Bioactive substances from Tea (*Camellia sinensis*)

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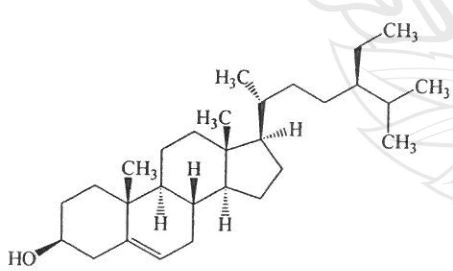
PREFACE

Plants have been used worldwide in traditional medicines for the treatment of diseases. It is estimated that even today approximately two-thirds to three-quarters of the world's population rely only on medicinal plants as their primary source of medicines. The genus Camellia with some 100 species was found mainly in Eastern and Southeastern Asia. As early as three thousand years ago, Chinese people started to use tea, initially as remedy for detoxification. Starting from West Han period (206 BC to 23 AD) tea has become a daily drink. Today tea is accepted as one of the three major beverages worldwide, and is widely cultivated in the warm regions. Tea from *Camellia sinensis* and *Camellia assamica* is a popular beverage due to the presence of bioactive substances such as alkaloids and catechins. Green tea and its constituents catechins are best known for their antioxidant properties, which has led to their evaluation in a number of diseases associated with reactive oxygen species, such as cancer, cardiovascular and neurodegenerative diseases. Moreover, the extracts of green tea have been reported in the literature as an antioxidant in animal and vegetable oils. In addition, the antitumor activity of *di-* and *tri-*terpenes has been reported. The polyphenolic compounds present in green tea show cancer chemopreventive effect both *in vivo* and *in vitro*. Therefore, the study of phytochemistry and biological activities are very important because the information from the study of bioactive compounds will be used for development and apply into related fields, for example cosmetics, agricultures and pharmacy. Finally, I hope that the information from this research might be helpful for other researcher who needs to use the information.

ABSTRACT

Study on the chemical constituents of the dried leaves of *Camellia sinensis* var. *assamica* resulted in isolation of a new compound: 13-methyl lansic acid (**2**) and eleven known compounds: *β*-sitosterol (**1**), lansionic acid (**3**), stigmasterol (**4**), (+)catechin (**5**), 21*R*-hydroxyonocera-8(2b),14-dien-3-one (**6**), lupeol (**7**), lupenone (**8**), (-)epigallocatechin gallate (**9**), (-)epicatechin gallate (**10**) and (-)epicatechin (**11**). Their structures were elucidated on the basis of UV, IR and NMR spectroscopic data.

The compounds with sufficient quantity were evaluated for their antioxidation and antibacterial activities. Compounds **5**, **9**, **10** and **11** exhibited stronger antioxidant activity (IC_{50} 0.60, 0.23, 0.27 and 0.07 mM, respectively) than that of ascorbic acid (IC_{50} 1.75 mM) and BHT (IC_{50} 3.03 mM). These four compounds also showed the moderate activities to inhibit the growth of *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Pseudomonas florescens* and *Salmonella typhimurium* with MIC 16-128 μg/mL compared to those of gentamycin and vancomycin (0.5 μg/mL).



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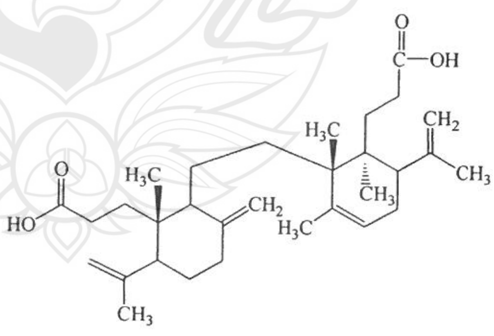

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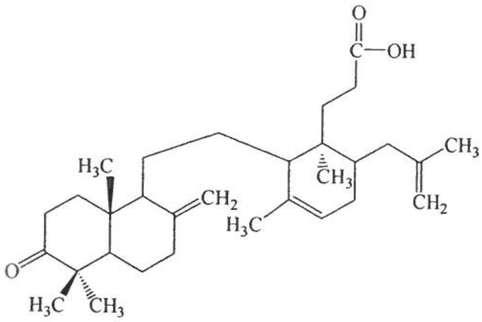

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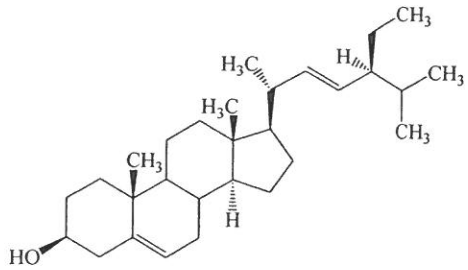

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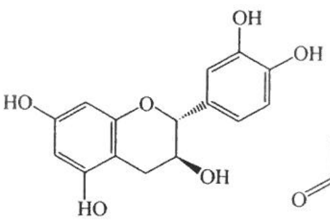

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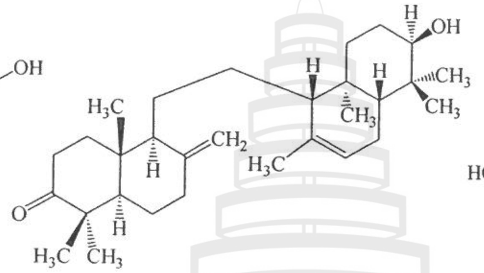

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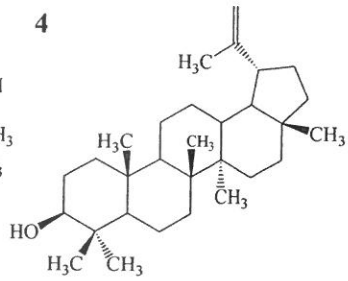

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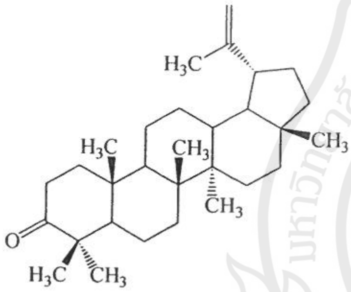

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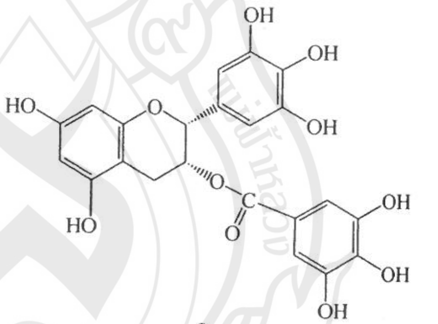

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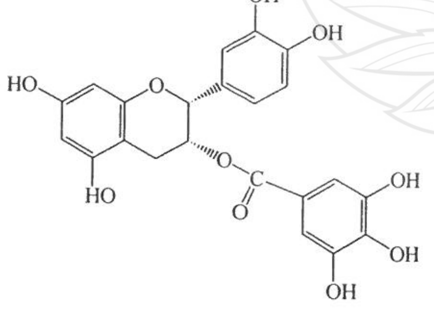

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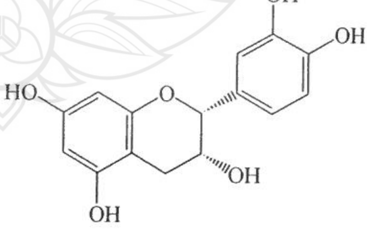

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Suwanna Deachathai

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INTRODUCTION

1.1 Statement and significance of the problem

Synthesis of many important drugs makes use of natural product starting materials. Researches are conducted in order to find major constituents with biological activity to be used as drugs or in synthesis of analog or derivatives. Pure compounds extracted from many plants and many parts of the plants are explored and tested for biological activities. However, elucidation of chemical constituents from natural products and biological activity testing are only the initial step in the process of study to find new compounds and acquire basic knowledge of biological activities. The important process is the application of the knowledge in pharmacology and medicine.

1.2 Objectives

Up to the present, reports revealed the isolation of the chemical constituents, antimicrobial and antioxidation activities of green tea leaves cultivated and processed in Chiang Rai, Thailand, have not been more investigated, it was therefore of interest to investigate the chemical constituents and evaluate the antimicrobial and antioxidation activities of isolated compounds.

1.3 Scope of study

This research focused on isolation, purification, structural determination of the chemical constituents from the leaves of *Camellia sinensis* var. *assamica*, as well as evaluation of antimicrobial and antioxidation activities of the crude extracts and pure compounds.

1.4 Benefit

Camellia sinensis var. *assamica* which is one of the medicinal plants, was investigated for the chemical constituents and biological activities. Therefore, some active compounds might be applied into the cosmetic, pharmacy or agriculture. This work might be published in international journals.

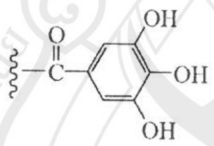
1.5 Abbreviations and Symbols

s	=	*singlet*
d	=	*doublet*
t	=	*triplet*
q	=	*quartet*
m	=	*multiplet*
dd	=	*doublet of doublet*
qd	=	*quartet of doublet*
ddd	=	*doublet of doublet of doublet*
br s	=	*broad singlet*
g	=	gram
kg	=	kilogram
mg	=	milligram
µg	=	microgram
mM	=	millimolar
mL	=	milliliter
h	=	hour
min	=	minute
%	=	percent
nm	=	nanometer
cm^3	=	cubic centimeter

m.p.	=	melting point
cm^{-1}	=	reciprocal centimeter (wave number)
δ	=	chemical shift relative to TMS
J	=	coupling constant
$[\alpha]_D$	=	specific rotation
λ_{max}	=	maximum wavelength
ν	=	absorption frequencies
ε	=	molar extinction coefficient
m/z	=	a value of mass divided by charge
°C	=	degree celsius
Hz	=	hertz
MHz	=	megahertz
ppm	=	part per million
c	=	concentration
rpm	=	revolutions per minute
EIMS	=	Electron Impact Mass Spectrometry
IR	=	Infrared
UV	=	Ultraviolet-Visible
NMR	=	Nuclear Magnetic Resonance
2D NMR	=	Two Dimentional Nuclear Magnetic Resonance
COSY	=	Correlated Spectroscopy
DEPT	=	Distortionless Enhancement by Polarization Transfer
HMBC	=	Heteronuclear Multiple Bond Correlation
HMQC	=	Heteronuclear Multiple Quantum Coherence
NOE	=	Nuclear Overhauser Effect Spectroscopy
CC	=	Column Chromatography
QCC	=	Quick Column Chromatography

TLC	=	Thin Layer Chromatography
PLC	=	Preparative Thin Layer Chromatography
CH_2Cl_2	=	dichloromethane
Me_2CO	=	acetone
MeOH	=	methanol
TMS	=	tetramethylsilane
DMSO	=	dimethyl sulfoxide
$CDCl_3$	=	deuterochloroform
Acetone-d_6	=	hexadeuteroacetone
MICs	=	Minimum inhibition concentration
IC_{50}	=	50% Inhibition concentration
DPPH	=	1,1-diphenyl-2-picrylhydrazyl radical
BHT	=	butylated hydroxy toluene

Gallate moiety=



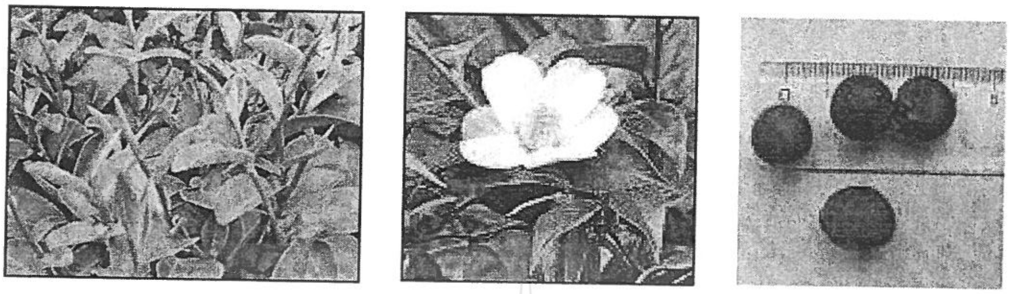
CHAPTER 2

LITERATURE REVIEWS

The genus Camellia with some 100 species was found mainly in Eastern and Southeastern Asia. As early as three thousand years ago, Chinese people started to use tea, initially as remedy for detoxification. Starting from West Han period (206 BC to 23 AD) tea has become a daily drink. Today tea is accepted as one of the three major beverages worldwide, and is widely cultivated in the warm regions (Xiao *et al.*, 2002). Tea from *Camellia sinensis* and *Camellia assamica* is a popular beverage due to the presence of bioactive substances such as alkaloids and catechins (Fernandez, *et al.*, 2003; Robb and Brown, 2001; Wang *et al.*, 2003).

2.1 General characteristics of *Camellia sinensis* var. *assamica*

Camellia sinensis var. *assamica* is widely distributed in northern, central and northeastern parts of Thailand. It has been known as "Assam" and belong to the family Theaceae. *C. sinensis* is a straggly shrub to 5 m, rarely becoming a small tree to 15 m. Bark is dark brown, smooth, thin, inner bark white. Leaves are dark green, 6-20 cm long and 2-6 cm broad, smooth and shiny, elliptic with a slightly pointed or tapering tip, blunt base and fine sharp teeth. Stalks are 0.5 cm, twigs dark brown, smooth and shiny. Flower axillary clusters of 1-3 large white or pale yellow flowers, 3.5-4.5 cm across with 5-6 spreading, rounded petals, many bright yellow stamens and a single 2-3 tiped style. Stalks woody, about 1 cm long. Fruits are 1.1-1.5 cm, rounded, splitting into 2-3 sections with many wingless seeds.



A B C

Figure 1 *Camellia sinensis* var. *assamica*:

(**A**) leaves, (**B**) flower and (**C**) seeds

2.2 Chemical constituents from the leaves of *Camellia sinensis* var. *assamica*

According to the information from NAPRALERT database developed by University of Illinois at Chicago and Chemical Abstracts, chemical constituents isolated from the leaves of *Camellia sinensis* var. *assamica* are presented in **Table 1**.

Table 1 Compounds isolated from the leaves of *Camellia sinensis* var. *assamica*

Compound	Structure	Reference
2,4-dimethylacetophenone	1	Vitzthum *et al*., 1975
3,4-dimethoxyacetophenone	2	
p-ethylacetophenone	3	
1,3-diacetylbenzene	4	
1,4-diacetylbenzene	5	
N-ethylaniline	6	
N-methylaniline	7	
benzothiazole	8	
2-methylbenzothiazole	9	

Table 1 (continued)

Compound	Structure	Reference
benzoxazole	10	Vitzthum *et al.*, 1975
N,N-dimethylbenzylamine	11	
N-ethyl propionamide	12	
ligustrazine	13	
2,3-dimethyl pyrazine	14	
2,5-dimethyl pyrazine	15	
2,6-dimethyl pyrazine	16	
2-ethyl-3,5-dimethyl pyrazine	17	
2-ethyl-3,6-dimethyl pyrazine	18	
2-ethyl-5-methyl pyrazine	19	
2-ethyl-6-methyl pyrazine	20	
ethyl pyrazine	21	
methyl pyrazine	22	
trimethyl pyrazine	23	
2,4-dimethyl propiophenone	24	
p-ethyl propiophenone	25	
2,5-dimethyl pyridine	26	
2,6-dimethyl pyridine	27	
2-acetyl pyridine	28	
2-ethyl pyridine	29	
2-methyl pyridine	30	
2-methyl-5-ethyl pyridine	31	
2-methyl-6-ethyl pyridine	32	
2-phenyl pyridine	33	
3-ethyl pyridine	34	
3-methoxy pyridine	35	
3-methyl pyridine	36	

Table 1 (continued)

Compound	Structure	Reference
3-*N*-butyl pyridine	37	Vitzthum *et al.*, 1975
3-phenyl pyridine	38	
4-methyl pyridine	39	
3-vinyl pyridine	40	
2,4-dimethyl quinoline	41	
2,6-dimethyl quinoline	42	
2-methyl quinoline	43	
3-*N*-propyl quinoline	44	
4,8-dimethyl quinoline	45	
4-*N*-butyl quinoline	46	
6-methyl quinoline	47	
2,4,5-trimethyl thiazole	48	
2,4-dimethyl thiazole	49	
2,5-dimethyl thiazole	50	
2,5-dimethyl-4-ethyl thiazole	51	
5-methyl thiazole	52	
o-toluidine	53	
dihydro actinidiolide	54	Etoh and Iguchi, 1980
1',2'-epoxy 1',2'-dihydro-β-ionone	55	
1',2'-*threo*-1',2'-dihydroxy-β-ionone	56	
loliolide	57	
3(*S*)-7-dimethylocta-1,5,7-trien-3-ol	58	
(+)-3(*S*)-7-dimethylocta-1,5-diene-3,7-diol	59	
heaspirone	-	
dehydro vomifoliol	-	
(-)-epi-afzelechin	60	Davis *et al.*, 1996; Hashimoto *et al.*, 1989(a)

Table 1 (continued)

Compound	Structure	Reference
3-*O*-gallate-epi-afzelechin	61	Hashimoto *et al.*, 1989(a)
assamicain A-C	62-64	
3,3'-di-*O*-gallate-epi-gallocatechin	65	
3,4'-di-*O*-gallate-epi-gallocatechin	66	
3,5-di-*O*-gallate-epi-gallocatechin	67	
3-*O*-caffeoate-epi-gallocatechin	68	
3-*O*-gallate-epi-gallocatechin	69	
3,5-di-*O*-gallate-epi-catechin	71	
(-)-3-*O*-gallate-epi-afzelechin	61	Hashimoto *et al.*, 1987; 1992
catechin-(4-α-8)-3-gallate-epi-gallocatechin	74	Hashimoto *et al.*, 1989(b)
epi-catechin-(4-β-8)-3-*O*-gallate-epi-gallocatechin	79	
3-*O*-gallate-epi-catechin-(4-β-8)-3-*O*-gallate-epi-gallocatechin	80	
3-*O*-gallate-epi-catechin-(4-β-6)-3-*O*-gallate-epi-gallocatechin	86	
3,3'-di-*O*-gallate-procyanidin B-5	87	
β-glucogallin	88	
3,3'-di-*O*-gallate-prodelphinidin B-2	89	
3'-*O*-gallate-prodelphinidin B-2	90	
3'-*O*-gallate-prodelphinidin A-2	-	
3,3'-di-*O*-gallate-prodelphinidin B-5	-	
oolonghomobisflavan A	-	
oolonghomobisflavan B	-	
apigenin	91	Cheng *et al.*, 1987
camellianin A	-	
camellianin B	-	

Table 1 (continued)

