TOTAL POLYPHENOL CONTENT, ANTI-OXIDANT AND ANTI-PANCREATIC LIPASE ACTIVITIES OF *Aquilaria* sp. LEAVES

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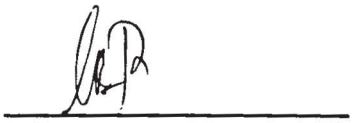

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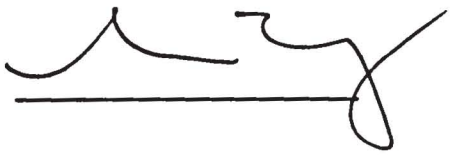

2. DEAN

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ABSTRACT

Obesity is considered as an overweight or excess body fat, which leads to health problem. Decreasing dietary fat absorption, through inhibition of pancreatic lipase activity has been reported to be one of the most effective ways for managing obesity. In this study, the effect of solvent (water and ethanol) on the percentage yields, High Performance Liquid Chromatography (HPLC) profiling, total polyphenol content (TPC), anti-oxidant activity and the anti-pancreatic lipase activity of gaharu leave samples were analyzed. The highest percentage yield was achieved by water extracts of teabag sample, which is 77.3 %. The extracts were then profiled using Reversed Phase-High Performance Liquid Chromatography (RP-HPLC). The chromatogram obtained showed the presence of a major compound at retention time 4.7 minutes, 5. 1 minutes, and 6.3 minutes. The TPC was determined using Folin-Ciocalteu method. The highest TPC was shown by the dried leaves of water and ethanol (1:1) mixture extract, 410.15 ± 7.32 mg GAE/g. Anti-oxidant activity was determined using DPPH (2,2-diphenyl-1-picryhydrazyl) assay, where the lowest IC_{50} was shown by the dried leaves of water and ethanol (1:1) mixture extract, 0.008 mg/ml. Most of the gaharu leaves samples showed higher anti-oxidant activity compared to the standard synthetic antioxidant 2,6-bis (1,1-dimethylethyl)-4-methylphenol (BHT). The percentage inhibition of gaharu leaves extract against the pancreatic lipase activity was determined using titration method, where the highest percentage inhibition of pancreatic lipase among the samples is 72.53 ± 0.06 %. The value is slightly lower than the standard drug, orlistat (77.73 % ± 0.00 %). The results of this study indicates the potential of the gaharu leaves to be used as the pancreatic lipase inhibitor to treat obesity.

JUMLAH KANDUNGAN POLIFENOL, AKTIVITI ANTI-OKSIDAN DAN AKTIVITI ANTI-LIPASE PANKREAS DALAM DAUN *Aquilaria* sp.

ABSTRAK

Obesiti dianggap sebagai masalah berat dan lemak badan yang berlebihan yang membawa kepada kemudaratan kesihatan. Mengurangkan penyerapan lemak, melalui perencatan aktiviti lipase pankreas telah dilaporkan sebagai salah satu cara yang paling berkesan untuk merawat obesiti. Dalam kajian ini, kesan pelarut (air dan etanol) di dalam hasil peratusan ekstrak, profil HPLC (kromatografi cecair prestasi tinggi fasa terbalik), jumlah kandungan polifenol (TPC), aktiviti anti-oksida dan aktiviti anti-lipase pankreas yang terdapat di dalam ekstrak daun gaharu telah dianalisis. Hasil peratusan tertinggi dicapai oleh ekstrak air sampel uncang daun gaharu sebanyak, 77.3%. Ekstrak kemudiannya dikaji menggunakan kromatografi cecair prestasi tinggi fasa terbalik (RP-HPLC). Kromatogram yang diperolehi menunjukkan kehadiran sebatian utama pada masa 4.7 minit, 5. 1 minit, dan 6.3 minit. Jumlah kandungan polifenol (TPC) telah ditentukan dengan menggunakan kaedah "Folin-Ciocalteu". TPC tertinggi telah ditunjukkan oleh daun kering gaharu daripada ekstrak campuran air dan etanol (1: 1), sebanyak 410. 15 ± 7.32 mg GAE / g. Aktiviti anti-oksida ditentukan dengan menggunakan kaedah DPPH , di mana kepekatan perencatan 50 (IC_{50}) terendah telah ditunjukkan oleh daun kering gaharu daripada ekstark campuran air dan etanol (1: 1) sebanyak, 0.008 mg / ml. Kebanyakan sampel daun gaharu menunjukkan aktiviti anti-oksida yang lebih tinggi berbanding dengan antioksidan sintetik rujukan, BHT. Perencatan peratusan gaharu daun ekstrak terhadap aktiviti lipase pankreas telah ditentukan dengan menggunakan kaedah titratan, di mana peratusan perencatan tertinggi lipase pankreas di antara sampel daun gaharu adalah 72.53% ± 0.06. Nilai adalah sedikit lebih rendah daripada bahan rujukan, orlistat (77.73% ± 0.00 %). Keputusan kajian ini menunjukkan potensi daun gaharu untuk digunakan sebagai perencat lipase pankreas untuk merawat obesiti.

