., Dutt, P., Suri, K.A. (2010). Modulation of Th1/Th2 cytokinase and inflammatory mediators by hydroxychavicol in adjuvant induced arthritic tissues. Cytokine+. 49:114-121.

Park, T.H., Kim, D.H., Kim, C.H., Jung, H.A., Choi, J.S., Lee, J.W., Chung, H.Y. (2004). Peroynitrite scavenging mode of alaternin isolated from *Cassia tora*. J. Pharmacy and Pharmacology. 56:1315-1321.

Parmar, V.S., Jain, S.C., Gupta, S. (1998). Polyphenols and alkaloids from Piper species. Phytochemistry. 49:1069-1078.

Patil, U.K. (2004). Hypolipidemic activity of seeds of *Cassia tora* Linn. J. Ethnopharmacol. 90:249-252.

Perry, M.L. (1980). Medicinal plants of East and Southeast Asia: attributed properties and uses. The MIT Press. England. 632 pages.

Perry, M.L., Metzger, J. (1895). Medicinal plants of East and Southeast Asia: Attributed Properties and uses. The MIT Press. England. 620 pages.

Phongpaichit, S., Pujenjob, N., Rukachaisirikul, V., Ongsakul, M. (2004). Antifungal activity from leaf extracts of *Cassia alata* L., *Cassia fistula* L. and *Cassia tora* L. Sonklanakarin J. SciTechnol. 26:741-748.

Pisha, E., Chai, H., Lee, I.S., Changwedera, T.E., Farnaworth, N.R., Cordel, G.A. (1995). Discovery of betulinic acid as a selective inhibitor of human melanoma that function by induction of apoptosis. Nat. Med.1046-1051.

Podojil, M., Sedmera, P., Vokoun, J., Betina, V., Barathova, H., Durackwa, Z., Horakova, K., Pemec, P., (2008). *Eurotium (Aspergillus) repens* metabolites and their biological activity. Filia Microbiologica. 23:438-443.

Poethke, W., Rao, D.A., Loescher, K.I. (1968). Chromatographic characterization of compnents of *Cassia tora* seeds. Pharm.Zentralh. 107:571-8. [Chemical abstract]

Premanathan, M., Kathiresan, K., Yamamoto, N., Nakashima, H. (1999). *In vitro* anti-human immunodeficiency virus activity of polysaccharide from *Rhizophora mucronata* Poir. Biosci. Biotechnol. Biochem..63:1187-91.

Price, L. and Prabakaran, P. (2011). Antifungal activity of medicinal plants against plant pathogenic fungus *Collectotrichum falcatum*. Asian Journal of Plant Science and Research. 1; 84-87.

Punyanubhab, T., Yodmani, S., Kongsiri, S. (1992). Medicinal plants of Thailand: past and present. Amarin Printing Co.Ltd. Thailand.

Radwan, H.M., Hassan, R.A. (2006). The flavonoids, lipids and protein contents of limoniastrum monopetatum L. growing in Egypt and their biological activity. Bulletin of the Faculty of Pharmacy (Cairo University). 44:93-101.

Raghukumar, R., Vali, L., Watson, D., Fearnley, J., Seidel, V. (2010). Antimethicillin-resistant *Stephylococcus aureus* (MRSA) cctivity of 'Pacific propolis' and isolated prenylflavanones. Phytoterapy Research. DOI. 10.1002/ ptr.3096.

Raihan, S.M.A. (1994). Gramrionea novel flavone from *Bruguiera gymnorhiza* Lam. ACGC Chemical Research Communications. 2:21-24.

Ramji, N., Iyer, R., Chandrasekaran, S. (2002). Phenolic antibacterials from *Piper betle* in the prevention of halitosis. J. Ethnopharmacol. 83:149-52.

Rathee, J.S., Pathro, B.S., Mula, S., Gamre, S., Chattopadhyay, S. (2006). Antioxidant activity of *Piper betel* leaf extract and its constituents. J. of Agricultural and Food Chemistry. 54:9046-9054.