Compound	Structure	Reference
6,8-di-*C*-β-D-arabinopyranosyl-apigenin	92	Chaboud *et al.*, 1986
2"-*O*-glucoside iso vitexin	-	
arbutin	93	Deisinger *et al.*, 1996
astragalin	94	Ozawa, 1982; Price *et al.*, 1998
avicularin	95	Price *et al.*, 1998
3-*O*-glucosyl-rhamnosyl-galactoside-kaemferol	96	
3-*O*-galactosyl-rhamnosyl-glucoside-kaempferol	97	
3-*O*-glucosyl-rhamnoside-kaempferol	98	
quercitrin	99	
trifolin	100	
1,2,3-trimethoxybenzene	101	Gong *et al.*, 1993
1,2,3-trimethoxy-5-ethylbenzene	102	
1,2,3-trimethoxy-5-methylbenzene	103	
1,2-dimethoxybenzene	104	
1,2-dimethoxy-4-ethylbenzene	105	
1,2-dimethoxy-4-methylbenzene	106	
2-methylbenzaldehyde	107	Renold *et al.*, 1974
4-methoxybenzaldehyde	108	
benzylbutyrate	109	
benzyl ethyl ketone	110	
carvacrol	111	
β-cyclocitral	112	
2,6,6-trimethyl cyclohex-2-en-1,4-dione	113	
2,6,6-trimethyl-cyclohex-2-en-1-one	114	
β-damascenone	115	
α-damascone	116	
β-damascone	117	

Table 1 (continued)

Compound	Structure	Reference
deca-*trans*-2-*cis*-4-dien-1-al	118	Renold *et al.*, 1974
deca-*trans*-2-en-1-al	119	
4-ethyl-7,11-dimethyl-dodeca-*trans*-2-*trans*-6,10-trien-1-al	120	
2-acetyl furan	121	
trans-geranic acid	122	
heptan-2-one	123	
5-isopropyl-heptan-2-one	124	
hepta-*trans*-2-en-1-al	125	
5-methyl-2-phenyl-hex-2-en-1-al	126	
trans-2-*cis*-4-hexadien-1-al	127	
hex-*cis*-3-en-1-ol formate	128	
cis-3-hexen-1-ol-*trans*-2-hexenoate	129	
hex-*cis*-3-hexen-1-ol propionate	130	
hex-*trans*-2-enyl acetate	131	
hex-*trans*-2-enyl butyrate	132	
hex-*trans*-2-enyl formate	133	
hex-*trans*-2-enyl hexanoate	134	
hex-*trans*-2-enyl propionate	135	
hex-*trans*-3-enyl butyrate	136	
hex-*trans*-3-enyl hex-*cis*-3-enoate	137	
hex-*trans*-3-enyl propionate	138	
hex-*trans*-3-enyl-2-methyl butyrate	139	
hexyl butyrate	140	
hexyl formate	141	

Table 1 (continued)

Compound	Structure	Reference
nonan-2-one	142	Renold *et al*., 1974
nona-*trans*-2-*cis*-4-dien-1-al	143	
nona-*trans*-2-*cis*-6-dien-1-al	144	
nona-*trans*-2-en-1-al	145	
nona-*trans*-2-*trans*-4-dien-1-al	146	
octan-2-one	147	
ethyl octanoate	148	
methyl octanoate	149	
octa-*trans*-2-enoic acid	150	
octa-*trans*-2-*cis*-4-dien-1-al	151	
octa-*trans*-2-*trans*-4-dien-1-al	152	
octa-*trans*-3-*cis*-5-dien-2-one	153	
4-methyl-2-phenyl pent-2-en-1-al	154	
pent-*cis*-3-en-1-al	155	
ethyl phenyl acetate	156	
hexyl phenyl acetate	157	
phenyl acetic acid	158	
safranal	159	
4-terpineol	160	
theaspirane	161	
thymol	162	
6,10-dimethyl undecan-2-one	163	
undeca-*trans*-2-en-1-al	164	
neral	165	
safrolephenylpropanoid	-	
6,7-epoxy dihydro theaspirane	-	
6-hydroxy dihydro theaspirane	-	

Table 1 (continued)

Compound	Structure	Reference
3-*O*-β-D-galactopyranosyl(1→2)-β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→3)-β-D-glucuronopyranosyl-21-*O*-cinnamoyl-16,22-di-*O*-acetyl barringtogenol C	-	Sagesaka *et al.*, 1994
1,2,4-trihydroxybenzene	166	Kaiser, 1967
1,2,5-trihydroxybenzene	167	
1,3,4-trihydroxybenzene	168	
1,3,5-trihydroxybenzene	169	
m-cresol	170	
o-cresol	171	
p-cresol	172	
brassinolide	173	Ikekawa *et al.*, 1984
24-ethylbrassinone	174	
28-norbrassinolide	175	Takatsuto *et al.*, 1982
6-keto-28-homo-brassinolide	176	
6-ketobrassinolide	177	
6-keto-28-norbrassinolide	178	
brassinone	179	Abe *et al.*, 1983; Ikekawa *et al.*, 1984
24(*S*)-ethylbrassinone	180	Abe *et al.*, 1983
butan-2-ol	181	Stalcup *et al.*, 1993
ethyl-3-hydroxy butyrate	182	
butyroin	183	
ethyl lactate	184	
2-methyl-tetrahydro-furan-3-one	185	
α-ionone	186	

Table 1 (continued)

Compound	Structure	Reference
limonene	187	Stalcup *et al.*, 1993
menthol	188	
α-pinene	189	
caffeine	190	Anon, 1976; Apostolides *et al.*, 1996; Ashihara *et al.*, 1997; Ashihara and Kubota, 1987; Bellakhdar *et al.*, 1993; Blauch and Tarka, 1983; Chen and Li, 1984; Dalluge *et al.*, 1998; Dulitzky *et al.*, 1984; Fujimori *et al.*, 1991; Fujimori and Ashihara, 1994; Gong *et al.*, 1993; Goto *et al.*, 1996; Hashimoto *et al.*, 1992; Higuchi *et al.*, 1995; Horie and Kohata, 1998; Ikegaya, 1985; Jeeraphan, 1951; Kato *et al.*, 1996; Lin *et al.*, 1998; Ma and Zhang, 1982; Maruyama *et al.*, 1990; Matsuda *et al.*, 1986; Matsura *et al.*, 1991; Min and Peigen, 1991; Miyagawa *et al.*, 1997; Nagata and Sakai, 1985; Ozawa, 1982; Poncini, 1979; Sakata *et al.*, 1991; Segawa *et al.*, 1988; Shervington *et al.*, 1998; Spiro *et al.*, 1992; Spiro, 1997; Suzuki and Takahashi, 1975; Suzuki and Takahashi, 1976(a); 1976(b); 1976(c); Tanizawa *et al.*, 1984; Tsunoda *et al.*, 1996; Valcic *et al.*, 1996; Yen and Chen, 1996; Yokozawa *et al.*, 1995; Zhang *et al.*, 1991; 1995; Zhu *et al.*, 1992
castasterone	191	Abe *et al.*, 1983; Ikekawa *et al.*, 1984
(+)-catechin	192	Ahn *et al.*, 1991; Anon, 1984; Cho *et al.*, 1993; Dalluge *et al.*, 1998; Davis *et al.*, 1996; Goto *et al.*, 1996; Hagiwara *et al.*, 1991; Hashimoto *et al.*, 1987; 1992; Koretskaya and Zaprometov, 1975; Lin *et al.*, 1996; 1998; Min and Peigen, 1991; Miyagawa *et al.*, 1997; Nakazato *et al.*, 1996; Namiki, 1990; Nonaka *et al.*, 1983

Table 1 (continued)

Compound	Structure	Reference
(+)-catechin	192	Otake *et al.*, 1991; Ozawa, 1982; Pearson *et al.*, 1998; Saijo, 1982; Sakanaka *et al.*, 1989(c); Sanq *et al.*, 1999; Tanizawa *et al.*, 1984; Yamane *et al.*, 1996; Yokozawa *et al.*, 1995; Zhang *et al.*, 1995; Zhu *et al.*, 1992
(+)-3-*O*-gallate-catechin (+)-3-*O*-gallate-gallocatechin	193 194	Goto *et al.*, 1996
(+)-3-*O*-gallate-gallocatechin	194	Dalluge *et al.*, 1998; Sakanaka *et al.*, 1995
(+)-3-*O*-gallate-catechin (+)-3-*O*-gallate-gallocatechin (-)-3-*O*-(3-*O*-methyl-gallate)-epi-gallocatechin	193 194 197	Davis *et al.*, 1996
(+)-gallocatechin	195	Ahn *et al.*, 1991; Cho *et al.*, 1993; Davis *et al.*, 1996; Goto *et al.*, 1996; Hagiwara *et al.*, 1991; Hashimoto *et al.*, 1992; Miyagawa *et al.*, 1997; Otake *et al.*, 1991; Saijo, 1982; Sakanaka *et al.*, 1989(c); Sanq *et al*, 1999; Shu *et al.*, 1991; Valcic *et al.*, 1996; Yokozawa *et al.*, 1995
(-)-3-*O*-(3-*O*-methyl-gallate)-epi-catechin (-)-3-*O*-(3-*O*-methyl-gallate)-epi-gallocatechin	196 197	Saijo, 1982
(-)-3-*O*-(3-*O*-methyl-gallate)-epi-gallocatechin (-)-3-*O*-(4-*O*-methyl-gallate)-epi-gallocatechin	197 198	Sanq *et al.*, 1999

Table 1 (continued)

Compound	Structure	Reference
3-*O*-gallate-epi-gallocatechin	69	Kakiuchi *et al*., 1985
(-)-3-*O*-gallate-epi-gallocatechin	69	Park and Boo, 1991; Ruch *et al*., 1989; Zhu *et al*., 1992
(-)-3-*O*-gallate-epi-gallocatechin	69	Hashimoto *et al*., 1987; Saijo, 1982
(-)-3-*O*-gallate-epi-gallocatechin	69	Lee and Lin, 1985
(-)-epi-gallocatechin	70	Hashimoto *et al*., 1989(a) ; Lee and Lin, 1985; Nonaka *et al*., 1983; Okada, 1978
(-)-epi-catechin	72	Ahn *et al*., 1991; Anon, 1984; 1985; Apostolides *et al*., 1996; Dalluge *et al*., 1998; Davis *et al*., 1996; Goto *et al*., 1996; Hagiwara *et al*., 1991; Hashimoto *et al*., 1987; 1989(a); 1992; Horie and Kohata, 1998; Kada *et al*., 1985; Katiyar *et al*., 1992; Katiyar and Bhatia, 1992; Lee and Lin, 1985; Li *et al*., 1992; Lin *et al*., 1996; 1998; Matsuda *et al*., 1986; Min and Peigen, 1991; Miyagawa *et al*., 1997; Nakazato *et al*., 1996; Namiki, 1990; Nonaka *et al*., 1983; Okada, 1978; Otake *et al*., 1991; Ozawa, 1982; Pearson *et al*., 1998; Roder, 1982; Ruch *et al*., 1989; Saijo, 1982; Sakanaka *et al*., 1989(b); 1989(c); 1995; Sakata *et al*., 1991; Sanq *et al*., 1999; Shiragami *et al*., 1995; Shu *et al*., 1991; Tanizawa *et al*., 1984; Ui *et al*., 1991; Valcic *et al*., 1996; Yamane *et al*., 1996; Yen and Chen, 1996; Yokozawa *et al*., 1995; Zhang *et al*., 1991; 1995; Zhu *et al*., 1992

Table 1 (continued)

Compound	Structure	Reference
(-)-3-*O*-gallate-epi-catechin	73	Ahn *et al.*, 1991; Anon, 1984; 985; Apostolides *et al.*, 1996; Cho *et al.*, 1993; Dalluge *et al.*, 1998; Davis *et al.*, 1996; Furukawa and Kawakita, 1987; Goto *et al.*, 1996; Hagiwara *et al.*, 1991; Hashimoto *et al.*, 1987; 1989(a); 1992; Horie and Kohata, 1998; Kada *et al.*, 1985; Katiyar *et al.*, 1992; Katiyar and Bhatia, 1992; Lee and Lin, 1985; Li *et al.*, 1992; Lin *et al.*, 1996; 1998; Matsuda *et al.*, 1986; Min and Peigen, 1991; Miyagawa *et al.*, 1997; Nakazato *et al.*, 1996; Nonaka *et al.*, 1983; Okada, 1978; Otake *et al.*, 1991; Ozawa, 1982; Ruch *et al.*, 1989; Saijo, 1982; Sakanaka *et al.*, 1989(a); 1989(c); Sakata *et al.*, 1991; Sanq *et al.*, 1999; Shiragami *et al.*, 1995; Tsunoda *et al.*, 1989; Valcic *et al.*, 1996; Yen and Chen, 1996; Yokozawa *et al.*, 1995; Zhang *et al.*, 1995; Zhu *et al.*, 1992
epi-gallo-catechin-(4-β-8)-3-*O*-galloyl-epi-catechin 1-*O*-galloyl-4-6-(-)-hexahydroxy-diphenoyl-β-D-glucose	81 -	Nonaka *et al.*, 1984
catechin-(4-α-8)-epi-gallocatechin gallocatechin-(4-α-8)-epi-catechin procyanidin B-2	75 76 83	Hashimoto *et al.*, 1989(a); 1989(b)
3,3'-di-*O*-gallate-procyanidin B-2	84	Hashimoto *et al.*, 1989(a); 1989(b); 1992; Nonaka *et al.*, 1983

Table 1 (continued)

Compound	Structure	Reference
3-*O*-gallate-epi-gallocatechin	69	Kada *et al.*, 1985; Okada, 1978
(-)-3-*O*-gallate-epi-gallocatechin	69	Ahn *et al.*, 1991; Anon, 1985; Apostolides *et al.*, 1996; Boo and Jeon, 1993; Chisaka *et al.*, 1988; Cho *et al.*, 1993; Dalluge *et al.*, 1998; Davis *et al.*, 1996; Goto *et al.*, 1996; Hagiwara *et al.*, 1991; Horie and Kohata, 1998; Katiyar *et al.*, 1992; Li *et al.*, 1992; Lin *et al.*, 1996; Matsuda *et al.*, 1986; Min and Peigen, 1991; Miyagawa *et al.*, 1997; Mukoyama *et al.*, 1991; Nakazato *et al.*, 1996; Oosu *et al.*, 1991; Otake *et al.*, 1991; Ozawa, 1982; Sakanaka *et al.*, 1989(c); Sakata *et al.*, 1991; Sanq *et al*, 1999; Shiragami *et al.*, 1995; Spiro, 1997; Tsunenari *et al.*, 1992; Tsunoda *et al.*, 1989; Unno *et al.*, 1996; Valcic *et al.*, 1996; Watanabe *et al.*, 1998; Yamane *et al.*, 1996; Yen and Chen, 1996; Yeo *et al.*, 1995; Yokozawa *et al.*, 1992; 1993; 1995; Yoshioka *et al.*, 1996; Yoshizawa *et al.*, 1987; Zhang *et al.*, 1991; 1995
epi-gallocatechin	70	Anon, 1984
camellia galactoglucan	-	Takeo *et al.*, 1992
camellia polysaccharide	-	Wang and Wang, 1991
deacyl camellia saponin B	-	Kitagawa *et al.*, 1995
camellia sinensis polysaccharidetsa	-	Fang *et al.*, 1991

Table 1 (continued)

Compound	Structure	Reference
(-)-epi-gallocatechin	70	Anon, 1985; Apostolides *et al*., 1996; Boo and Jeon, 1993; Cho *et al*., 1993; Davis *et al*., 1996; Goto *et al*., 1996; Hagiwara *et al*., 1991; Hashimoto *et al*., 1987; 1992; Horie and Kohata, 1998; Katiyar *et al*., 1992; Katiyar and Bhatia, 1992; Li *et al*., 1992; Lin *et al*., 1998; Matsuda *et al*., 1986; Min and Peigen, 1991; Miyagawa *et al*., 1997; Otake *et al*., 1991; Ozawa, 1982; Roder, 1982; Ruch *et al*., 1989; Saijo, 1982; Sakanaka *et al*., 1989(c); 1995; Sakata *et al*., 1991; Sanq *et al*, 1999; Shu *et al*., 1991; Valcic *et al*., 1996; Yen and Chen, 1996; Yokozawa *et al*., 1995; Zhang *et al*., 1991; 1995; Zhu *et al*., 1992
(-)-3,3'-di-*O*-gallate-epi-gallocatechin (-)-3,4'-di-*O*-gallate-epi- gallocatechin (-)-3-*O*-gallate-epi-gallocatechin 3'-*O*-gallate-procyanidin B-2 3-*O*-*p*-coumaroae-(-)-epi-gallocatechin	65 66 69 85 -	Nonaka *et al*., 1983
(-)-3,3'-di-*O*-gallate-epi-gallocatechin (-)-3,4'-di-*O*-gallate-epi-gallocatechin (-)-3-*O*-(3-*O*-methyl-gallate)-epi-catechin (-)-3-*O*-(4-*O*-methyl-gallate)-epi-catechin	65 66 196 198	Hashimoto *et al*., 1987

Table 1 (continued)

Compound	Structure	Reference
(-)-3-*O*-cinnamate-epi-gallocatechin (-)-3-*O*-*p*-coumaroate-epi-gallocatechin (-)-3-*O*-*p*-hydroxy-benzoate-epi-catechin	- - -	Hashimoto *et al.*, 1987
(-)-3-*O*-gallate-epi-gallocatechin 3-*O*-gallate-epi-gallo-catechin-(4-β-8)-gallate -epi-catechin (-)-3-*O*-(3-*O*-methyl-gallate)-epi-catechin (-)-3,5'-di-*O*-gallate-epi-gallocatechin 3-*O*-gallate-epi-theaflagallin theaflavonin degalloyl theaflavonin 1,4,6-tri-*O*-galloyl-β-D-glucose (-)-3-*O*-*p*-coumaroate-epi-gallocatechin	69 82 196 199 200 201 202 - -	Hashimoto *et al.*, 1992
gallate-catechin	203	Sakanaka *et al.*, 1995
(-)-3-gallate-gallocatechin	204	Taniguchi *et al.*, 1988
(-)-3-gallate-gallocatechin	204	Katiyar and Bhatia, 1992; Lin *et al.*, 1998
(-)-3-gallate-gallocatechin	204	Yokozawa *et al.*, 1995
(-)-gallocatechin	205	Lin *et al.*, 1998
chlorogenic acid	206	Ozawa, 1982; Zaprometov *et al.*, 1976
diphenylamine	207	Karawya *et al.*, 1984; 1986
trans,trans-α-farnesene	-	Nobumoto *et al.*, 1990
4-hydroxy-2'-methoxy angular-furocoumarin	-	Banerjee and Ganguly, 1997
citric acid	-	Ding *et al.*, 1997

Table 1 (continued)