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LIST OF SYMBOLS, ABBREVIATIONS AND EQUATIONS

%	percentage
µg/m	microgram per meter
µg/ml	microgram per milliliter
µm	micrometer
µmol	micromole
^{0}C	degree celcius
A.	*Aquilaria*
AchE	Acetylcholinesterase
AD	Alzheimer's disease
BMI	body mass index
CID	collision-induced dissociation
cm	centimeter
CMC-Na	carboxylmethylcellulose sodium
CPE	crude polyphenol extract
DCM	dichloromethane
DIO	diet-induced obese
DPPH	2,2'-diphenyl-1-picrylhydrazyl
ECG	andepicatechin gallate
EGCG	epigallocatechin gallate
EIS	electrochemical impedance spectroscopy
EMA	european Medicine Act
ESI	electrospray ionization
FDA	food and drug administration
FRAP	ferric reducing anti-oxidant power
g	gram
GCMS	gas liquid mass spectrometry
GES-1	gastric epithelial cell
HCl	hydrochloric acid
HPLC	high Performance liquid chromatography
HUVEC	human umbilical vein endothelial cells
I_3C	indole-3-carbinol

IC_{50}	half-inhibition concentration
IT-TOF	ion trap time-of-fligh
K	Kelvin
LC	Liquid chromatography
LPS	lipopolysaccharide
m	meter
M	molar
mg	milligram
mg/kg	milligram per kilograme
mg/mL	milligram per milliliter
MIC	minimum inhibitory concentration
mL	milliliter
mm	millimeter
mM	millimolar
MS	mass spectrometry
nm	nanometer
NO	nitric oxide
pNPP	para-nitrophenyl palmitate
PPL	Porcine Pancreatic Lipase
RAM	radial arm maze
RCT	randomised controlled trials
RM	Ringgit Malaysia
SD	standard deviation
SEM	scanning electron microscopy
Sp.	species
TAG	triacylglycerols
TF	total flavanol
TFC	total flavonoid content
TLC	thin layer chromatography
TPC	total phenolic content
UV	ultraviolet
v/v	volume per volume
WRL-68	human normal hepatic cell line
α	alpha

β	beta
GAE	gallic acid equivalents

LIST OF EQUATIONS

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND STUDY

Obesity is defined as a body mass index (BMI) of 30 or more. Overweight and obesity are diseases in which an overabundance of body fat has collected in the body which can have many health problems (Kopelman et al., 2003). Lipases (triacylglycerol hydrolase) are enzymes that catalyze the hydrolysis of ester bonds of triacylglycerols (fats and oils) to produce free fatty acids, diacylglycerols, monoglycerols and glycerol. In the small intestine of mammals, the digestion of dietary triacylglycerols (TAG) is basically due to the activity of pancreatic lipase. The end products after they have been absorbed by the body are responsible for the development of obesity. Therefore, if the hydrolysis of TAG, is stopped or minimized, the risk of obesity can be reduced. For this reason, an inhibitor of digestive lipases could become a useful anti-obesity agent. There are four types of anti-obesity synthetic drugs available on the market such as orlistat, sibutramine, phentermine and diethylpropion. Orlistat reduces the intestinal fat absorption through the inhibition of pancreatic lipase. The side effects of this drug are liquid oily stools, fecal urgency, flatulence, less frequently abdominal and rectal pain, headache, menstrual irregularities, anxiety, fatigue, and rarely hepatitis (Kiortsis *et al.*, 2005). While, sibutramine suppresses the appetite (Yun, 2010) by the inhibition of the re-uptake of the noradrenaline and serotonin. Sibutramine is not licensed for use longer than one year. It also have side effects such as constipation, anorexia, dry mouth, insomnia, nausea, tachycardia, palpitations, hypertension, vasodilation, light-headedness, paresthesia, headache, anxiety, sweating, taste disturbance and blurred vision (Leung *et al.*, 2003) Phenthermine and diethylpropion drugs are an anorectic agents, however the licenses for marketing these drugs have been withdrawn by the European Court on 2002 due to the absence of information about their longer-term