Rejiya, C.S., Cibin, T.R., Abraham, A., (2009). Leaves of *Cassia tora* as a novel cancer therapeutic-an *In vitro* study. Toxicology in Vitro. 23:1034-1038.

Rimando, A.M., Han, B.H., Park, J.H., Cantoria, M.C. (1986). Studies on the constituents of Philippine *Piper betel* leaves. Arch. Pharm. Res**.** 9:93-97.

Robertson, S.A. (1989). Flowering plants of Seychelles. Royal botanic gardens. Great Britain. 344 pages.

Romeo, J.T. and Dixon, R.A. (2002). Phytochemistry in the genomics and post-genmics eras. Pergamon.Academic press. United Kingdom. 268 pages.

Roopashree, T.S., Raman D., Shobha, R.R., Narendra, C. (2008). Antibacterial activity of antipsoriatic herbs: *Casia tora, Momordica charantia* and *Calendula officinalis.* International Journal of Applied Research in Natural Products. 1; 20-28.

Ross, T.T., Hayes, C.E., Goldstein, I.J. (1976). Carbohydrate-binding properties of an immobi=Hd -D-galactopyranosyl-binding protein (lectin) from the seeds of *Bandeirea simplicifolia.* Carbohydr.Res. 47:91-7. [Chemical abstract]

Row, L.-C.M. and Ho, J.-C. (2009). The Antimicrobial activity, mosquito larvicidal activity, antioxidant property and tyrosinase inhibition of *Piper betel.* J. of the Chinese Chemical Society. 56:653-658.

Rudjiman (1992). *Brufuiera gymnorrhiza* (L.) Saving. In: Prosea, plant resource of Southeast Asia 3. Dye and tannin producing plants. Indonesia press. Indinesia. 53-55.

Runzhen, F., (2010). Method for detecting content of total flavones in leaves og *Bruguiera gymnorhiza*. Faming Zhuanli Shenqing Gongkai Shuomingshu (patent)

Russell, B. (2005). Searching for anticancer natural products from the rainforest plants of Suriname and Madargascar. Dissertation. Faculty of Virginia Institutute and State University. Blacksbury, Virginia, USA.

Santos de Sa, M., Costa, J.F., o., Krettli, A.U., Zalis, M.G. (2009). Antimalarial activity of betulinic acid and derivatives *in vitro* against *Plasmodium falciparum* and *in vivo* in *P. berghi*-infected mice. Parasitol Res. 105:275-279.

Sakurai, S., Yaguchi, Y., Inoue, T., (1987). Triterpenoids from *Myrica rubra*., Phytochemistry. 26:217-217.

Saralamp, P., Temsiririrkkul, R., Chuakul, W., Riewpaiboon, A., Prathanturarug, S., Suthisisang, C. and Pongcharoensuk, P. (1996). Medicinal plants in Siri Ruckhachati. Amarin Printing and Publishing public Co. Ltd.. Bangkok. Thailand. 257 pages.

Sarkar, A, Ganguly, S.N. (1978). Gymnorhizol, a new triterpene alcohol from *Bruguiera gymnorhiza*. Indian Journal of Chemistry, Section B: organic Chemistry Including Medical Chemistry.16B:742.

Sarkar, D., Saha, P., Gamre, S., Bhattacharjee, S., Hariharan, C. (2008). Anti-inflammatory effect of allylpyrocatechol on LPS- induced macrophages is mediated by suppression of iNOS and COX-2 via the NF-$_k$B pathway. International Immunopharmacology. 8:1264-1271.

Savaspun, K., Kongkathio, N., Kongkathip, B., Phonnakha, S. (2003). Extraction and isolation of antifungal and antibacterial constituents from the Thai betel (*Piper betle* Linn.). Master Thesis. Graduation school. Kasetsart University. Bangkok. [Chemical abstract].

Schmeda-Hirschmann, G., Papastergiou, F. (2003). Naphthoquinone derivatives and lignans from the Paraguayan crude drug “Tayï Pytá”(*Tabebuia heptaphylla*, Bignoniaceae). Z.Naturforsch. 58: 495-501.