Compound	Structure	Reference
farnesol	208	Lin *et al.*, 1984
N-hexadecane	209	
hex-*cis*-3-en-1-ol acetate	210	
hex-*cis*-3-en-1-ol butyrate	211	
hex-*cis*-3-en-1-ol caproate	212	
jasmonic acid methyl ester	213	
myrcene	214	
2,6,10,14-tetramethyl pentadecane	215	
linalool oxide A	-	
linalool oxide B	-	
linalool oxide C	-	
gallic acid	216	Apostolides *et al.*, 1996; Gong *et al*, 1993; Hashimoto *et al.*, 1992; Lin *et al.*, 1998; Maruyama *et al.*, 1992; Okada, 1978; Ozawa, 1982; Zhang *et al.*, 1991
geraniol	217	Lee and Lin, 1985; Lin *et al.*, 1982; Morita *et al.*, 1994
hyperoside	218	Imperato, 1980; Mikaberidze *et al.*, 1974(a); Price *et al.*, 1998
indole-3-methyl-ethanoate	219	Roy and Ganguly, 1997
β-ionone	220	Lin *et al.*, 1982
oxide *trans*-linalool	221	
jasmone	222	Lin *et al.*, 1982; 1984
cis-jasmone	223	
(-)-methyl ester (1*R*,2*R*) jasmonic acid	224	Wang *et al.*, 1996
(+)-methyl ester (1*R*,2*S*) jasmonic acid	225	

Table 1 (continued)

Compound	Structure	Reference
kaempferitrin nicotiflorin thearubigin tricetinidin	226 227 228 229	Ozawa, 1982
(*S*)-methyl methionine	230	Ohtsuki *et al.*, 1984
methylamine	231	Neurath *et al.*, 1977
kaempferol	232	De Vries *et al.*, 1998; Hertog *et al.*, 1993; Mikaberidze *et al.*, 1974(a); Price *et al.*, 1998
geraniol-6-*O*-*β*-D-xylopyranosyl- *β*-D-glucopyranoside linalool-6-*O*-*β*-D-xylopyranosyl-*β*-D-glucopyranoside geraniol-*β*-bicianoside	- - -	Nishikitani *et al.*, 1996
geranyl-6-*O*-*β*-D-xylopyranosyl-*β*-D-glucopyranoside	-	Guo *et al.*, 1993
8-hydroxy-geranyl-*β*-primeveroside hex-*cis*-enyl-*β*-D-glucopyranoside *cis*-linalool-3-7-oxide glycoside *trans*-linalool-3-7-oxide glycoside salicylic acid-6-*O*-*β*-D-primeveroside	- - - - -	Moon *et al.*, 1996
N-*p*-coumaryl-glutamic acid kaempferol-3-*O*-*β*-D-rutinoside	- -	Imperato, 1980
indole *β*-sesquiphellandrene	- -	Lee and Lin, 1985
morin	233	Wang *et al.*, 1995

Table 1 (continued)

Compound	Structure	Reference
myricetin quercetin-fructosyl-glucoside	234 -	Hertog *et al.*, 1993; Mikaberidze *et al.*, 1974(a)
quercetin	235	De Vries *et al.*, 1998; Hertog *et al.*, 1993; Mikaberidze and Moniava, 1974(a); Price *et al.*, 1998; Zhu *et al.*, 1992
kaempferol-3-glucosyl(1→3)-rhamnosyl(1→6)-galactoside quercetin-3-glucosyl(1→3)-rhamnosyl(1→6)galactoside	236 237	Finger *et al.*, 1991
linalool	238	Gong *et al.*, 1993; Lee and Lin, 1985; Lin *et al.*, 1982; 1984; Morita *et al.*, 1994; Owuoe, 1989
(*R*)-linalool	239	Wang *et al.*, 1994
oxide(furanoid) *cis*-linalool oxide(pyranoid) *cis*-linalool oxide(furanoid) *trans*-linalool oxide(pyranoid) *trans*-linalool	240 241 242 243	Morita *et al.*, 1994; Wang *et al.*, 1994
nerolidol	244	Lin *et al.*, 1984; Lee and Lin, 1985
nicotine	245	Davis *et al.*, 1991
6-*O*-*β*-D-xylopyranosyl-*β*-D-glucopyranoside *cis*-linalool-3,6-oxide	-	Moon *et al.*, 1994; Nishikitani *et al.*, 1996
6-*O*-*β*-D-xylopyranosyl-*β*-D-glucopyranoside *trans*-linalool-3,6-oxide	-	Moon *et al.*, 1994
naringenin-fructosyl-glucoside	-	Imperato, 1976

Table 1 (continued)

Compound	Structure	Reference
theasinensisin A theasinensisin B	247 248	Hashimoto *et al.*, 1988; 1989(a); 1992; Nonaka *et al.*, 1983
theasinensisin C theasinensisin D theasinensisin E theasinensisin F theasinensisin G oolongtheanin	246 249 250 - - -	Hashimoto *et al.*, 1988
oxalic acid	251	Nakahara, 1974
2-phenylethanol	252	Lin *et al.*, 1984; Morita *et al.*, 1994
pipecolic acid hydroxyl proline	253 -	Higuchi *et al.*, 1995
pedunculagin	254	Ooishi *et al.*, 1994
pheophorbide A	255	Kohata *et al.*, 1998
prodelphinidin B-4 procyanidin B-3 procyanidin B-4	77 256 258	Hashimoto *et al.*, 1989(a); 1989(b); 1992
3-*O*-gallate-procyanidin B-3	257	Cho *et al.*, 1993
3'-*O*-gallate-prodelphinidin B-2 3'-*O*-gallate-procyanidin B-4	90 259	Nonaka *et al.*, 1983
prunasin	260	Guo *et al.*, 1998
3'-*O*-gallate-prodelphinidin B-4 strictinin procyanidin C-1	78 261 262	Hashimoto *et al.*, 1989(b); 1992
umbelliferone quercimeritrin	263 -	Mikaberidze and Moniava , 1974(b)

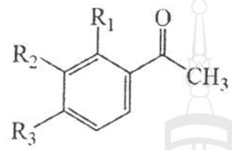
Table 1 (continued)

Compound	Structure	Reference
iso quercitrin	-	Imperato, 1980; Ozawa, 1982; Price *et al.*, 1998
(-)-quinic acid	264	Sakata *et al.*, 1986
succinic acid tartaric acid	265 266	Ding *et al.*, 1997
rutin	267	Humphreys, 1964; Ozawa, 1982; Price *et al.*, 1998
tannic acid	268	Savolainen, 1992; Wheeler, 1978
teasterone typhasterol teasaponin B-2 teasaponin B-3 teasaponin B-4	269 270 - - -	Akagi *et al.*, 1997
α-terpineol	271	Gong *et al.*, 1993; Stalcup *et al.*, 1993
theaflavin	272	Apostolides *et al.*, 1996; Davis *et al.*, 1995; Hashimoto *et al.*, 1989(b); 1992; Honda and Hara, 1993; Mukoyama *et al.*, 1991; Nonaka *et al.*, 1986; Okada *et al.*, 1977; 1978; Ozawa, 1982; Roder, 1982; Shiragami *et al.*, 1995; Shiraki *et al.*, 1994; Vijaya *et al.*, 1995; Yang *et al.*, 1997
theaflavin digallate	273	Apostolides *et al.*, 1996; Davis *et al.*, 1995; Honda and Hara, 1993; Mukoyama *et al.*, 1991; Okada, 1978; Ozawa, 1982; Shiragami *et al.*, 1995; Shiraki *et al.*, 1994
theaflavate B 3'-*O*-gallate isotheaflavin 3'-*O*-gallate neotheaflavin	- - -	Lewis *et al.*, 1998
epi-theaflavic acid gallate-epi-theaflavic acid	- -	Cattell and Nursten, 1976

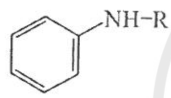
Table 1 (continued)

Compound	Structure	Reference
theaflagallin epi-theaflagallin	- -	Nonaka *et al.*, 1986
theaflavin monogallate	-	Davis *et al.*, 1995; Mukoyama *et al.*, 1991; Namiki, 1990; Okada, 1978; Ozawa, 1982
theaflavin monogallate A theaflavin monogallate B	- -	Honda and Hara, 1993; Shiragami *et al.*, 1995; Shiraki *et al.*, 1994
3-*O*-gallate-epi-theaflagallin	-	Hashimoto *et al.*, 1989(b); Nonaka *et al.*, 1986
3,3'-di-*O*-gallate-theaflavin	274	Yang *et al.*, 1997
3,3'-di-O-gallate-theaflavin 3'-*O*-gallate-theaflavin 3-*O*-gallate-theaflavin	274 275 276	Hashimoto *et al.*, 1989(b); 1992; Nonaka *et al.*, 1986
3'-gallate-theaflavin 3-gallate-theaflavin	275 276	Apostolides *et al.*, 1996; Yang *et al.*, 1997
theanine	277	Chu *et al.*, 1997; Feldheim *et al.*, 1986; Horie and Kohata, 1998; Tsushida, 1987; Yokozawa *et al.*, 1995; Zhu, 1986
theasaponin	278	Shcheglov *et al.*, 1979
theasaponin B-1	279	Kitagawa *et al.*, 1995
teasaponin B-1	279	Akagi *et al.*, 1997; Sagesaka *et al.*, 1996
theobromine	280	Ashihara *et al.*, 1997; Blauch *et al.*, 1983; Fujimori and Ashihara, 1994; Lin *et al.*, 1998; Nagata and Sakai, 1985; Suzuki and Takahashi, 1975; Zhang *et al.*, 1995
theophylline	281	Ito *et al.*, 1997; Lin *et al.*, 1998; Zhang *et al.*, 1995
theogallin	282	Hashimoto *et al.*, 1989(b); 1992; Ozawa, 1982
triacontan-1-ol	283	Narayan *et al.*, 1988
theacitrin A	284	Davis *et al.*, 1997

2.3 Structure of compounds from the leaves of *Camellia sinensis* var. *assamica*

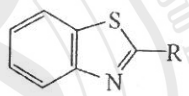


R_1	R_2	R_3	
CH_3	H	CH_3	**1 :** 2,4-dimethylacetophenone
H	OCH_3	OCH_3	**2 :** 3,4-dimethoxyacetophenone
H	H	CH_2CH_3	**3 :** *p*-ethylacetophenone
H	$COCH_3$	H	**4 :** 1,3-diacetylbenzene
H	H	$COCH_3$	**5 :** 1,4- diacetylbenzene



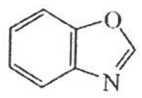
$R = CH_2CH_3$ **6 :** *N*-ethylaniline

$R = CH_3$ **7 :** *N*-methylaniline

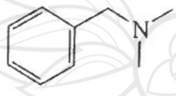


R = H **8 :** benzothiazole

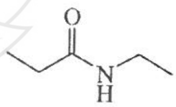
$R = CH_3$ **9 :** 2-methylbenzo thiazole



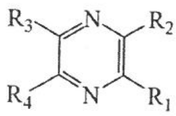
10 : benzoxazole



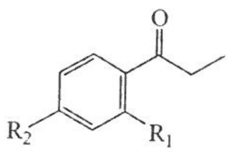
11 : *N,N*-dimethylbenzylamine



12 : *N*-ethyl propionamide

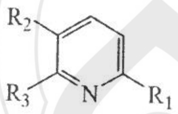


R_1	R_2	R_3	R_4	
CH_3	CH_3	CH_3	CH_3	**13 :** ligustrazine
CH_3	CH_3	H	H	**14 :** 2,3-dimethyl pyrazine
CH_3	H	CH_3	H	**15 :** 2,5-dimethyl pyrazine
CH_3	H	H	CH_3	**16 :** 2,6-dimethyl pyrazine
CH_2CH_3	CH_3	CH_3	H	**17 :** 2-ethyl-3,5-dimethyl pyrazine
CH_2CH_3	CH_3	H	CH_3	**18 :** 2-ethyl-3,6-dimethyl pyrazine
CH_2CH_3	H	CH_3	H	**19 :** 2-ethyl-5-methyl pyrazine
CH_2CH_3	H	H	CH_3	**20 :** 2-ethyl-6-methyl pyrazine
CH_2CH_3	H	H	H	**21 :** ethyl pyrazine
CH_3	H	H	H	**22 :** methyl pyrazine
CH_3	CH_3	CH_3	H	**23 :** trimethyl pyrazine

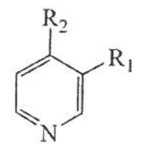


$R_1 = CH_3$, $R_2 = CH_3$ **24 :** 2,4-dimethyl propiophenone

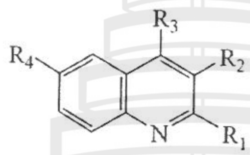
$R_1 = H$, $R_2 = CH_2CH_3$ **25 :** *p*-ethyl propiophenone



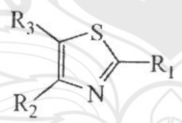
R_1	R_2	R_3	
CH_3	CH_3	H	**26 :** 2,5-dimethyl pyridine
CH_3	H	CH_3	**27 :** 2,6-dimethyl pyridine
$COCH_3$	H	H	**28 :** 2-acetyl pyridine
CH_2CH_3	H	H	**29 :** 2-ethyl pyridine
CH_3	H	H	**30 :** 2-methyl pyridine
CH_3	CH_2CH_3	H	**31 :** 2-methyl-5-ethyl pyridine
CH_3	H	CH_2CH_3	**32 :** 2-methyl-6-ethyl pyridine
C_6H_5	H	H	**33 :** 2-phenyl pyridine



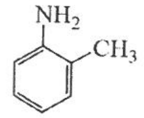
R_1	R_2	
CH_2CH_3	H	**34 :** 3-ethyl pyridine
OCH_3	H	**35 :** 3-methoxy pyridine
CH_3	H	**36 :** 3-methyl pyridine
NHC_4H_9	H	**37 :** 3-*N*-butyl pyridine
C_6H_5	H	**38 :** 3-phenyl pyridine
H	CH_3	**39 :** 4-methyl pyridine
$CH=CH_2$	H	**40 :** 3-vinyl pyridine



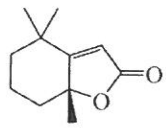
R_1	R_2	R_3	R_4	
CH_3	H	CH_3	H	**41 :** 2,4-dimethyl quinoline
CH_3	H	H	CH_3	**42 :** 2,6-dimethyl quinoline
CH_3	H	H	H	**43 :** 2-methyl quinoline
H	NHC_3H_7	H	H	**44 :** 3-*N*-propyl quinoline
H	H	CH_3	H	**45 :** 4,8-dimethyl quinoline
H	H	NHC_4H_9	H	**46 :** 4-*N*-butyl quinoline
H	H	H	CH_3	**47 :** 6-methyl quinoline



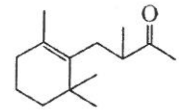
R_1	R_2	R_3	
CH_3	CH_3	CH_3	**48 :** 2,4,5-trimethyl thiazole
CH_3	CH_3	H	**49 :** 2,4-dimethyl thiazole
CH_3	H	CH_3	**50 :** 2,5-dimethyl thiazole
CH_3	CH_2CH_3	CH_3	**51 :** 2,5-dimethyl-4-ethyl thiazole
H	H	CH_3	**52 :** 5-methyl thiazole



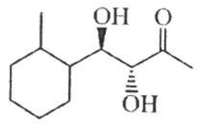
53 : *o*-toluidine



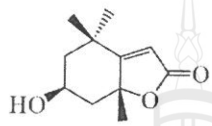
54 : dihydro actinidiolide



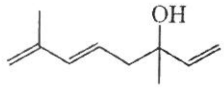
55 : 1',2'-epoxy 1',2'-dihydro-β-ionone



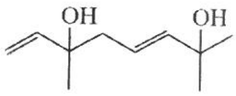
56 : 1',2'-*threo*-1',2'-dihydroxy-β-ionone



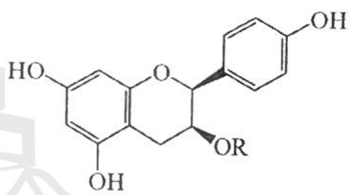
57 : loliolide



58 : 3(*S*)-7-dimethylocta-1,5,7-trien-3-ol

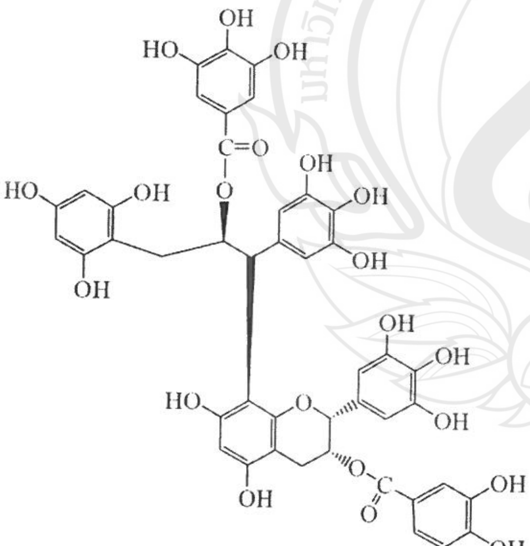


59 : (+)-3(*S*)-7-dimethylocta-1,5-diene-3,7-diol

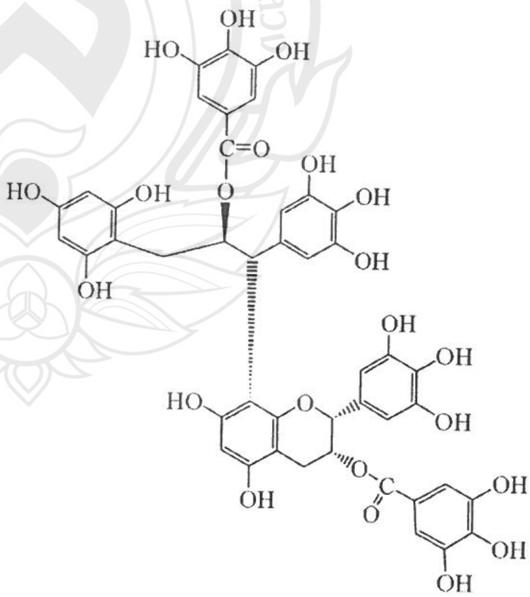


R = H **60 :** (-)-epi-afzelechin

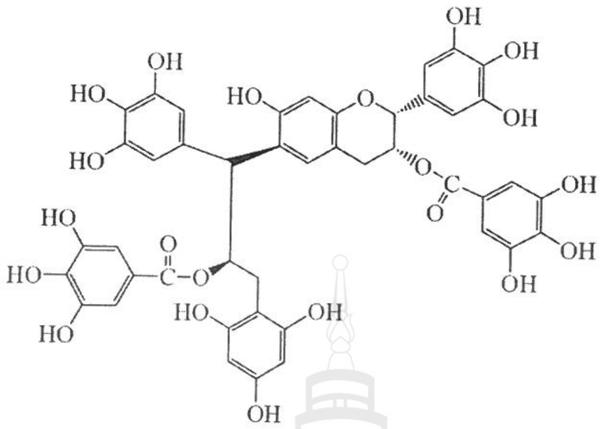
R = gallate **61 :** 3-*O*-gallate-epi-afzelechin