efficacy and safety. Since these synthetic drugs possess bad side effects, there is a need to develop a natural product for the treatment of obesity because medicinal plants provide safe, natural and cost effective alternatives than synthetic drugs (Garza *et al.*, 2011).

There are several plants that have been studied related to their anti-obesity activity. The research carried out by Changhyun *et al.* (2012) shows that methyl gallate a bioactive compound from HemoHIM potently inhibits lipid formation. HemoHIM is composed of the herbal mixtures from *Angelica radix, Cnidium rhizoma*, and *Cnidium radix*. Other anti-obesity plants are tea saponin, *Salacia reticulata*, grape seed extract, soy proteins, *Morinda citrifolia* fruit (Sahib et al., 2012), *Panax japonicus*, *Platycodi radix*, *Salacia reticulata*, *Nelumbo nucifera* and so on. Carbohydrates such as chitin/ chitosan also showed pancreatic lipase inhibitory effect (Yun, 2010). Oolong tea (Nakai *et al.*, 2003) and leaves of *Acanthopanax sessiliflorus* (Yoshizumi *et al.*, 2006) also have inhibitory effects on pancreatic lipase activity. There also studies on the antidiabetic activity of Malaysian agarwood/ gaharu leaves extract (Nur Liyana Zulkifle *et al.*, 2013). A plant that has antidiabetic activity also usually shows an anti-obesity activity (Lim *et al.*, 2009).

Agarwood or gaharu tree belongs to Thymeleaceae family from *Aquilaria* sp. Gaharu which is in high demands due to its resin commercial value. The gaharu market price is between RM 10,000 to RM 38,000 per kilogram. The known chemical componds in gaharu leaves includes iriflophenone-2-O-a-L-rhamnopyranosyl-(1-4)-O-a-L-rhamnopyranoside, iriflophenone 2-O-b-D fucopyranosyl-(1-4)-O-a-L-rhamnopyranoside, iriflophenone 2-O-b-D quinovopyranosyl-(1-4)-O-a-L-rhamnopyranoside, iriflophenone 2-O-b-D xylopyranosyl-(1-4)-O-a-L-rhamnopyranoside, iriflophe-none 2-O-a-L-(4"-acetyl) rhamnopyranoside (Lim *et al.*, 2009), viz-aquilarisinin, aquilarisin, hypolaetin 5-O-b-D glucuronopyranoside, aquilarixanthone, mangiferin, iriflophenone 3-C-b-D-glucoside and iriflophenone 3,5-C-b-D-diglucopyranoside (Feng *et al.*, 2011). Gaharu also has important medicinal value. The aqueous extract of *Aquilaria crassna* leaves possesses an *in vitro* antibacterial activity against *Staphylococcus epidermidis*, with no sign of acute oral toxicity in mice, by interfering with bacterial cell wall synthesis and inhibiting biofilm formation (Kamonwannasit *et al*, 2013). Besides that, *Aquilaria sinensis* leaves have been confirmed to exert potent analgesic and anti-

inflammatory actions (Zhou *et al.*, 2008). Gaharu also possesses a panoply of effects such as aphrodisiac, sedative, cardiatonic, carminative, and to relieve gastric problems, coughs, asthma, high fever (Naef, 2011). Burkill (1966) reported that Malaysians used gaharu mixed with coconut oil to treat rheumatism. Gaharu is also used as complex ointment for smallpox, and as a tonic taken particularly during pregnancy, after childbirth and diseases of female genital organs (Chakrabarty, 1994). However, there is still lack of scientific information on the anti-obesity activity of the gaharu while the gaharu leaves anti-pancreatic lipase activity has never been studied before.