Schwarzbach, A.E., McDade, L.A. (2002). Phylogenetic relationships of the mangrove family Acanthaceae based on chloplast and nuclear ribisomal DNA sequences. Systematic Botanic. 27:84-98.

Sefidkun, F., Shaabani, A. (2003). Analysis of the oil of *Hippomarathrum microcarpum* (M.B.) B. Fedsch. from Iran. J. of Essential Oil Reasearch. [Chemical abstract].

Serra, S., Fugguti, C. (2005). Aromatic annulation on the p-menthane monoterpenes: enantiospecific synthesis of *trans* and *cis* isomers of calamenene and 8-hydroxy calamenene. Tetrahedron Letters. 46:4769-4772.

Seshadri, T.R., Venkataramani, B., (1959). Leucocyanidin from mangroves. Journal of Scientific& Industrial Research. 18B:261-2.

Setzer, W.N., Shen, X., Bates, R.B., Burns, J.R., Kelly, J., Zhang, P., Moriarity, D.M., Lawton, R.O. (2000). A phytochemical investigation of *Alchornea latifolia*. Fitoterapia. 71:195-198.

Shang, S. and Lang, S. (2008). Bruganin, a new syn-2,3-dihydrobenzofuran neolignan diolate from the mangrove *Bruguiera gymnorhiza*. Chemistry of Natural Compounds. 44:186-189.

Sharma, S., Khan, I.A., Ali, I., Ali, F., Kumar, M., Kumar, A., Johri, R. (2009). Evaluation of the antimicrobial, antioxidant, and anti-inflammatory activities of hydroxychavicol for its potential use as an oral care agent. Antimicrobial Agents and Chemotherapy. 53:216-222.

Shen, X., Guo, Y., Wang, Q., Liu, H., (2008). Process for preparation of bruguiesulfurol and derivatives for treating diabetes mellitus. Faming Zhuanli Shenqing Gongkai Shuomingshu, 24pp. (patent).

Shi, Y.-Q., Fukai, T., Sakagami, H, Kuroda, J., Miyaoka, R., Tamura, M., Yoshida, N., Nomura, T. (2001). Cytotoxic and DNA damage-inducing activities of low molecular weight phenols from rhubarb. Anticancer Research. 21:2847-2853.

Shibato, S., Morishita, E., Kaneda, M., Kimura, Y., Takido, M., Takahashi, S. (1969). Chemical studies on the oriental plant drugs.XX.The constituents of *Cassia tora* L. The structure of torachrysone. Chem.Pharm.Bull. 17:454-457. [Chemical abstract]

Shinoda, Y., Iwata, S., Tajima, T., (1987). Chemical composition of mangroves. III. The bark. Gifu Daigaku Nogakubu Kenkyu Hokoku. 52:147-158.

Shinoda, Y., Ogisu, M., Iwata, S., Tajima, T. (1985). Chemical composition of mangroves. Gifu Daigaku Nogakubu Kenkyu Hokoku. 50:155-65.

Silva, D., Alves, M., Coelho, F.P. (1950). The natural tannins of Portugal. I. Some properties of solutions of tannins of *Acacia dealbata* (Link) and of *Bruguiera gymnorhiza* (Lam.). Rev. faculdade cienc., Univ. Coimbra. 19:151-61.

Singh, M., Shakya, S., Soni, V., Kumar, D., A., Kumar, N., Bhattacharya, S.-M. (2009). The n-hexane and chloroform fractions of *Piper betel* L. trigger different arms of immune responses in BALB/c mice and exhibit antifilarial activity against human lymphatic filarid *Brugia malayi*. International Immunophamacology. 9:716-728.

Smitinand, T. (1976). The Botany of mangrove forest in Thailand. Proceedings of the first Thai national seminar on mangrove ecology, Phuket. 10-15 Jan 1976. National Research Council of Thailand. Part 2. 1:222-256.

Srimani, P., Mandal, G., Ganguly, S., Saha, P., Sen, R., De, R. (2009). Antioxidant effect of ethanolic extract of *Piper betel* Linn. (Paan) on erythrocytes from patients with HbE-beta thalassemia. Indian J. of Biochemistry&Biophysics. 46:241-246.