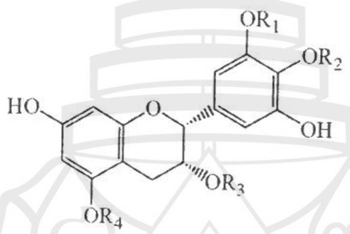
62 : assamicain A



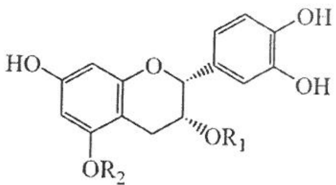
63 : assamicain B



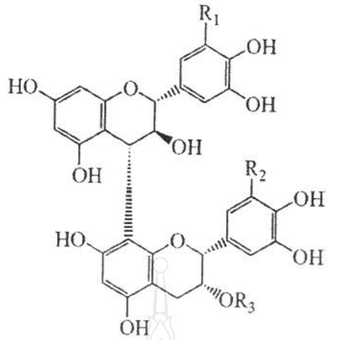
64 : assamicain C



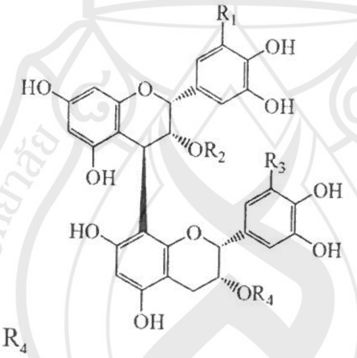
R_1	R_2	R_3	R_4	
gallate	H	gallate	H	**65 :** 3,3'-di-*O*-gallate-epi-gallocatechin
H	gallate	gallate	H	**66 :** 3,4'-di-*O*-gallate-epi-gallocatechin
H	H	gallate	gallate	**67 :** 3,5-di-*O*-gallate-epi-gallocatechin
H	H	caffeoate	H	**68 :** 3-*O*-caffeoate-epi-gallocatechin
H	H	gallate	H	**69 :** 3-*O*-gallate-epi-gallocatechin
H	H	H	H	**70 :** (-)-epi-gallocatechin



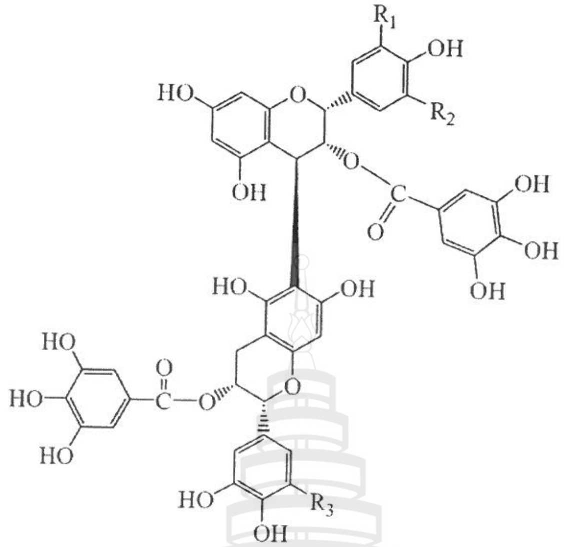
R_1	R_2	
gallate	gallate	**71 :** 3,5-di-*O*-gallate-epi-catechin
H	H	**72 :** (-)-epi-catechin
gallate	H	**73 :** (-)-3-*O*-gallate-epi-catechin



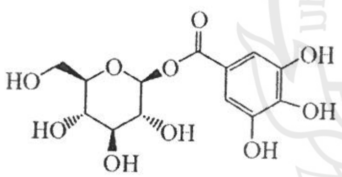
R_1	R_2	R_3	
H	OH	gallate	**74 :** catechin-(4-α-8)-3-gallate-epi-gallocatechin
H	OH	H	**75 :** catechin-(4-α-8)-epi-gallocatechin
OH	H	H	**76 :** gallocatechin-(4-α-8)-epi-catechin
OH	OH	H	**77 :** prodelphinidin B-4
OH	OH	gallate	**78 :** 3'-*O*-gallate-prodelphinidin B-4

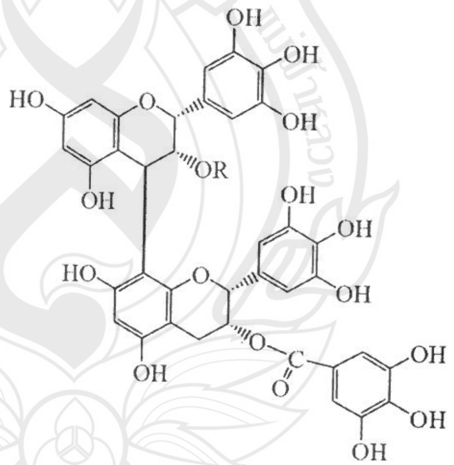


R_1	R_2	R_3	R_4	
H	H	OH	gallate	**79 :** epi-catechin-(4-β-8)-3-*O*-gallate-epi-gallocatechin
H	gallate	OH	gallate	**80 :** 3-*O*-gallate-epi-catechin-(4-β-8)-3-*O*-gallate -epi-gallocatechin
OH	H	H	gallate	**81 :** epi-gallo-catechin-(4-β-8)-3-*O*-galloyl -epi-catechin
OH	gallate	H	gallate	**82 :** 3-*O*-gallate-epi-gallo-catechin-(4-β-8)-gallate -epi-catechin
H	H	H	H	**83 :** procyanidin B-2
H	gallate	H	gallate	**84 :** 3,3'-di-*O*-gallate- procyanidin B-2
H	H	H	gallate	**85 :** 3'-*O*-gallate- procyanidin B-2



R_1	R_2	R_3		
H	OH	OH	**86 :**	3-*O*-gallate-epi-catechin-(4-*β*-6)-3-*O*-gallate-epi-gallocatechin
OH	H	H	**87 :**	3,3'-di-*O*-gallate-procyanidin B-5

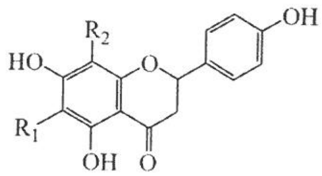




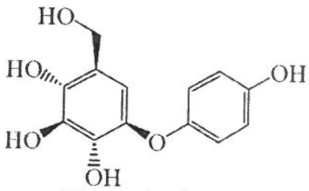
88 : *β*-glucogallin

R = gallate **89 :** 3,3'-di-*O*-gallate-prodelphinidin B-2

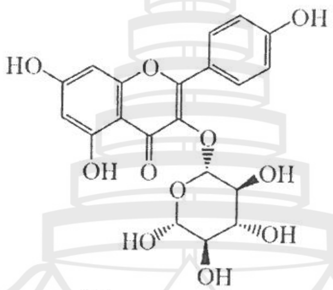
R = H **90 :** 3'-*O*-gallate-prodelphinidin B-2



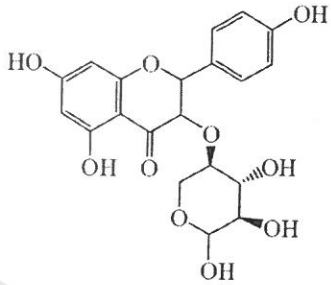
R_1	R_2	
H	H	**91 :** apigenin
Arabinosyl	pyranosyl	**92 :** 6,8-di-*C*-*β*-D-arabinopyranosylapigenin



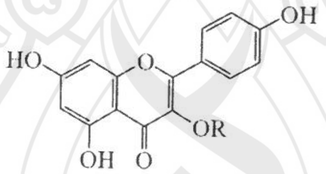
93 : arbutin



94 : astragalin



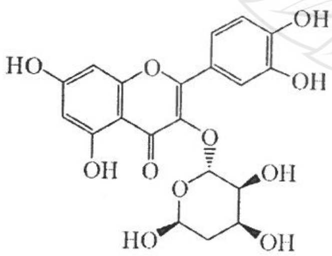
95 : avicularin



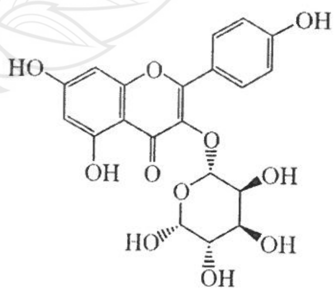
R = glu-rham-gal **96 :** 3-*O*-glucosyl-rhamnosyl galactoside-kaemferol

R = gal-rham-glu **97 :** 3-*O*-galactosyl-rhamnosyl-glucoside-kaempferol

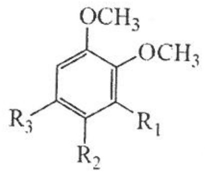
R = glu-rham **98 :** 3-*O*-glucosyl-rhamnoside-kaempferol



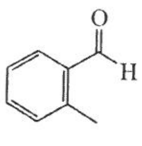
99 : quercitrin



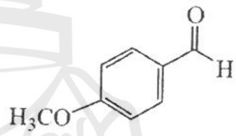
100 : trifolin



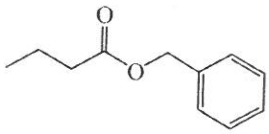
R_1	R_2	R_3			
OCH_3	H	H	**101**	:	1,2,3-trimethoxybenzene
OCH_3	H	CH_2CH_3	**102**	:	1,2,3-trimethoxy-5-ethylbenzene
OCH_3	H	CH_3	**103**	:	1,2,3-trimethoxy-5-methylbenzene
H	H	H	**104**	:	1,2-dimethoxybenzene
H	CH_2CH_3	H	**105**	:	1,2-dimethoxy-4-ethylbenzene
H	CH_3	H	**106**	:	1,2-dimethoxy-4-methylbenzene



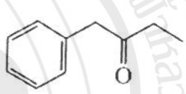
107 : 2-methylbenzaldehyde



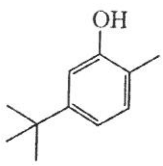
108 : 4-methoxybenzaldehyde



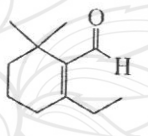
109 : benzylbutyrate



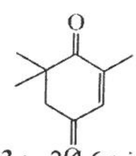
110 : benzyl ethyl ketone



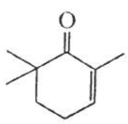
111 : carvacrol



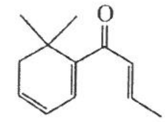
112 : β-cyclocitral



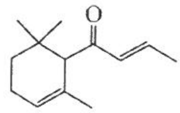
113 : 2,6,6-trimethyl cyclohex-2-en-1,4-dione



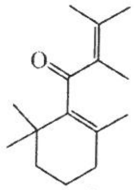
114 : 2,6,6-trimethyl cyclohex-2-en-1-one



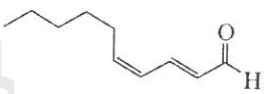
115 : *β*-damascenone



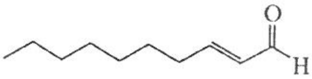
116 : *α*-damascone



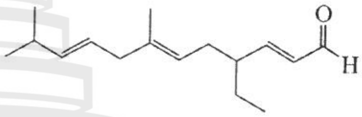
117 : *β*-damascone



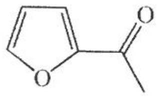
118 : deca-*trans*-2-*cis*-4-dien-1-al



119 : deca-*trans*-2-en-1-al

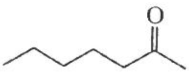


120 : 4-ethyl-7,11-dimethyl-dodeca-*trans*-2-*trans*-6,10-trien-1-al

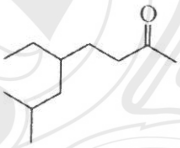


121 : 2-acetyl furan

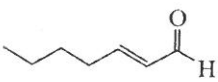
122 : *trans*-geranic acid



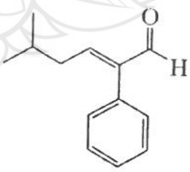
123 : heptan-2-one



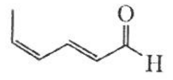
124 : 5-isopropyl-heptan-2-one



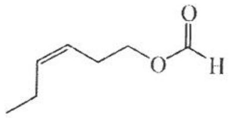
125 : hepta-*trans*-2-en-1-al



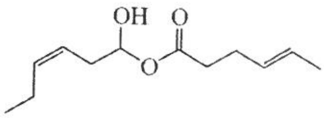
126 : 5-methyl-2-phenyl-hex-2- en-1-al



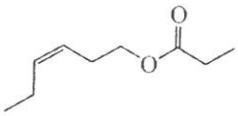
127 : *trans*-2-*cis*-4-hexadien-1-al



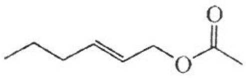
128 : hex-*cis*-3-en-1-ol formate



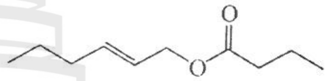
129 : *cis*-3-hexen-1-ol-*trans*-2-hexenoate



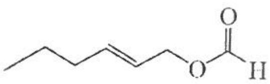
130 : hex-*cis*-3-hexen-1-ol propionate



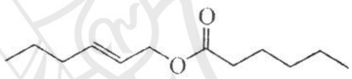
131 : hex-*trans*-2-enyl acetate



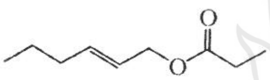
132 : hex-*trans*-2-enyl butyrate



133 : hex-*trans*-2-enyl formate

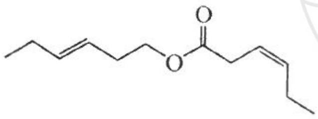


134 : hex-*trans*-2-enyl hexanoate



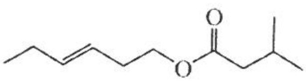
135 : hex-*trans*-2-enyl propionate

136 : hex-*trans*-3-enyl butyrate

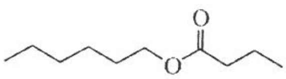


137 : hex-*trans*-3-enyl-hex-*cis*-3-enoate

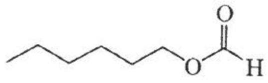
138 : hex-*trans*-3-enyl propionate



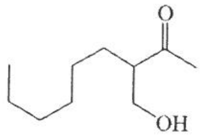
139 : hex-*trans*-3-enyl-2-methyl butyrate



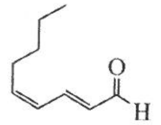
140 : hexyl butyrate



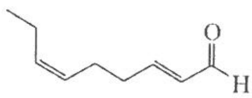
141 : hexyl formate



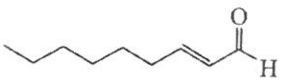
142 : nonan-2-one



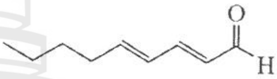
143 : nona-*trans*-2-*cis*-4-dien-1-al



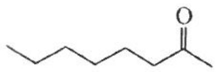
144 : nona-*trans*-2-*cis*-6-dien-1-al



145 : nona-*trans*-2-en-1-al



146 : nona-*trans*-2-trans-4-dien-1-al

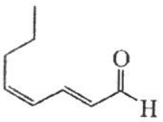


147 : octan-2-one

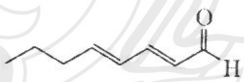
$R = C_2H_5$ **148 :** ethyl octanoate

$R = CH_3$ **149 :** methyl octanoate

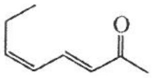
$R = H$ **150 :** octa-*trans*-2-enoic acid



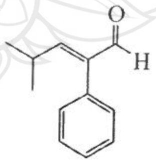
151 : octa-*trans*-2-*cis*-4-dien-1-al



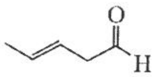
152 : octa-*trans*-2-*trans*-4-dien-1-al



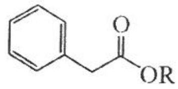
153 : octa-*trans*-3-*cis*-5-dien-2-one



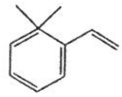
154 : 4-methyl-2-phenyl pent-2-en-1-al



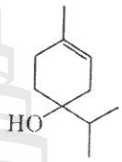
155 : pent-*cis*-3-en-1-al



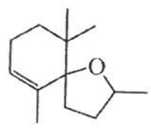
R = CH_2CH_3	**156 :** ethyl phenyl acetate
R = $CH_2(CH_2)_4CH_3$	**157 :** hexyl phenyl acetate
R = H	**158 :** phenyl acetic acid



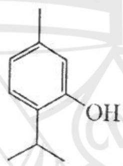
159 : safranal



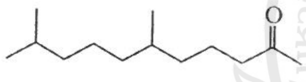
160 : 4-terpineol



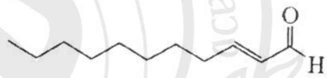
161 : theaspirane



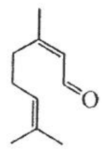
162 : thymol



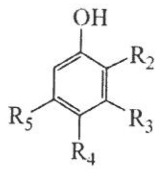
163 : 6,10-dimethyl undecan-2-one



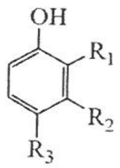
164 : undeca-*trans*-2-en-1-al



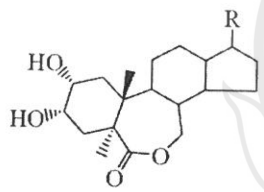
165 : neral

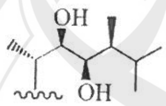


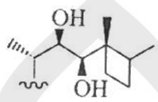
R1	R2	R3	R4	
OH	H	OH	H	**166 :** 1,2,4-trihydroxybenzene
OH	H	H	OH	**167 :** 1,2,5-trihydroxybenzene
H	OH	OH	H	**168 :** 1,3,4-trihydroxybenzene
H	OH	H	OH	**169 :** 1,3,5-trihydroxybenzene

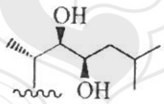


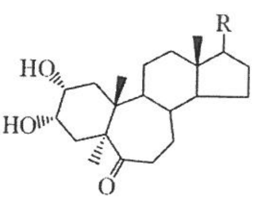
R1	R2	R3	
H	CH_3	H	**170 :** *m*-cresol
CH_3	H	H	**171 :** *o*-cresol
H	H	CH_3	**172 :** *p*-cresol

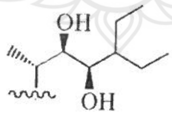


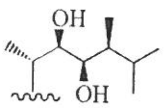
R =  **173 :** brassinolide

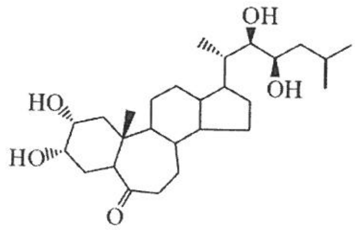
R =  **174 :** 24-ethylbrassinone

R =  **175 :** 28-norbrassinolide

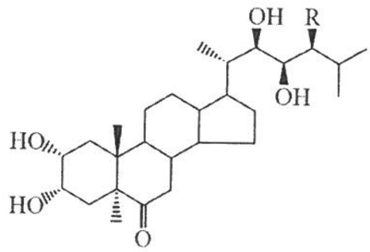


R =  **176 :** 6-keto-28-homo-brassinolide

R =  **177 :** 6-ketobrassinolide

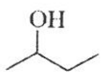


178 : 6-keto-28-norbrassinolide

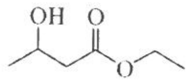


R = H **179 :** brassinone

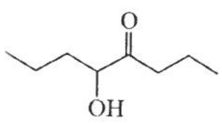
R = CH_2CH_3 **180 :** 24(*S*)-ethylbrassinone