1.2 PROBLEM STATEMENT

The synthetic drugs to inhibit the activity of the pancreatic lipase such as the orlistat have several side effects. There is a significant need to search for an anti-pancreatic lipase synthetic drug substitutes from natural resources. The gaharu leaves have never been studied on its ability to inhibit the pancreatic lipase activity which can be used as an anti-obesity treatment.

1.3 OBJECTIVES OF THE STUDY

The objectives of this study are:

a) To prepare the extracts of gaharu fresh leaves, gaharu dried leaves and gaharu tea using different types of solvent.
b) To profile the gaharu leaves extract.
c) To evaluate the Total Polyphenol Content (TPC) & the anti-oxidant activity of the gaharu leaves extracs.
d) To evaluate the anti-pancreatic lipase activity of the gaharu leaves extracts.

1.4 SCOPE OF THE STUDY

The parameter used in this study is the different type of solvents which are the hot distilled water solvent and the ethanol solvent. The part of the plant being extracted was the leaves. The High Performance Liquid Chromatography (HPLC) instrument is used to profile the chemical compounds of the gaharu leaves extract through the analysis of the peak and the retention time (Ito *et al.*, 2012). The assay of the pancreatic lipase activity was carried out by titration method (Muhammad Abu Bakar Ado *et al.*, 2013). The Total Polyphenol Content was determined by Folin- Ciocalteau reagent method according to Sharma *et al.* (2015), with slight modification. The anti-oxidant activity was determined using DPPH radical scavenging activity according to Tay *et al.* (2014).

CHAPTER 2

LITERATURE REVIEW

2.1 Gaharu

Gaharu is classified as a non-wood forest product from the species in the *Aquilaria* genus (Lata, 2007). Gaharu produces resin within its heartwood which has high commercial value and medicinal value. There are many names given to this resinous wood, including agar, agarwood, kalamabak, eaglewood, aloe wood and agalocha. The term 'gaharu' is commonly used in Malaysia. There are 25 species of *Aquilaria* genera that are thought to produce resin. The species are *A. beccariana*, *A. hirta*, *A. microcarpa*, *A. cumingiana*, *A. audata*, *A. brachyantha*, *A. urdanetensis*, *A. citrianaecarpa*, *A. apiculata*, *A. parvifolia*, *A. rostrata*, *A. crassna*, *A. rigosa*, *A. banaense*, *A. khasiana*, *A. subintegra*, A. acuminata, *A. yunnanensis*, and *A. malaccensis*. The scientific classification of gaharu is at table 2.1.

Table 2.1 Scientific classification of gaharu (Source: Adelina *et al.*, 2004)

Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliosida
Order	Myrtales
Family	Thymelaeaceae
Genus	Aquilaria

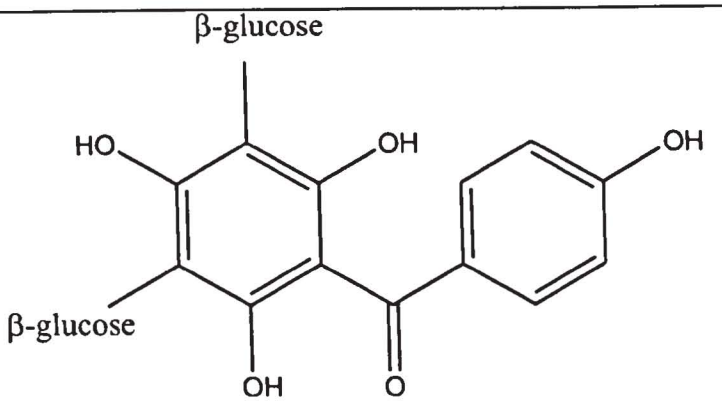
2.1.1 Morphology

Gaharu is a medium-sized tree on average 15–25 meter tall, but some of the species such as *A. malaccensis* can reach heights of 40 meters (Hou, 1960). It is a relatively slow-growing tree. Their stem is moderately straight and it can achieve a diameter around 250 cm, although some species remain considerably smaller and more shrub like, such as *A. khasiana.* Most gaharu species have smooth, thin, pale gray bark with dense, dark foliage of the shiny elliptical to oblong leaves about 7.5–12 cm long and 2.5–5.5 cm wide (Irnayuli *et al.*, 2011). Gaharu regenerates under natural conditions as seedlings around the mother tree or sprouts from the stumps of harvested trees. The occurrence of the tree itself does not guarantee the presence of the resin. Only 10% of the gaharu trees in the forest may contain resin (Frankie, 1994). The resin is formed in response to wounding or fungal infection (Sepiah, 2011), and is found in many parts of the gaharu tree. The resin is commonly found in trees about 20 years or older under natural condition (Chakrabarty *et al.*, 1994), whereas the tree ages around 50 years old shows the highest concentration of resin (Soehartono & Mardiastuti, 1997).