Subhalakshmi, B., Ghosh, A., Hazra, B., (2005). Evaluation of the antibacterial activity of *Ventilago madraspatana* Gaerth, *Rubia cordifolia* Linn. And *Lantana*

camara Linn.: isolation of emodin and physcion as active antibacterial agents. Phytotherapy Research. 19, 888-894.

Subrahmanyam, C., Rao, B., V., Ward, R., Hursthouse, M.B., Hibbs, D. (1999). Diterpenes from the marine mangrove *Bruguiera gymnorhiza*. Phytochemistry.51:83-90.

Sun, X., Zhou, X., Zhu, L., Wei, B. (1999). Influence of different active componaents of *Rumex japonicus* Hott on agonist-induced contraction of thoracic Aorta. Nanjing Yike Daxue Xuebao. 19:488-490.

Sun, Y.-Q., Guo, Y.-W., (2004). Gymnorrhizol, an unusual macroclyclic polydisulfide from the Chinese mangrove *Bruguiera gymnorhiza*. Tetrahedron Letters. 45:5533-5535.

Suchitta, P. (1989). In culture and environment in Thailand, a symposium of the Siam society. The Society. Bangkok. Thailand. 558 pages.

Tabata, M., Hiraoka, N., Ikenoue, M., Sano, Y., konoshima, M. (1975). The production of antraquinones in callus cultures of *Cassia tora*. Lloydia. 38:131-4. [Chemical abstract]

Takei, T., Iijima, T., Higaki, M., Fukuzumi, T. (1994). The properties and chemical components of mangroves. V. Lignin carbohygrate complexes from *Bruguiera gymnorhiza* wood. Mokuzai Gakkaishi. 40:868-873.

Tang, D.W., Chang, K.W., Chi, C.W., Lin, T.Y. (2004). Hydroxychavicol modulates benzo[a]pyrene-induced genotoxicity through induction of dihydrodiol dehydrogenase. Toxicol lett. 152:235-43.

Tao, G., Irie, Y., Li, D.-J., Keung., M. (2005). Eugenol and its structure analogs inhibit monoamine oxidase A and exhibit antidepressant-like activity. Bioorganic&Medicinal Chemistry. 13:4777-4788.

Tewari, S.N., Premalatha, D.A. (1984). Indian Phytopathology (Indian Phytopathol). 37:458-461. [Chemical abstract]

Tewtrakul, S., Subhadhirasakul, S., Kummee, S. (2006). Anti-HIV-1 integrase activity of medicinal plants used as self medication by AIDS patients. Songklanakarin J. Sci. Technol. 28:785-790.

Thanh, L. (1996/1997). Combination of capillary GC, GC/MS and ^{13}C-NMR for the characterization of the rhizome oil of *Piper betle* L. (Piperaceae) from Vietnam, Spectroscopy. 13:131-136.

Tomlinson, P.B. (1995). The botany mangroves (Cambridge Tropical Biology Series). The Press Syndicate of the University of Cambridge. U.K. 419 pages.

Tripathi, V.R., Kumar, S., Garg, S., K. (2011). A study on trypsin, *Aspergillus flavus* and *Bacillus sp*. protease inhibitory activity in *Cassia tora* (L.) syn *Senna tora* (L.) Roxb. seed extract. MBC Complementary and Alternative Medicine. 11; 56.

Tsao, C.-C., Shen, Y.-C., Su, C.-R., Li, C.-Y., Liou, M.-J., Dung, N.-X., Wu, T.-S. (2008). New diterpenoids and the bioloactivity of *Erythrophleum fordii*. Bioorganic&Medicainal Chemistry. 16:9867-9870.

Vadhanasin, S., Singkhonrat, J., Pojjanapimol, S. (2007). Screening for natural antimicribials from Thai medicinal plants. Planta Med. DOI: 10.1055/S 2007-986988.

Vadlapudi, V. and Naidu, K., C. (2009). Bioactivity of marine plant *Avicennia alba* on selected plant and oral pathogens. International J. of Chem. Tech Research. 1:1213-1216.