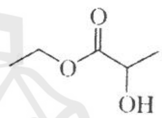
181 : butan-2-ol



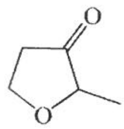
182 : ethyl-3-hydroxy butyrate



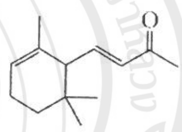
183 : butyroin



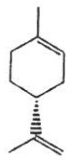
184 : ethyl lactate



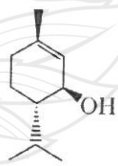
185 : 2-methyl-tetrahydro-furan-3-one



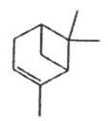
186 : *α*-ionone



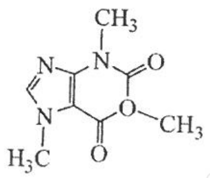
187 : limonene



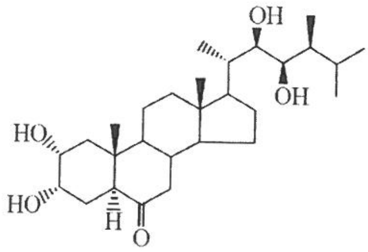
188 : menthol



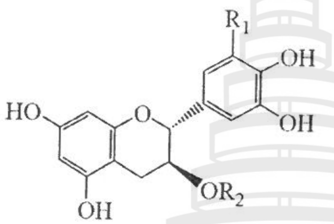
189 : *α*-pinene



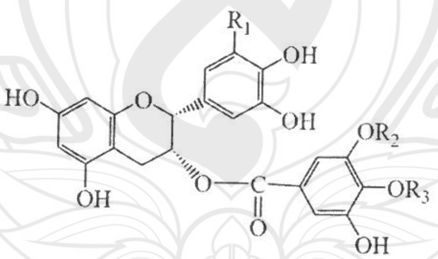
190 : caffeine



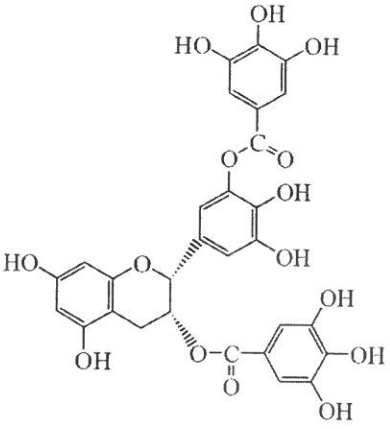
191 : castasterone

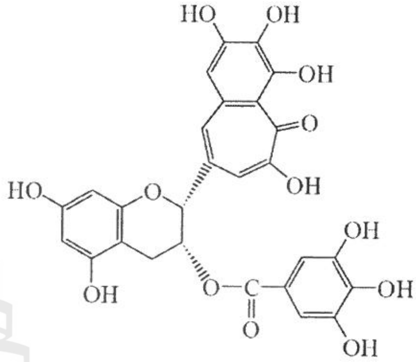


R_1	R_2	
H	H	**192 :** (+)-catechin
H	gallate	**193 :** (+)-3-*O*-gallate-catechin
OH	gallate	**194 :** (+)-3-*O*-gallate-gallocatechin
OH	OH	**195 :** (+)-gallocatechin



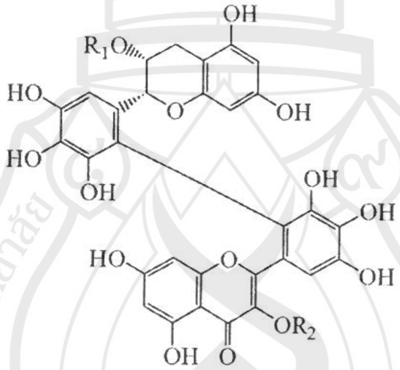
R_1	R_2	R_3	
H	CH_3	H	**196 :** (-)-3-*O*-(3-*O*-methyl-gallate)-epi-catechin
OH	CH_3	H	**197 :** (-)-3-*O*-(3-*O*-methyl-gallate)-epi-gallocatechin
H	H	CH_3	**198 :** (-)-3-*O*-(4-*O*-methyl-gallate)-epi-catechin



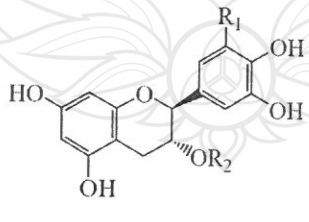


199 : (-)-3,5'-di-*O*-gallate-epi-gallocatechin

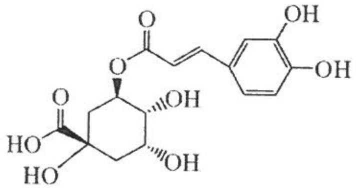
200 : 3-*O*-gallate-epi-theaflagallin



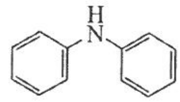
R_1	R_2		
gallate	β-D-glucopyranosyl	**201 :**	theaflavonin
H	β-D-glucopyranosyl	**202 :**	degalloyl theaflavonin



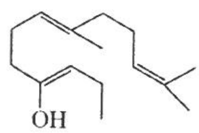
R_1	R_2		
H	gallate	**203 :**	gallate-catechin
OH	gallate	**204 :**	(-)-3-gallate-gallocatechin
OH	H	**205 :**	(-)-gallocatechin



206 : chlorogenic acid



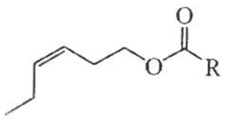
207 : diphenylamine



208 : farnesol

$H_3C—(CH_2)_{14}—CH_3$

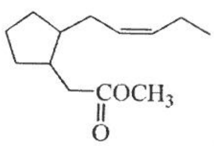
209 : *N*-hexadecane



$R = CH_3$ **210 :** hex-*cis*-3-en-1-ol acetate

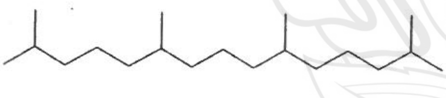
$R = CH_2CH_2CH_3$ **211 :** hex-*cis*-3-en-1-ol butyrate

$R = CH_2(CH_2)_2CH_3$ **212 :** hex-*cis*-3-en-1-ol caproate

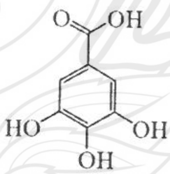


213 : jasmonic acid methyl ester

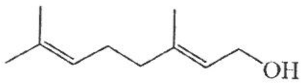
214 : myrcene



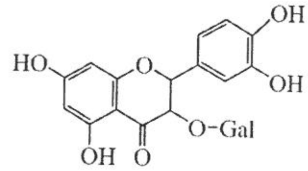
215 : 2,6,10,14-tetramethyl pentadecane



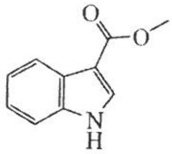
216 : gallic acid



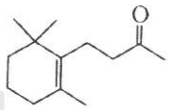
217 : geraniol



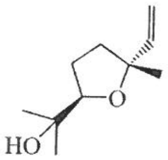
218 : hyperoside



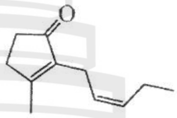
219 : indole-3-methyl-ethanoate



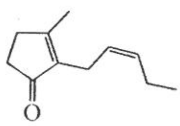
220 : *β*-ionone



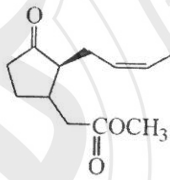
221 : oxide *trans*-linalool



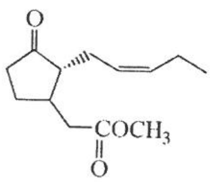
222 : jasmone



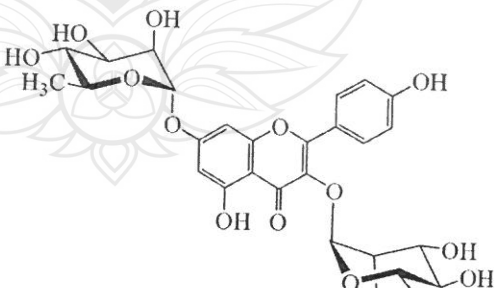
223 : *cis*-jasmone



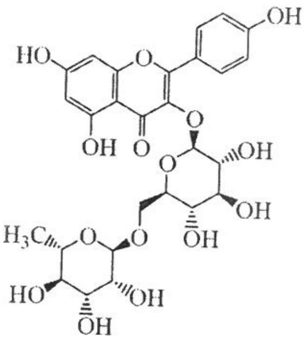
224 : (-)-methyl ester (1*R*,2*R*) jasmonic acid



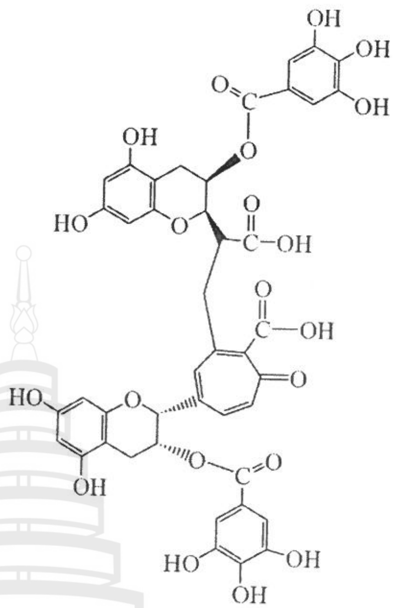
225 : (+)-methyl ester (1*R*,2*S*) jasmonic acid



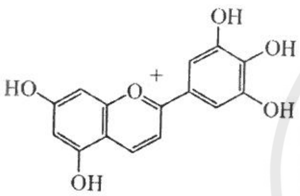
226 : kaempferitrin



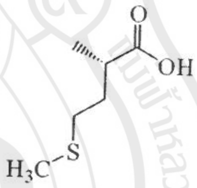
227 : nicotiflorin



228 : thearubigin



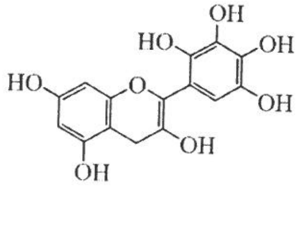
229 : tricetinidin



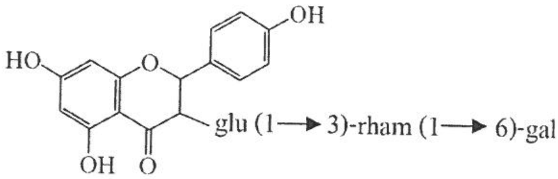
230 : (*S*)-methyl methionine

$H_3C—NH_2$

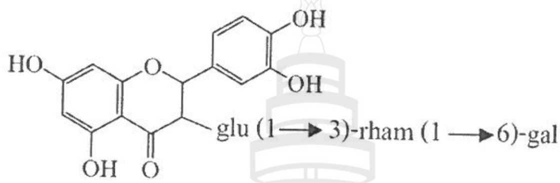
231 : methylamine



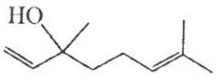
R_1	R_2	R_3	
H	H	H	**232 :** kaempferol
H	H	OH	**233 :** morin
OH	OH	H	**234 :** myricetin
H	OH	H	**235 :** quercetin



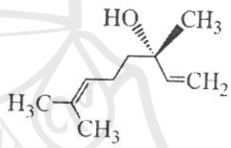
236 : kaempferol-3-glucosyl(1→3)-rhamnosyl(1→6)-galactoside



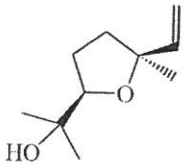
237 : quercetin-3-glucosyl(1→3)-rhamnosyl(1→6)galactoside



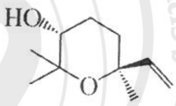
238 : linalool



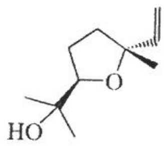
239 : (*R*)-linalool



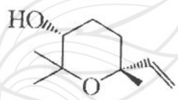
240 : oxide(furanoid) *cis*-linalool



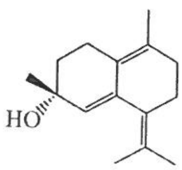
241 : oxide(pyranoid) *cis*-linalool



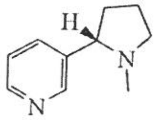
242 : oxide(furanoid) *trans*-linalool



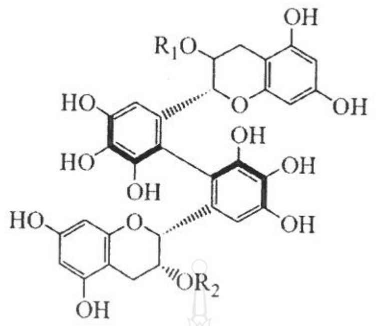
243 : oxide(pyranoid) *trans*-linalool



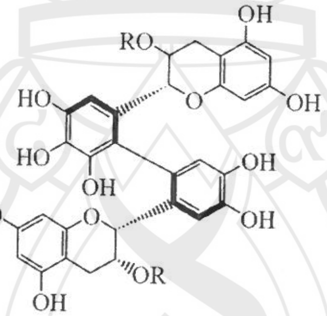
244 : nerolidol



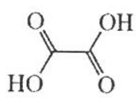
245 : nicotine



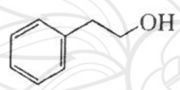
R_1	R_2	
H	H	**246 :** theasinensisin C
gallate	gallate	**247 :** theasinensisin A
H	gallate	**248 :** theasinensisin B



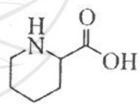
R = gallate	**249 :** theasinensisin D
R = H	**250 :** theasinensisin E



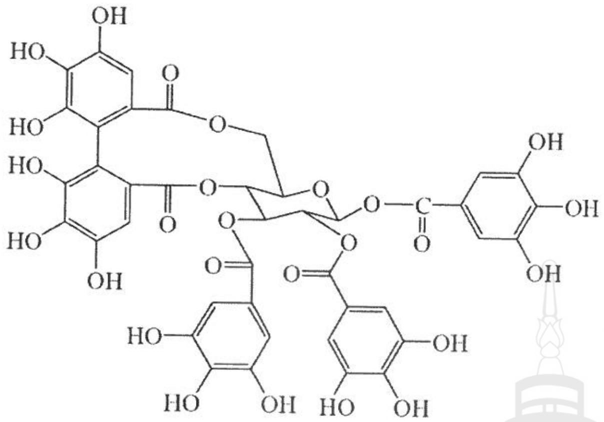
251 : oxalic acid



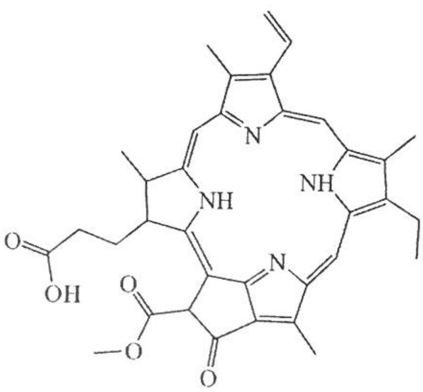
252 : 2-phenylethanol



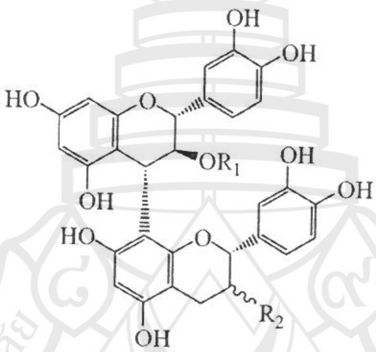
253 : pipecolic acid



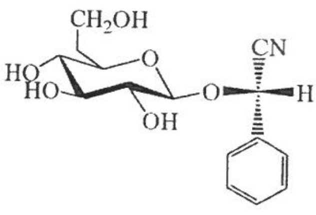
254 : pedunculagin



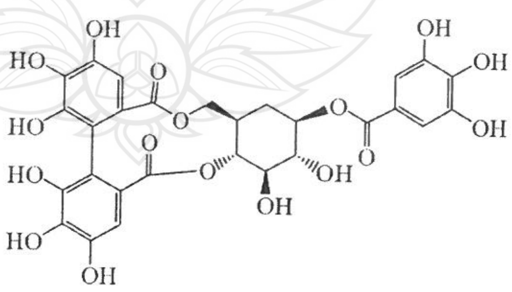
255 : pheophorbide A



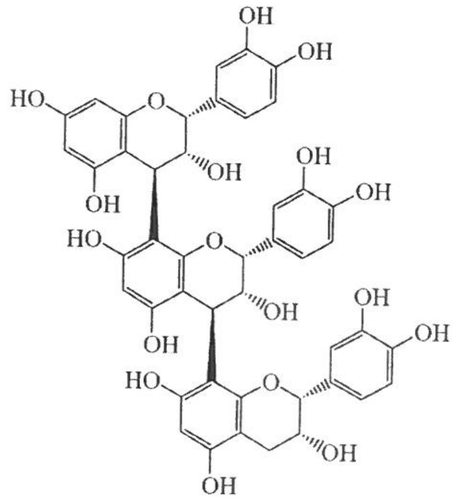
R_1	R_2	
H	OH (wedge)	**256 :** procyanidin B-3
gallate	OH (wedge)	**257 :** 3-*O*-gallate-procyanidin B-3
H	OH (hashed)	**258 :** procyanidin B-4
H	Ogallate (hashed)	**259 :** 3'-*O*-gallate-procyanidin B-4