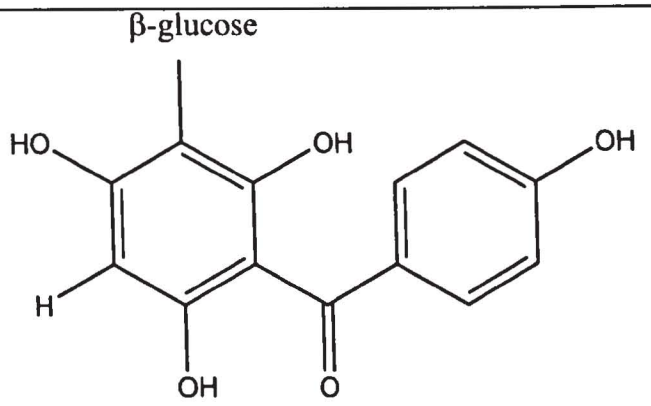
2.1.2 Chemical content in gaharu

Ito *et al.* (2012), extracted *Aquilaria crassna* leaves with aqueous ethanol and water and analyzed the extracts via liquid chromatography diode array detection and electrospray ionization mass spectrometry (LC-ESI-MS) methods. The phenolic compounds are separated using semi-micro HPLC and are identified as iriflophenone 3,5-*C*-β-diglucoside (1), iriflophenone 3-*C*-β-glucoside (2), mangiferin (3), iriflophenone 2-*O*-α-rhamnoside (4), genkwanin 5-*O*-β-primeveroside (5), genkwanin 5-*O*-β-glucoside (6), genkwanin 4'methyl ether 5-*O*-β-primeveroside (7), and genkwanin (8) via a comparison with authentic samples as shown in Table 2.2. The hybrid ion trap time-of-flight (IT-TOF) mass spectrometry is used to detect the collision-induced dissociation (CID) -MS/MS spectra of the polyphenols compounds and the unknown chromatographic peaks.

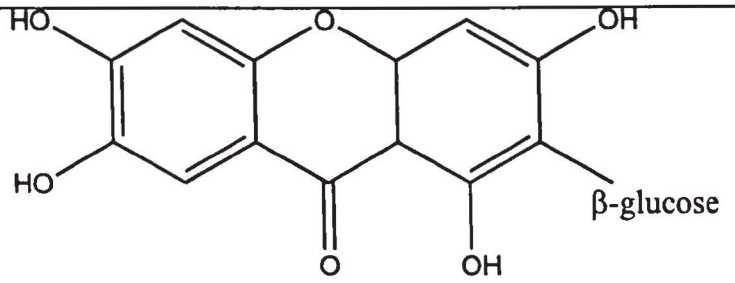
Table 2.2 Chemical compounds in *Aquilaria crassna* leaves (Ito *et al.*, 2012)