Vaghasiya, Y., Nair, R., Chanda, S. (2007). Investigation of some Piper species for anti-bacterial and anti-inflammatory property. International J. of Pharmacology. 3:400-405.

Vali, L., Davies, S., Lai, L., Dave, J., Amyes, S.G.B. (2008). Frequency of biocide resistance genes, antibiotic resistance, and the effect of chlorhexidine exposure on clinical ,MRSA isolates. J. Antimicrob. Chemother. 61; 524-532.

Vang, L., Inomata, S.-I., Kinjo, M., Komai, F., Ando, T. (2005). Sex pheromones of five Olethreutine species Lepidoptera: Tortricidae associated with the seedlings and fruits of mangrove plants in the Ryukyu Islands, Japan: identification and field evaluation. Journal of Chemical Ecology. 31:859-878.

Varshney, S.C., Rizvi, S.A.I., Gupta, P.C. (1973). Structure of a polysaccharide from *Cassia tora* seeds.I. J. Agr. Food Chem. 21:222-226.

Wairt, C. (2002). Medicinal Plants of SouthEast Asia, second edition. Prentice Hall. Malaysia. 300 pages.

Wang, G., Li, X., Huang, F., Zhao, J., Ding, H., Cunningham, C., Coad, J., E., Flynn, D., C., Reed, E., Li, Q.Q. (2005). Antitumor effect of beta-elemene in non –small-cell lung carcinogent cells is mediated via induction of cell cycle arrest and apoptotic cell death. Cell Mol. Life Sci. 62:881-93.

Wang, J., Li, M., Zhang, Y.-H., Yang, S.-C. (2008). HPLC analysis of ABA and GA3 in reproductive organs of *Bruguiera gymnorhiza*. Ziran Kexueban. 47:752-756.

Wang, K., Li, Z., Chen, Y., Su, C. (2005). The Pharmacokinetics of an anti-tumor agent, B-elemene, in sprague-dawley rats. Biopharm. Drug Dispos. 36:301-307.

Wang, S.-K., Hsu, C.-H., Dai, C.-F., Duh, C.-Y. (2009). Novel sesquiterpenes and norergosterol from the soft Corals *Nephthea erectu* and *Naphthea chabroli*. Tetrahedron Letters. 50:802-806.

Watson, L. and Dallwitz, M.J. (2002). The families of flowering plants: description; illustrations, identification and information retrieval version 20th May 2010. http://deta-intkey.com. 20th July 2010.

Watt, J.M., Breyer-Brandwijk, M.G. (1962). The Medicinal and poisonous plants of Southern and Eastern Africa, E.&S. Livingston Ltd. Edinburgh and London.

Wenyuan, Y., Tianyong, W. (1997). Simultaneous determination of aloe emodin and emodin in *Cassia tora* L. by reversed-phase high-performance liquid chromatography. Fenxi Shiyanshi. 16:55-57. [Chemical abstract]

Widowati, W., Mozef, T., Risdian, C., Ratnawati, H., Tjahjani, S., Sandra, F. (2011). The comparison of antioxidative and proliferation inhibitor properties of *Piper betle* L., *Catharanthus roseus* (L.) G. Don, *Dendrophtoe petandra* L., *Curcuma magga* Val. extracts on T47D Cancer cell line. International Research Journal of Biochemistry and Bioinformatics. 1; 022-028.

Williams, D.H., Fleming, I. (1995). Spectroscopic methods in organic chemistry. The McGraw-Hill Higher Education. London. 304 pages.

Wit, H. C.D. (1963). Plants of the World: The higher plants I, A. Sijthoff. N. V. Duntton. Netherlands. 374 pages.

Woelfle, D., Schmutte, C., Westendorf, J., Marquardt, H (1990). Hydroxyanthraquinones as tumor promotes: enhancement of malignant

transformation of C3H mouse fibroblasts and growth stimilation of primary rat hepatocytes. Cancer Research. 50: 6540-6544.