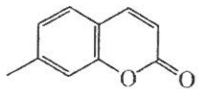
260 : prunasin



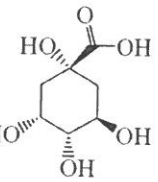
261 : strictinin



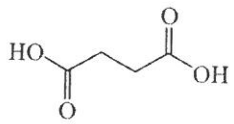
262 : procyanidin C-1



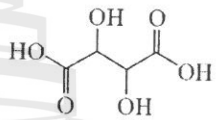
263 : umbelliferone



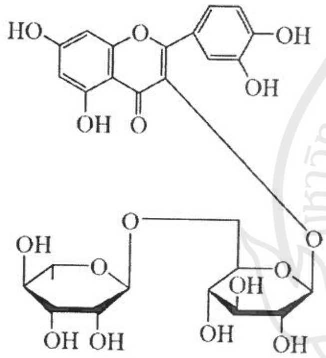
264 : (-)-quinic acid



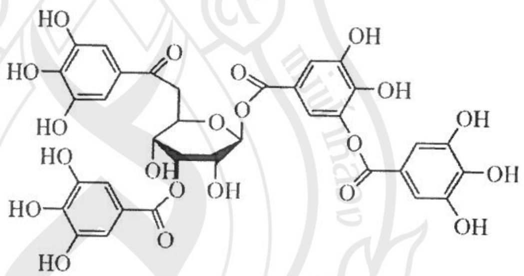
265 : succinic acid



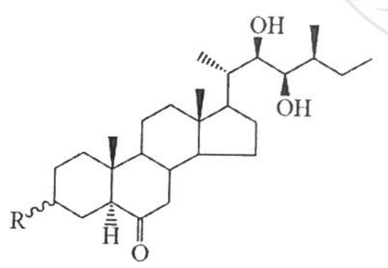
266 : tartaric acid



267 : rutin

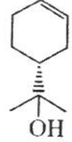


268 : tannic acid

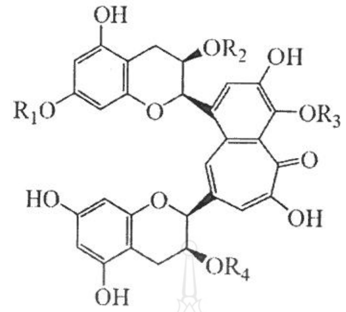


R = OH **269 :** teasterone

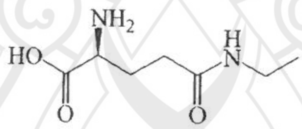
R = OH **270 :** thyphasterol



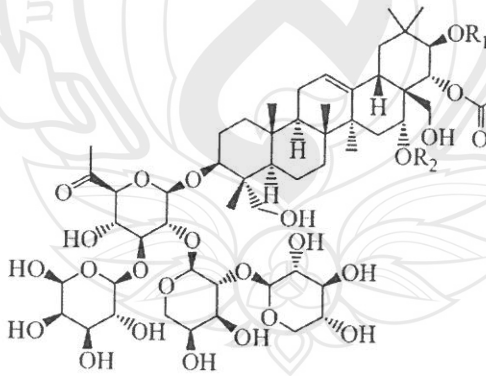
271 : α-terpineol

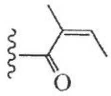


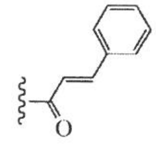
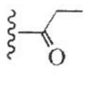
R_1	R_2	R_3	R_4	
H	H	H	H	**272 :** theaflavin
gallate	H	gallate	H	**273 :** theaflavin digallate
H	gallate	H	gallate	**274 :** 3,3'-di-*O*-gallate-theaflavin
H	H	H	gallate	**275 :** 3'-*O*-gallate-theaflavin
H	gallate	H	H	**276 :** 3-*O*-gallate-theaflavin

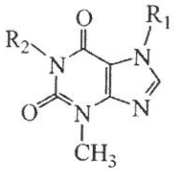


277 : theanine

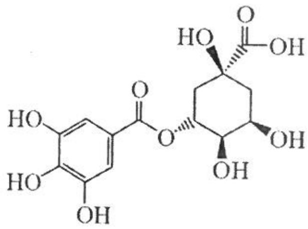


R_1 =  R_2 = H **278 :** theasaponin

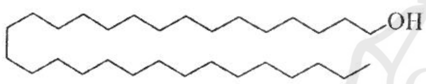
R_1 =  R_2 =  **279 :** theasaponin B-1



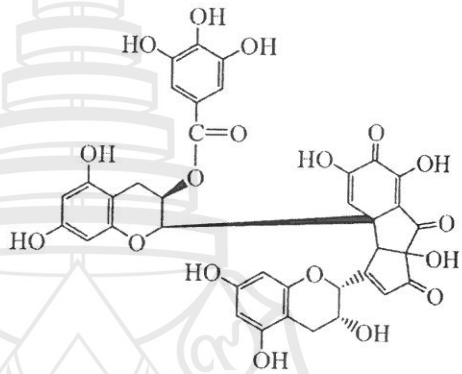
	R1	R2	
	CH_3	H	**280 :** theobromine
	H	CH_3	**281 :** theophylline



282 : theogallin



283 : triacontan-1-ol



284 : theacitrin A

2.4 Biological activities of *Camellia sinensis* var. *assamica*

Since ancient times, antipyretic, anti-inflammatory, antimicrobial and antioxidative properties of tea have been recognized (Siddiqui *et al.*, 2005; Ghosh *et al.*, 2006). Green tea, the dried leaf of *Camellia sinensis*, contains a variety of biologically active compounds such as polyphenols, methylxanthines, essential oils, proteins, vitamins, and amino acids. Most of their biological actions, such as lowering of plasma lipid levels, anti-inflammatory effects, antimicrobial, anticancer, and antioxidant activities, are related to the polyphenol fraction, namely, tea catechins. A widely used approach that has increasingly been used recently is to search for new drugs from natural sources due to its success in the detection of compounds for the treatment of some parasitic diseases. Extracts as well as pure compounds obtained from *C. sinensis* have been reported to possess significant antiprotozoan activities with no side effects (Paveto *et al.*, 2004).

Green tea and its constituents catechins are best known for their antioxidant properties, which has led to their evaluation in a number of diseases associated with reactive oxygen species, such as cancer, cardiovascular and neurodegenerative diseases (Zaveri, 2006). Moreover, the extract of green tea have been reported in the literature as an antioxidant in animal and vegetable oils (Farhoosh *et al.*, 2007). In addition, the antitumor activity of di- and tri-terpenes has been reported. The polyphenolic compounds present in green tea show cancer chemopreventive effect both *in vivo* and *in vitro* (Ghosh *et al.*, 2006).

CHAPTER 3

METHODOLOGY

3.1 General methods

Quick column chromatography (QCC) was performed on silica gel 60H (Merck). Column chromatography (CC) utilized silica gel 100 (70-230 Mesh ASTM) (Merck). Precoated TLC aluminum sheets of silica gel 60 GF_{254} (20 × 20 cm, layer thickness 0.2 mm) (Merck) were used for analytical purposes and the compounds were visualized under UV light and spray with vanillin sulfuric acid reagent. Preparative thin-layer chromatography (PLC) was carried out on glass plates using silica gel 60 GF_{254} (20 × 20 cm, layer thickness 0.2 mm) (Merck). Bands were detected by exposure to short wavelength ultraviolet light. All organic solvents for extraction and chromatography were distillated at their boiling point ranges perior to use. ^{1}H and ^{13}C nuclear magnetic resonance spectra were recorded on a Brüker AVANCE 300 MHz or Varian UNITY INOVA 500 MHz NMR. Spectra were recorded in $CDCl_3$ or acetone-d_6 solution with tetramethylsilanes (TMS) as internal standard. The spectra were recorded as chemical shift parameter (δ) value in ppm down field from TMS. Melting points were measured in celsius (°C) and are uncorrected. They were measured on a digital Electrothermal BÜCHI Melting Point B-540 Melting Point Apparatus. The analytical grade of absolute ethanol,1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (Fluka), ascorbic acid (Fluka) and butylated hydroxy toluene (BHT, Fluka) were used for antioxidation activity testing. The absorptions of the test solution were measured with a Thermo Spectronic Genesys 20 spectrophotometer. The nutrient agar (CRITERION dehydrated culture media) and dimethyl sulfoxide (DMSO) were used for antimicrobial activity testing against 6 strains of microorganism (*Escherichia coli*, *Bacillus cereus Pseudomonas aeruginosa*, *Pseudomonas florescens*, *Staphylococcus aureus* and *Salmonella typhimorium*). Antibiotic paper discs and drugs (vancomycin, gentamycin and streptomycin) were used for control maker of antimicrobial activity.

3.2 Plant material and microorganism culture materials

Leaves of *Camellia sinensis* var. *assamica* were collected on August 2007 from Doi Mae Salong, Chiang Rai province in the northern part of Thailand.

Three microorganism cultures (*Bacillus cereus* TISTR 678, *Pseudomonas fluorescens* TISTR 358, *Salmonellae typhimurium* TISTR 292) were collected from TISTR in Mae Fah Luang University. And four microorganisms (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC25932 and methicillin-resistant strain MRSA SK1) were supported by Department of Microbiology, Faculty of Science, Prince of Songkla University.

3.3 Extraction and Isolation

The dried leaves of *Camellia sinensis* var. *assamica* (3.0 kg) were chopped and extracted at room temperature with hexane, CH_2Cl_2 and MeOH (7 days), respectively. Removal of the solvents from each extract under reduced pressure gave dark green viscous liquid of hexane (136.38 g), dichloromethane (216.20 g) and methanolic (246.20 g) extracts. Overall process of extraction was shown in **Figure 2**.

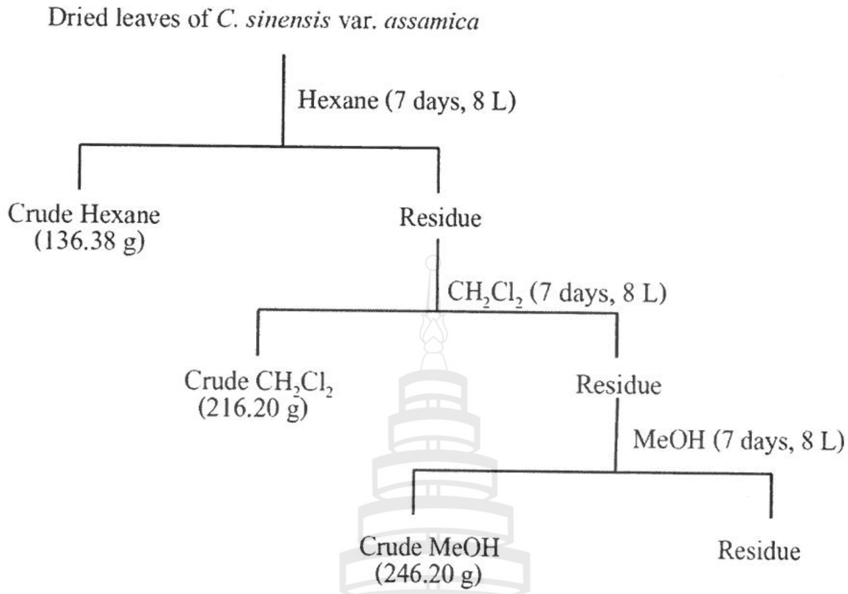


Figure 2 Extraction of crude extracts from the leaves of *C. sinensis* var. *assamica*

3.4 Purification

3.4.1 Purification of crude CH_2Cl_2 extract

Crude CH_2Cl_2 extract (13.0519 g) was fractionated by CC using hexane, hexane-CH_2Cl_2, CH_2Cl_2, CH_2Cl_2-Me_2CO, Me_2CO, Me_2CO-MeOH and MeOH as an eluents. The fractions containing similar components were combined into thirty-one fractions (CAD1-CAD31) (**Table 2**) and selected fractions were further purified to afford compounds **1-4** (**Figure 3**).

Table 2 Physical characteristic and weight of fractions obtained from CC of crude CH_2Cl_2 extract

Fraction	Weight (g)	Physical Characteristic
CAD1	0.2260	white needles mixed with deep green viscous liquid
CAD2	0.1150	white needles mixed with deep green viscous liquid
CAD3	0.2332	white needles mixed with deep green viscous liquid
CAD4	0.2742	yellow viscous liquid
CAD5-CAD6	0.1421	deep yellow viscous liquid
CAD7	0.2048	deep yellow viscous liquid
CAD8	0.0476	deep yellow viscous liquid
CAD9	0.0973	deep yellow viscous liquid
CAD10	0.2370	purple viscous liquid
CAD11	0.1050	deep purple viscous liquid
CAD12	0.7086	white solid mixed with deep green viscous liquid
CAD13	0.1152	white solid mixed with deep green viscous liquid
CAD14	0.6431	white needles mixed with deep green viscous liquid
CAD15	0.2335	white needles mixed with deep green viscous liquid
CAD16	0.3353	deep green viscous liquid
CAD17	1.0899	deep green viscous liquid
CAD18-CAD19	0.5319	deep green viscous liquid
CAD20	0.2038	white needles mixed with deep green viscous liquid
CAD21-CAD22	0.3683	white needles mixed with deep green viscous liquid
CAD23	0.1782	white needles mixed with deep green viscous liquid
CAD24	0.0195	white needles mixed with deep green viscous liquid
CAD25	0,0997	deep green viscous liquid
CAD26	0.3353	white solid mixed with deep green viscous liquid
CAD27	0.2060	white solid mixed with deep green viscous liquid
CAD28	0.1450	white solid mixed with deep green viscous liquid
CAD29-CAD30	1.2086	deep green viscous liquid
CAD31	0.1531	deep green viscous liquid

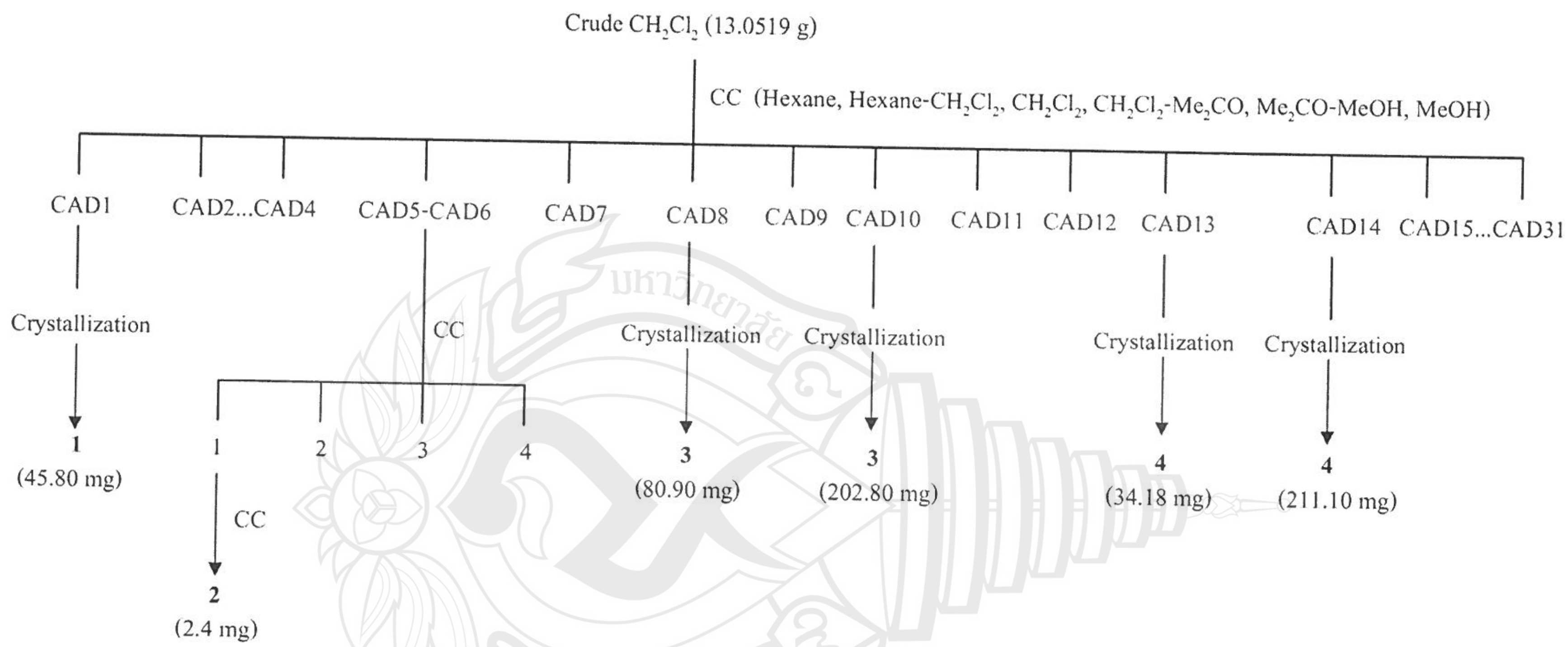


Figure 3 Isolation of compounds **1-4**

Isolation of compound 1

Fraction CAD1 (0.2260 g) was crystallized in MeOH to give colorless needles of compound **1** (45.80 mg).

Isolation of compound 2

On the basis of TLC characteristic fractions CAD5 and CAD6 were combined and further purified by CC on silica gel, eluting with hexane to give four subfractions. The first subfraction was subjected to CC and eluted with 100% hexane to obtain **2** (2.4 mg) as a white solid.

Isolation of compound 3

Fractions CAD8 (0.0476 g) and CAD10 (0.2370 g) were crystallized in MeOH to give a colorless amorphous solid of **3** (80.90 mg) and (202.80 mg), respectively.

Isolation of compound 4

Fractions CAD13 (0.1152 g) and CAD14 (0.6431 g) were crystallized in MeOH to give a white powder of **4** (34.18 mg) and (211.10 mg), respectively.

3.4.2 Purification of crude MeOH extract

Crude methanolic extract (60.75 g) was subjected to QCC using silica gel as a stationary phase and eluted with hexane, hexane-CH_2Cl_2, CH_2Cl_2, CH_2Cl_2-Me_2CO, Me_2CO, Me_2CO-MeOH and then MeOH. On the basis of their TLC characteristic, the collected fractions (250 mL each) which contained the same major components were combined, fractions CAM1-CAM17 were obtained (**Table 3**). The selected fractions were further purified to obtain seven pure compounds as shown in **Figure 4**.