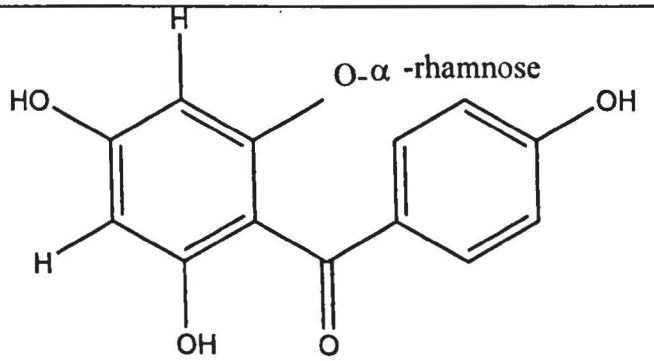
(1) iriflophenone 3,5-C-β-diglucoside



(2) iriflophenone 3,5-C-β-glucoside

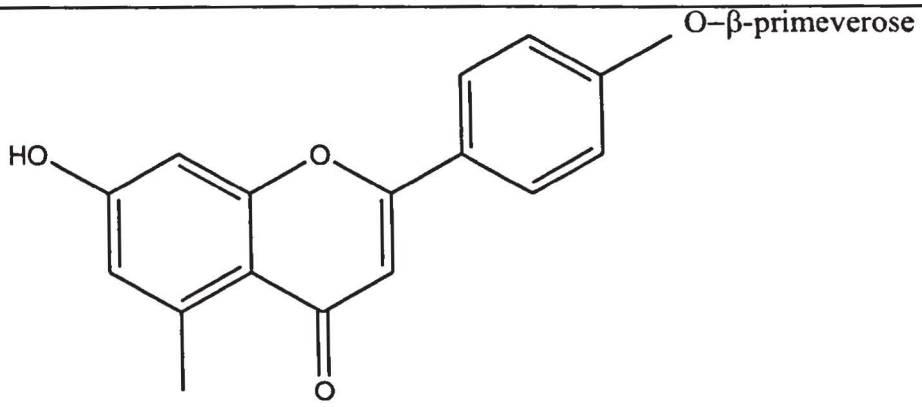


(3) mangiferin

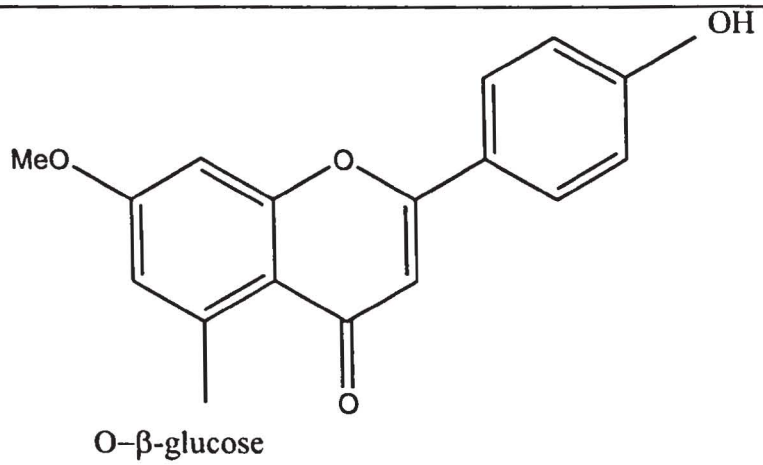


(4) iriflophenone 2,O-α-rhamnoside

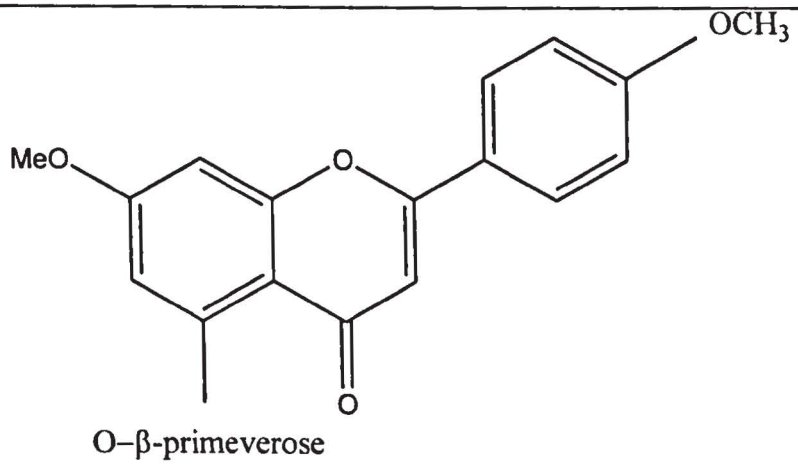
Table 2.2 (continue)