Wong, R.H., Hu, C.W., Yeh, C.Y. (2007). Sulfotranaferase 1A1 and glutathione S-transferase P1 genetic polymorphisms modulate the leaves of urinary 8-hydroxy- 2'-deoxyguanosine in betel quid chewers. Arch. Toxicol. 3. [Chemical abstract]

Wong, S.M., Wong, M.M., Seligmann, O., Wagner, H. (1989). New antihepatotoxic naphthopyrone glycosides from the seeds of *Cassia tora*. Planta Med. 55:276-80. [Chemical abstract]

Xuhong, Y., Huaibiao, L., Hong, C., Ping, L., Boping, Y. (2008). Chemical constituents in the leaf of *Rhizophora styosa* L. and their biological activities. Yaoxue Xuebao. 43:974-978.

Yadava, R.N. and Satnami, D.K. (2008). New flavone glycoside from *Cassia tora* Linn. International J. of Chemical Sciences. 6:2130-2135.

Yadov, S. and Chattopadhyay, S. (2008). *Piper betel*-a gold mine for antioxidative and anti- ulcer drug. Proceeding of the Biennial Meeting of the Society for Free Radical Research International. 14th. Beijing.China. Oct. 18-22.

Yang, X., Yang, L., Wang, S., Yu, D., Ni, H., (2007). Synergistic interaction of physcion and chrysophanol on plant powdery mildew. Pest Management Science. 63:5111-515.

Yen, G.C., Chen, H.W., Duh, P.D. (1998). Extraction and identification of an antioxidative component from Jue Ming Zi (*Cassia tora* L.). J.Agric.Food.Chem. 46:820-824.

Yen, G.C., Duh, P.D., Chuang, D.-Y. (2000). Antioxidant activity of anthraquinones and anthrone. Food Chemistry. 70:437-441.

Yenjit, P., Issarakraisila, M., Intana, W., Chantrapromma, K. (2009). Fungicidal activity of compounds extracted from the pericarp of *Areca catechu* against colletotrichum gloeosporioides *in vitro* and in mango fruit. Posthavest Biology and Technology. 55:129-132.

Yuk, J.E., Woo, J.S., Yun, C.-Y., Lee, J.-S., Kim, J.-H., Song, G.-Y. (2007). Effectes of lactose-β-sitosterol and β-sitosterol on ovalbumin-induced lung inflammation in activity sensitized mice. International Immunopharmacology. 7:1517-1527.

Zeng, H.W., Jiang, Y.Y. (1997). Piperbetol, methylpiperbetol, piperrol A and piperol B: a new series of highly specific PAF receptor antagonists from *Piper betle*. Planta Med. 63:296-8.

Zhang, L.-L. and Lin, Y.-M., (2008). Preparation and antioxidant activity of cyaniding from condensed tannin extracted from calyx of *Bruguiera gymnorhiza*. Linchan Huaxue Yu Gongye. 28:1-5.

Zhang, L.-L. and Lin, Y.-M., (2009). Analysis of products of degradation and side reactions of proanthocyanidin by high performance liquid chromatography-diode array detection. Fenxi Huaxue 36:1281-1284.

Zhang, Y., Wei, D., Zhang, X., Guo, S., Chen, F. (2007). The extraction and GC-MS analysis of the volatile oil constituents from *Cassia tora* L. Huaxue Yu Shengwu Gongcheng. 24:70-74.

Zhang, Y.-F., Wei, D., Guo, S.-Y., Liang, S.-Z. (2009). Separation of active Components from *Cassia tora* L. using high-speed countercurrent chromatography. Ziran Kexueban. 37:129-134.

Zheng, Y.-Y., and Zhang, S.-H., Liu, R., Wang, M., (2008). Principal component analysis of environmental factors on the production of total flavonoids in mangrove plant, *Bruguiera gymnorhiza*. Zhongguo Tianran Yaowu. 6:362-366.

Zhu, Y.-Z., Tan, B., K.-H., Bay, B.-H., B., Liu, C.-H. (2007). Natural products: essential resources for human survival. World Scientific Publishing Co. Plc. Ltd. Singapore. 454 pages.

Zomlefer, W.B. (1994). Guide to flowering plant families. University of North Carolina Press. United States of America. 430 pages.