Table 3 Physical characteristic and weight of fractions obtained from QCC of crude MeOH extract

Fraction	Weight (g)	Physical Characteristic
CAM1-CAM6	0.0008	colorless
CAM7	0.0529	deep green viscous liquid
CAM8	0.0878	deep green viscous liquid
CAM9	0.0164	deep green viscous liquid
CAM10	0.0620	deep green viscous liquid
CAM11	0.0213	deep green viscous liquid
CAM12	0.0615	white needles mixed with deep green viscous liquid
CAM13	0.0531	white needles mixed with deep green viscous liquid
CAM14	0.0718	white solid mixed with deep green viscous liquid
CAM15	0.2901	colorless needles mixed with deep green viscous liquid
CAM16	0.3418	deep green viscous liquid
CAM17	0.7856	green solid mixed with green viscous liquid

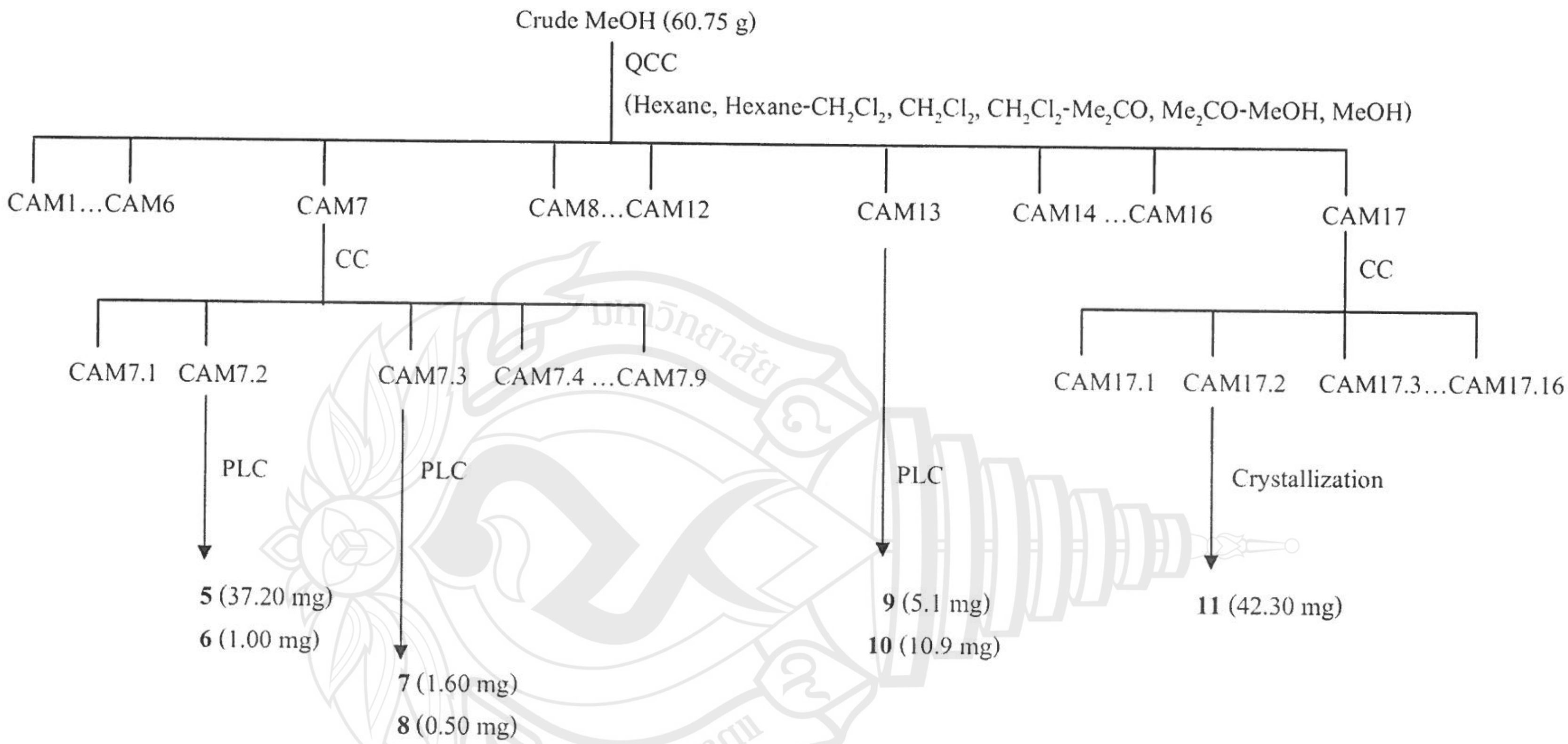


Figure 4 Isolation of compounds **5-11**

Isolation of compounds 5-8

Fraction CAM7 (0.0529 g) was further separated by CC on silica gel and eluted with 50% CH_2Cl_2-hexane. All fractions were collected and combined on the basis of TLC characteristic to give nine subfractions (CAM7.1-CAM7.9). Subfraction CAM7.2 was further purified by preparative TLC and eluted with 30% CH_2Cl_2-hexane yielded two isolated bands. The component from the first band was obtained as a cream solid of **5** (37.20 mg). A white solid **6** (1.00 mg) was obtained from the second band. Subfraction CAM7.3 was further purified by preparative TLC, using 50% CH_2Cl_2-hexane as an eluent. A white solid of **7** (1.60 mg) and **8** (0.50 mg) were obtained from the first and third isolated bands.

Isolation of compounds 9-10

Fraction CAM12 (0.0615 g) was further purified by CC and eluted with CH_2Cl_2 to obtain **9** (10.9 mg) and **10** (5.1 mg) as a white solid.

Isolation of compound 11

Fraction CAM17 (0.7856 g) was rechromatographed on CC and eluted with 50% CH_2Cl_2-hexane. All fractions were collected and combined on the basis of TLC characteristic to give sixteen subfractions (CAM17.1-CAM17.16). Subfraction CAM17.2 was crystallized in CH_2Cl_2 to give **11** as a white powder (42.30 mg).

3.5 DPPH radical scavenging assay

The potential antioxidant activities of the crude extracts and pure compounds isolated from the leaves of *Camellia sinensis* var. *assamica* were assessed on the basis of scavenging activity of the stable 1,1-diphenyl-2-picrylhydrayl (DPPH) free radical. The DPPH assay is one of the methods used for evaluation of antioxidative activity. The following assay procedure was modified from those described in previous reports (Subhadhirasakul and Khumfang, 2000). The test solution in absolute ethanol (50 μL) was mixed with 3 mL of 0.05 mM DPPH solution in ethanol. The absorbance (A) was then measured at 517 nm on a spectrophotometer. BHT and ascorbic acid were used as a positive control. The measurements were performed at least in triplicate. The result expressed as percentage inhibition. The concentration of the sample at 50% inhibition (IC_{50}) was obtained by linear regression analysis of dose-response curve, which was plotted between % inhibition and concentration (Subhadhirasakul and Khumfang, 2000).

$$\% \text{ inhibition} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

3.5.1 Screening on the free radical scavenging activity of crude extracts

The crude material was dissolved in absolute ethanol to prepare the solution with concentration of 6.1 mg/mL. The solution of each sample (50 μL) was mixed with 0.05 mM DPPH ethanolic solution (3 mL) in a cuvette to give the solution with the final concentration of 100 μg/mL. The trapping effect was assessed by measuring the absorbance change of the solution at 517 nm against 0.05 mM DPPH ethanolic solution after 15, 30, 45 and 60 min. Ascorbic acid and BHT were used as a positive control. The measurements were performed at least in triplicate. The degree of loss of color implied the activity.

3.5.2 Evaluation of 50% inhibition concentration (IC_{50}) of crude extracts

Crude CH_2Cl_2 and MeOH extracts showed the strong activity, they were then selected for further study. The solution of DPPH (0.05 mM, 3mL) was mixed with the sample at concentrations of 5.0, 4.0, 3.0, 2.0, 1.0, 0.5, 0.25 and 0.125 mg/mL. The absorbances were measured at 517 nm for 30 minute. The concentration that needed to decrease % inhibition of DPPH solution to 50% inhibition (IC_{50}) was obtained by linear regression analysis of dose-response curve.

3.5.3 Screening on the free radical scavenging activity of pure compounds

The testing was performed as in **3.5.1** except the concentrations of samples were made at 0.61 mM (final concentration 10 μM).

3.5.4 Evaluation of 50% inhibition concentration (IC_{50}) of pure compounds

Compounds **5**, **9**, **10** and **11** showed the strong activity, they were then selected for further study. The solution of DPPH (0.05 mM, 3 mL) was mixed with the sample at concentrations of 0.61, 0.5, 0.4, 0.3, 0.2, 0.1, 0.05, 0.025 and 0.0125 mM. The absorbances were measured at 517 nm for 30 min. The concentration that needed to decrease percentage inhibition of DPPH solution to 50% inhibition concentration (IC_{50}) was obtained by linear regression analysis of dose-response curve.

3.5.5 Evaluation of 50% inhibition concentration (IC_{50}) of standard antioxidants

The evaluation of IC_{50} of standard antioxidants, ascorbic acid and BHT, were study as in **3.5.4** except the standard solution concentrations were at 3.0, 2.0, 1.0, 0.5, 0.25 and 0.125 mM.

3.6 Antimicrobial activity assays

The paper disc diffusion method (Lorian, 1996) was used to screen the antimicrobial activity. Minimum inhibition concentrations (MICs) were determined by broth microdilution method (CLSI M7-A4, 2002) for bacterial assay.

3.6.1 Paper disc diffusion method

The paper disc diffusion method (Lorian, 1996) was used to screen the antimicrobial activity of the crude extracts. Three-five colonies of microbial cultures are transferred to nutrient broth and incubated for 3 hrs at 35 $^{\circ}$C, 150 rpm shaking incubator. The turbidity of microbial suspension was adjusted with 0.85% NaCl (normal saline solution, NSS) compared to 0.5 McFarland standard. The cell culture is determined using total plate count. Spread the cultures into agar plates with sterile cotton swab. Filter papers were placed on the agar plates, containing tested microorganisms, then 10 μL of crude extracts were dropped on the filter paper. Plates were kept in the incubator at 35 $^{\circ}$C for 18 h. This performed in duplicate for each extracts. The clear zone shown on the plate indicating in antimicrobial activity. Hexane, CH_2Cl_2 and MeOH are used for testing markers for crude hexane, CH_2Cl_2 and MeOH extracts of *C. sinensis* var. *assamica*. Antibiotic paper discs are used for control markers of antimicrobial activity.

3.6.2 Broth microdilution method

3.6.2.1 Screening of pure compounds

Test samples were dissolved in dimethyl sulfoxide (DMSO) and mixed with melted Mueller Hinton Broth (MHB) in microtiter plates. Add 50 μL of inoculum suspensions in each well. Final concentrations were 200 μg/mL. The inoculated plate were incubated at 35 ºC for 16-18 h. Then drop 0.18% resazurin 10 μL in microtiter plate and incubated in 35 ºC for 2-3 h. The blue color showed sample can inhibit microbial growth and pink color shown sample can not inhibit microbial growth. The test was performed in triplicates for each sample. Vancomycin, gentamycin and Streptomycin were used as a positive control drug.

3.6.2.2 Determination of minimum inhibition concentration

Minimum inhibition concentrations (MICs) were determined by the Broth microdilution method (CLSI M7-A4, 2002) for bacteria. Test samples were dissolved in dimethyl sulfoxide (DMSO). Serial 2-fold dilutions of the test samples were mixed with melted Mueller Hinton Broth (MHB) in microtiter plates. Final concentrations of the test sample in broth ranged from 1280–2.5 μg/mL. 50 μL of inoculum suspensions were added in each well (final concentration $1x10^4$ CFU/well). The inoculated plate were incubated at 35 ºC for 16-18 h. A 0.18% resazurin solution (10 μL) was dropped in microtiter plate and incubated in 35 ºC for 2-3 h. The blue color shown demonstrating inhibition of microbial growth while the pink color indicated no activity. MICs were recorded by reading the lowest concentration that inhibited visible growth. The test was performed in triplicates. Vancomycin, gentamycin and streptomycin were used as positive control drugs. Growth controls were performed on agar containing DMSO.

CHAPTER 4

RESULTS AND DISCUSSION

The leaves of *Camellia sinensis* var. *assamica* were collected from Doi Mae Salong, Chiang Rai, Thailand. The dried leaves of *Camellia sinensis* var. *assamica* were extracted with hexane, dichloromethane and methanol at room temperature, respectively. Upon chromatrographic separation the crude dichloromethane extract yielded a new compound: 13-methyl lansic acid (**2**) and three known compounds: *β*-sitosterol (**1**), lansionic acid (**3**) and stigmasterol (**4**). Separation of methanolic extract produced seven known compounds: (+)catechin (**5**), 21*R*-hydroxyonocera-8(26),14-dien-3-one (**6**), lupeol (**7**), lupenone (**8**), (-)epigallocatechin gallate (**9**), (-)epicatechin gallate (**10**) and (-) epicatechin (**11**). Their structures were determined by spectroscopic evidences, especially 1D and 2D NMR spectral data.

4.1 Spectroscopic data of pure compounds

Compound 1

Melting point : 139-142 $^{\circ}$C

IR (KBr) ν (cm^{-1}) : 3425, 2924, 2854

^{1}H NMR (500 MHz) ($CDCl_3$) (δ ppm) : 5.36 (1H, *m*, H-6), 3.53 (1H, *m*, H-3), 2.31 (1H, *m*, H-4), 2.24 (1H, *m*, H-4), 2.03 (*m*, H-12), 1.98 (2H, *m*, H-7), 1.96 (*m*, H-2), 1.86 (*m*, H-16), 1.84 (*m*, H-1), 1.83 (*m*, H-16), 1.66 (*m*, H-25), 1.59 (*m*, H-2), 1.57 (*m*, H-11), 1.50 (*m*, H-8), 1.29 (*m*, H-22), 1.28 (*m*, H-12, H-20), 1.25 (*br s*, H-28), 1.16 (*m*, H-23), 1.15 (*m*, H-15, H-17), 1.08 (*m*, H-1, H-15), 1.02 (*m*, H-11, H-14), 1.01 (3H, *s*, H-19), 1.00 (*s*, H-22), 0.93 (*m*, H-9, H-24), 0.92 (3H, *d*, *J* = 6.5 Hz, H-21), 0.85 (3H, *t*, *J* = 8.0 Hz, H-29), 0.84 (3H, *d*, *J* = 6.5 Hz, H-26), 0.81 (3H, *d*, *J* = 6.5 Hz, H-27), 0.68 (3H, *s*, H-18)

Compound 2

Melting point : 182-183.5 °C

$[\alpha]_D^{29}$ -25.30 ° (c = 0.02, CH_3OH)

IR (KBr) ν (cm^{-1}) : 3465, 1730, 1650, 1520, 1400, 898

^1H NMR (500 MHz) ($CDCl_3$) (δ ppm) : 5.38 (*m*, H-15), 4.89 (*s*, H-26), 4.87 (*s*, H-23), 4.83 (*s*, H-29), 4.79 (*s*, H-29), 4.69 (*s*, H-23), 4.66 (*s*, H-26), 2.49 (*m*, H-19), 2.45 (*m*, H-1), 2.36 (*m*, H-7), 2.29 (*m*, H-1, H-2), 2.26 (*m*, H-17), 2.25 (*m*, H-5), 2.22 (*m*, H-16), 2.18 (*m*, H-19), 1.99 (*dt*, *J* = 12.5, 4.5 Hz, H-7), 1.89 (*m*, H-13), 1.85 (*m*, H-2), 1.84 (*s*, H-31), 1.81 (*m*, H-16), 1.81 (*s*, H-27), 1.79 (*m*, H-9), 1.77 (*m*, H-11, H-20), 1.77 (*s*, H-30), 1.72 (*s*, H-24), 1.68 (*m*, H-6), 1.66 (*m*, H-20), 1.61 (*m*, H-6), 1.46 (*m*, H-12), 1.29 (*t*, *J* = 10.5 Hz, H-11), 1.16 (*t*, *J* = 12.5 Hz, H-12), 0.80 (*s*, H-28), 0.72 (*s*, H-25)

^{13}C NMR (125 MHz) ($CDCl_3$) (δ ppm) : 181.6, 181.5, 147.9, 147.3, 147.0, 135.7, 121.9, 114.0, 113.7, 107.2, 51.6, 50.6, 48.7, 47.5, 41.5, 38.6, 38.0, 32.9, 31.9, 30.4, 29.5, 29.2, 29.0, 28.5, 27.8, 27.4, 23.5, 23.1, 22.7, 17.7, 15.9

DEPT 135° ($CDCl_3$) (δ ppm) : 29.2, 23.5, 23.1, 22.7, 17.7, 15.9 (CH_3); 114.0, 113.7, 107.2, 38.0, 32.9, 31.9, 30.4, 29.5, 29.0, 27.8, 28.5, 27.4 (CH_2); 121.9, 51.6, 50.6, 47.5, 48.7 (CH); 181.6, 181.5, 147.9, 147.3, 147.0, 135.7, 41.5, 38.6 (C)

EIMS *m/z* (% relative intensity) : under investigation

HRMS *m/z* : under investigation

Compound 3

Melting point : 82.6-83.1 °C

IR (KBr) ν (cm^{-1}) : 3400-2800, 1710, 1640, 1460, 1380, 890

^1H NMR (500 MHz) ($CDCl_3$) (δ ppm) : 5.40 (1H, *br s*, H-15), 4.91 (1H, *s*, H_{eq}-26), 4.89 (1H, *s*, H_{eq}-29), 4.80 (1H, *s*, H_{ax}-29), 4.69 (1H, *s*, H_{ax}-26), 2.68 (1H, *m*, H_{eq}-2), 2.45 (1H, *m*, H_{eq}-20), 2.42 (2H, *m*, H-7), 2.37 (1H, *m*, H_{ax}-2), 2.29 (1H, *m*, H_{ax}-

20), 2.20 (1H, *m*, H-17), 2.09 (1H, *m*, H_{eq}-16), 2.03 (1H, *m*, H_{eq}-1), 1.87 (1H, *m*, H_{ax}-16), 1.83 (1H, *m*, H-13), 1.79 (3H, *s*, H-30), 1.75 (3H, *m*, H-27), 1.69 (1H, *m*, H_{eq}-6), 1.68 (3H, *m*, H-5, H-19), 1.67 (1H, *m*, H_{eq}-11), 1.61 (1H, *m*, H-9), 1.50 (1H, *m*, H_{ax}-1), 1.48 (1H, *m*, H_{ax}-6), 1.40 (1H, *m*, H_{ax}-11), 1.25 (2H, *m*, H-12), 1.09 (3H, *s*, H-23), 1.05 (1H, *m*, H-1), 1.02 (3H, *s*, H-25), 0.86 (3H, *s*, H-24), 0.85 (3H, *s*, H-28)

^{13}C NMR (125 MHz) ($CDCl_3$) (δ ppm) : 217.2, 179.7, 147.5, 147.5, 135.8, 121.8, 113.4, 107.6, 57.5, 55.0, 49.1, 48.2, 47.7, 39.3, 38.6, 37.8, 37.5, 34.7, 32.6, 29.3, 28.9, 27.2, 26.2, 26.0, 25.1, 22.9(x2), 21.7, 16.3, 14.1

Compound 4

Melting point : 156-157 $^{\circ}$C

$[\alpha]_D^{29}$ + 20° (c = 0.03 in CH_3OH)

IR (KBr) ν(cm^{-1}) : 3433, 2959, 2869

^{1}H NMR (500 MHz) ($CDCl_3$) (δ ppm) : 5.36-5.33 (*m*), 5.16 (*dd*), 5.02 (*dd*), 3.56-3.48, 1.05, 1.02, 0.86, 0.82, 0.80, 0.69

Compound 5

Melting point : 169.7-170.3 $^{\circ}$C

$[\alpha]_D^{29}$ + 20° (c = 0.03 in CH_3OH)

^{1}H NMR (500 MHz) (Acetone-d_6) (δ ppm) : 28.30 (1H, *br s*, OH), 8.11 (1H, *br s*, OH), 8.00 (1H, *br s*, OH), 7.94 (1H, *br s*, OH), 6.90 (1H, *d*, *J* = 2.0 Hz, H-2′), 6.80 (1H, *d*, *J* = 8.0 Hz, H-5′), 6.76 (1H, *dd*, *J* = 8.0, 2.0 Hz, H-6′), 6.03 (1H, *d*, *J* = 2.5 Hz, H-6), 5.88 (1H, *d*, *J* = 2.5 Hz, H-8), 4.56 (1H, *d*, *J* = 7.5 Hz, H-2), 3.99 (1H, *m*, H-3), 3.03 (1H, *br s*, OH-3), 2.90 (1H, *dd*, *J* = 16.0, 5.0 Hz, H_a-4), 2.53 (1H, *dd*, *J* = 16.0, 8.5 Hz, H_b-4)