(5) genkwanin 5,O-β-primeveroside

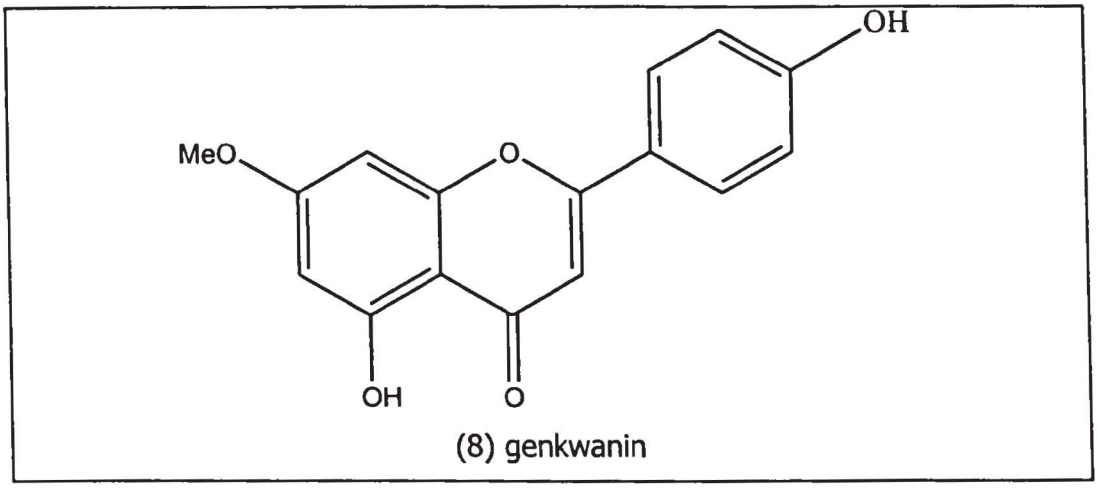


(6) genkwanin 5-O-β-glucoside



(7) genkwanin 4′-methyl ether-5-O-β-glucoside

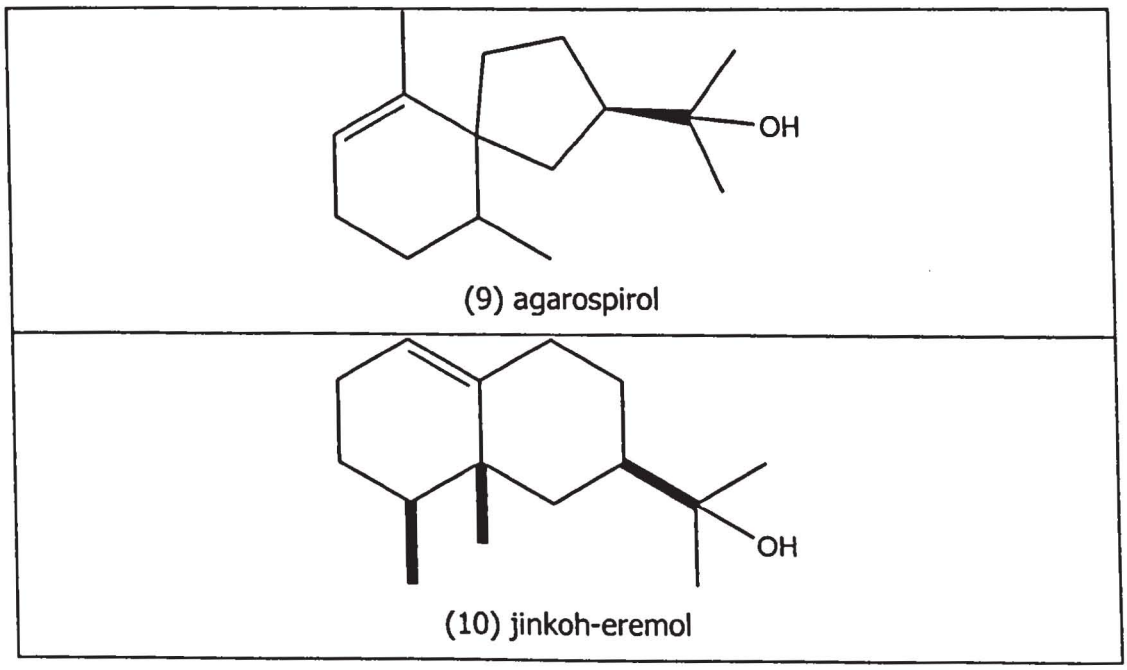
Table 2.2 (continue)



(8) genkwanin

Meier *et al.* (2003), also has analyzed the essential oil of *Aquilaria agallocha* from Assam, India using GC/MS. The main constituents are found to be agarospirol (12.1%) and jinkoheremol (10.0%) as shown in Table 2.3. Anisyl acetone is isolated as a minor constituent and fully characterized from a commercial sample of agarwood oil (Meier *et al.*, 2003).

Table 2.3 Chemical compounds in the essential oil of gaharu (Meir *et al.*, 2003).



(9) agarospirol

(10) jinkoh-eremol

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