^{13}C NMR (125 MHz) (Acetone-d_6) (δ ppm) : 157.8, 157.2, 156.9, 145.7, 145.6, 132.1, 120.1, 115.7, 115.2, 100.6, 96.1, 95.4, 82.7, 68.3, 28.9

Compound 6

Melting point : 101-102 °C

IR (KBr) ν(cm^{-1}) : 3400-2800, 1710

^{1}H NMR (500 MHz) ($CDCl_3$) (δ ppm) : 5.42 (1H, *br s*, H-15), 4.86 (1H, *s*, H_a-26), 4.55 (1H, *s*, H_b-26), 3.27 (1H, *dd*, *J* = 7.3, 3.0 Hz, H-21), 2.41 (2H, *qd*, H-2), 2.40 (2H, *m*, H-7), 2.04 (1H, *m*, H-1), 1.95 (1H, *m*, H-11), 1.94 (1H, *m*, H-1), 1.90 (1H, *m*, H-11), 1.76 (1H, *m*, H-19), 1.72 (3H, *s*, H-27), 1.69 (1H, *m*, H-12), 1.60 (3H, *m*, H-9, H-20), 1.59 (2H, *m*, H-5), 1.58 (1H, *m*, H-13), 1.54 (1H, *m*, H-12), 1.40 (2H, *m*, H-16), 1.38 (2H, *m*, H-6), 1.13 (1H, *m*, H-17), 1.09 (3H, *s*, H-24), 1.06 (1H, *m*, H-19), 1.05 (3H, *s*, H-23), 1.00 (3H, *s*, H-30), 0.94 (3H, *s*, H-25), 0.77 (3H, *s*, H-29), 0.67 (3H, *s*, H-28)

^{13}C NMR (125 MHz) ($CDCl_3$) (δ ppm) : 217.2, 148.0, 135.7, 121.6, 106.9, 78.3, 56.5, 54.6, 54.3, 51.5, 47.5, 39.2, 39.1, 38.1, 37.2, 36.4, 34.7, 38.0, 28.2, 27.9, 25.5, 24.9, 24.8, 23.9, 24.0, 22.2, 22.1, 15.4, 14.6, 13.3

Compound 7

Melting point : 213-215 °C

IR (KBr) ν(cm^{-1}) : 3235, 1640, 1490, 1382, 1185, 1105, 1040, 984, 943

^{1}H NMR (300 MHz) ($CDCl_3$) (δ ppm) : 4.68 (*d*, *J* = 2.4 Hz, H-29), 4.56 (1H, *m*, H-29), 3.39 (1H, *dd*, *J* = 5.7, 1.5 Hz, H-3), 2.39 (*ddd*, *J* = 5.7, 5.7, 5.4 Hz, H-19), 1.95 (*m*, H-21), 1.70 (*m*, H-2, H-12), 1.68 (3H, *s*, H-30), 1.65 (*m*, H-13), 1.52 (*m*, H-15), 1.50 (2H, *m*, H-1), 1.45 (*m*, H-6, H-11), 1.40 (*m*, H-5, H-16, H-22), 1.35 (*m*, H-18), 1.20 (*m*, H-7, H-9, H-22), 1.03 (3H, *s*, H-26), 0.96 (3H, *s*, H-23), 0.93 (3H, *s*, H-27), 0.84 (3H, *s*, H-24), 0.82 (3H, *s*, H-25), 0.78 (3H, *s*, H-28)

^{13}C NMR (75 MHz) ($CDCl_3$) (δ ppm) : 151.1, 109.3, 76.3, 50.2, 49.0, 48.3, 48.0, 43.0, 42.9, 41.1, 40.0, 38.0, 37.6, 37.3, 35.6, 34.2, 33.1, 29.9, 28.3, 27,4, 25.4, 25.1, 22.2, 20.8, 19.3, 18.3, 18.0, 16.0, 15.9, 14.7

DEPT 135° ($CDCl_3$) (δ ppm) : 28.3, 22.2, 19.3, 18.0, 16.0, 15.9, 14.7 (CH_3); 109.3, 40.0, 35.6, 34.2, 33.1, 29.9, 27.4, 25.4, 25.1, 20.8, 18.3 (CH_2); 76.3, 50.2, 49.0, 48.3, 48.0, 38.0 (CH); 151.1, 43.0, 42.9, 41.1, 37.6, 37.3 (C)

Compound 8

Melting point : 168-171 °C

IR (KBr) ν(cm^{-1}) : 1701, 1280, 1139, 1085, 1062, 830, 785

^{1}H NMR (300 MHz) ($CDCl_3$) (δ ppm) : 4.70 (1H, *d*, *J* = 2.4 Hz, H_a-29), 4.58 (1H, *m*, H_b-29), 2.40 (*m*, H-2), 2.35 (1H, *m*, H-19), 1.90 (*m*, H-2, H-21, H-22), 1.72 (*m*, H-30), 1.71 (*m*, H-12, H-15), 1.66 (*m*, H-13), 1.48 (*m*, H-16), 1.43 (*m*, H-6, H-7, H-11), 1.42 (*m*, H-1), 1.36 (*m*, H-9, H-18), 1.35 (*m*, H-16), 1.32 (*m*, H-5), 1.26 (*m*, H-11), 1.21 (*m*, H-1), 1.08 (3H, *s*, H-23), 1.07 (3H, *s*, H-26), 1.00 (3H, *s*, H-24), 0.95 (3H, *s*, H-27), 0.94 (3H, *s*, H-25), 0.79 (3H, *s*, H-28)

^{13}C NMR (75 MHz) (CDCl3) (δ ppm) : 218.3, 151.0, 109.5, 55.2, 49.7, 48.3, 48.0, 47.5, 43.2, 42.9, 41.0, 40.0, 39.5, 38.5, 36.9, 36.1, 34.0, 33.8, 29.9, 27.8, 26.5, 25.3, 22.0, 21.0, 20.1, 19.4, 17.9, 16.0, 15.7, 14.5

Compound 9

Melting point : 254-255 °C

$[\alpha]_D^{29}$ – 181° (c = 0.3 in CH_3OH)

^{1}H NMR (500 MHz) (Acetone-d_6) (δ ppm) : 7.02 (1H, *s*, H-2'',6''), 6.62 (1H, *s*, H-6'), 6.04 (1H, *s*, H-2'), 6.02 (1H, *d*, *J* = 2.5 Hz, H-6), 5.54 (1H, *m*, H-2), 5.05 (1H, *br s*, H-1), 3.03 (1H, *dd*, J = 17.5, 4.5 Hz, H_a-4), 2.90 (1H, *dd*, J = 17.1, 2.0 Hz, H_b-4)

^{13}C NMR (125 MHz) (Acetone-d_6) (δ ppm) : 165.9, 157.7, 157.3, 156.2, 147.3, 147.2, 146.3, 138.7, 133.5, 131.8, 123.2, 108.1, 110.0, 107.2, 100.2, 96.9, 96.4, 79.2, 70.0, 26.1

Compound 10

Melting point : 257-258 $^{\circ}$C

$[\alpha]_D^{29}$ – 180 $^{\circ}$ (c = 0.3 in CH_3OH)

^{1}H NMR (500 MHz) (Acetone-d_6) (δ ppm) : 7.06 (1H, *d*, J = 1.5 Hz, H-2'), 7.02 (2H, *s*, H-2'',6''), 6.89 (1H, *dd*, J = 8.5, 1.5 Hz, H-6'), 6.76 (1H, *d*, J = 8.5 Hz, 5'), 6.05 (1H, *d*, J = 2.0 Hz, H-6), 6.03 (1H, *d*, J = 2.0 Hz, H-8), 5.54 (1H, *br s*, H-1), 5.12 (1H, *m*, H-2), 3.04 (1H, *dd*, J = 17.5, 5.0 Hz, H-4), 2.91 (1H, *dd*, 7.5, 2.5 Hz, H-4)

^{13}C NMR (125 MHz) (Acetone-d_6) (δ ppm) : 170.2, 156.1, 156.0, 155.2, 144.0, 143.8, 143.8, 137.5, 130.3, 121.1, 109.2, 118.0, 114.5, 113.2, 97.9, 95.1, 94.8, 75.5, 68.0, 26.2

Compound 11

Melting point : 239.4 – 240.5 $^{\circ}$C

$[\alpha]_D^{29}$ – 70 $^{\circ}$ (c = 0.03 in CH_3OH)

UV (MeOH) λ_{max}(log ε) : 282, 233

IR (KBr) ν(cm^{-1}) : 3323, 2921, 1631, 1606, 1519, 1469, 1281, 1142, 1090, 1060, 824, 792

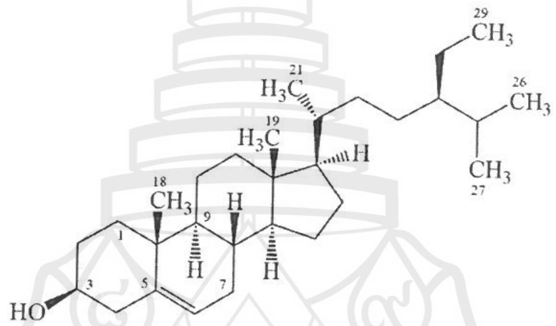
^{1}H NMR (500 MHz) (Acetone-d_6) (δ ppm) : 7.06 (1H, *d*, J = 1.5, Hz, H-2'), 6.84 (1H, *dd*, J = 8.0, 1.5, Hz, H-6'), 6.79 (1H, *d*, J = 8.0 Hz, H-5'), 6.02 (1H, *d*, J = 2.5 Hz, H-

6), 5.92 (1H, *d*, J = 2.5 Hz, H-8), 4.88 (1H, *s*, H-2), 4.20 (1H, *m*, H-3), 2.87 (1H, *dd*, J = 12.0, 4.5 Hz, H_a-4), 2.74 (1H, *dd*, J = 12.0, 4.5 Hz, H_b-4)

^{13}C NMR (125 MHz) (Acetone-d_6) (δ ppm) : 157.6, 157.5, 157.2, 145.4, 145.3, 132.3, 119.4, 115.5, 115.3, 99.8, 96.1, 95.7, 79.4, 66.9, 29.0

4.2 Structural determination

Compound 1: Stigmast-5-en-3β-ol (β-Sitosterol)

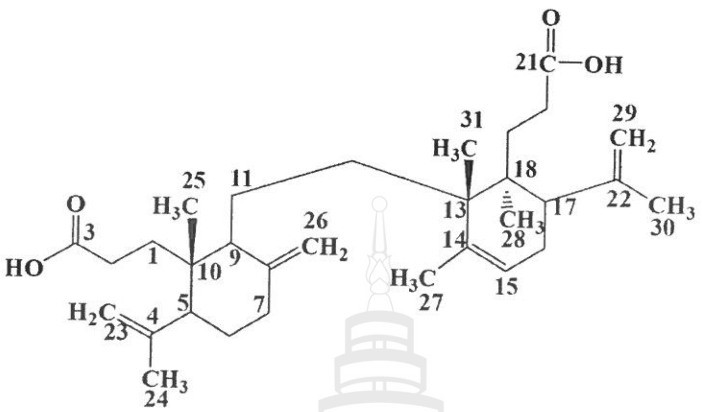


Compound **1** was colorless needles, m.p. 139-142 °C. The IR spectrum showed the absorption bands of O-H stretching (3425 cm^{-1}) and C-H stretching (2924, 2854 cm^{-1}). The ^{1}H NMR spectral data (**Table 4**) revealed the presence of an olefinic proton at δ5.36 (1H, *m*, H-6) and an oxymethine proton at δ3.53 (1H, *m*, H-3). The signals of six methyl groups were shown at δ0.68 (*s*, H-18), 0.81 (*d*, J = 6.5 Hz, H-27), 0.84 (*d*, J = 6.5 Hz, H-26), 0.85 (*t*, J = 8.0 Hz, H-29), 0.92 (*d*, J = 6.5 Hz, H-21) and 1.01 (*s*, H-19). Accordingly the structure of **1** was proposed to be stigmast-5-en-3β-ol. It was known as β-sitosterol (Nguyen *et al.*, 2004).

Table 4 NMR spectral data of compound **1**

Position	δ_H (*multiplicity*, J_{Hz})	
	Compound **1**	*β*-Sitosterol
1	1.08 (*m*), 1.84 (*m*)	1.06 (*m*), 1.85 (*m*)
2	1.59 (*m*), 1.96 (*m*)	1.61 (*m*), 1.95 (*m*)
3	3.53 (1H, *m*)	3.54 (1H, *m*)
4	2.24 (1H, *m*), 2.31 (1H, *m*)	2.27 (1H, *m*), 2.36 (1H, *m*)
6	5.36 (1H, *m*)	5.38 (1H, *m*)
7	1.98 (2H, *m*)	1.98 (2H, *m*)
8	1.50 (*m*)	1.52 (*m*)
9	0.93 (*m*)	0.93 (*m*)
11	1.02 (*m*), 1.57 (*m*)	1.02 (*m*), 1.56 (*m*)
12	1.28 (*m*), 2.03 (*m*)	1.18 (*m*), 2.02 (*m*)
14	1.02 (*m*)	1.01 (*m*)
15	1.08 (*m*), 1.15 (*m*)	1.08 (*m*), 1.12 (*m*)
16	1.83 (*m*), 1.86 (*m*)	1.83 (*m*), 1.86 (*m*)
17	1.15 (*m*)	1.12 (*m*)
18	0.68 (3H, *s*)	0.68 (3H, *s*)
19	1.01 (3H, *s*)	1.00 (3H, *s*)
20	1.28 (*m*)	1.36 (*m*)
21	0.92 (3H, *d*, 6.5)	0.92 (3H, *d*, 6.4)
22	1.00 (*s*), 1.29 (*m*)	1.00 (*s*), 1.34 (*m*)
23	1.16 (2H, *m*)	1.18 (2H, *m*)
24	0.93 (*m*)	0.95 (*m*)
25	1.66 (*m*)	1.66 (*m*)
26	0.84 (3H, *d*, 6.5)	0.82 (3H, *d*, 6.8)
27	0.81 (3H, *d*, 6.5)	0.84 (3H, *d*, 6.8)
28	1.25 (*br s*)	1.26 (*br s*)
29	0.85 (3H, *t*, 8.0)	0.84 (3H, *t*, 7.6)

Compound 2: 13-methyl lansic acid



Compound **2** was obtained as a white solid, m.p. 182-183.5 °C, $[\alpha]_D^{29}$ -25.30 ° (c = 0.02, CH_3OH). The IR spectrum exhibited absorption bands of hydroxyl groups at 3465 cm^{-1}, two carbonyl groups at 1710 and 1650 cm^{-1} and exomethylene groups at 898 cm^{-1}. The ^1H NMR spectral data (**Table 5**) showed *singlet* resonances of three tertiary methyl groups at δ 0.72 (H-25), 0.80 (H-28) and 1.84 (H-31), three olefinic methyl groups at δ 1.72 (H-24), 1.77 (H-30) and 1.84 (H-31). Seven olefinic protons were observed at δ 5.38 (*br s*, H-15), 4.80, 4.70 (*s*, H-23), 4.89, 4.66 (*s*, H-26) and 4.83, 4.79 (*s*, H-29). The characteristic signals of methylene protons H-11 and H-12 were resonated at δ 1.78 (*m*), 1.29 (*t*, J = 10.0 Hz) and 1.47 (*m*), 1.16 (*t*, J = 12.0 Hz), respectively. ^{13}C NMR spectrum (**Table 5**) revealed the presence of a trisubstituted olefin carbon at δ 135.7 (C-14) and 121.9 (C-15), three exomethylene carbons at δ 147.0 (C-4), 147.9 (C-8), 147.3 (C-22), 113.7 (C-23), 107.2 (C-26) and 114.0 (C-29) and two carboxyl carbons at δ 181.6 (C-3) and 181.5 (C-21). This characterization is consistent with the bicyclic triterpene skeleton (Kiang, *et al.*, 1967). The location of six methyl groups were supported by HMBC correlations between H-24 to C-23, C-5 and C-4; H-25 to C-10, C-9 and C-1; H-27 to C-15, C-14, C-13; H-28 to C-18, C-17, C-13; H-30 to C-29, C-22, C-17 and H-31 to C-18, C-13, C-12. The spectral data of **2** were similar to those of lansic acid (Tanaka *et al.*,

2002) except there was an additional methyl group located at C-13. Thus 13-methyl lansic acid was assigned for **2**. It is a new bicyclic triterpene derivative.

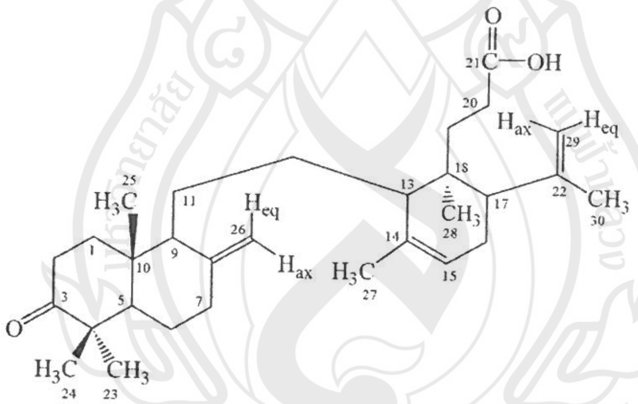
Table 5 NMR spectral data of compound **2**

Position	δ_H (*multiplicity*, J_{Hz})	δ_C	DEPT	HMBC
1	2.50 (*m*), 2.28 (*m*)	29.0	CH_2	C-2, C-3
2	1.85 (*m*), 2.28 (*m*)	31.9	CH_2	-
3	-	181.6	C	-
4	-	147.0	C	-
5	2.27 (*m*)	50.6	CH	C-10
6	1.67 (*m*), 1.60 (*m*)	30.4	CH_2	-
7	2.37 (*m*), 1.99 (*m*)	38.0	CH_2	C-6, C-9, C-26
8	-	147.9	C	-
9	1.79 (*m*)	51.6	CH	-
10	-	41.5	C	-
11	1.78 (*m*), 1.29 (*t*, 10.0)	27.4	CH_2	-
12	1.47 (*m*)1.16 (1H, *t*, 12.0)	27.8	CH_2	C-8, C-11
13	-	47.5	C	-
14	-	135.7	C	-
15	5.38 (*br s*)	121.9	CH	C-9, C-17, C-27
16	2.24 (*m*), 1.81 (*m*)	29.5	CH_2	-
17	2.27 (*m*)	48.7	CH	C-19
18	-	38.6	C	-
19	2.49 (*m*), 2.18 (*m*)	28.5	CH_2	C-17, C-20, C-21
20	1.77 (*m*), 1.66 (*m*)	32.9	CH_2	-
21	-	181.5	C	-
22	-	147.3	C	-
23	-	113.7	CH_2	C-4, C-5, C-24
24	4.87 (*s*), 4.69 (*s*)	23.5	CH_3	C-4, C-5, C-23

Table 5 (continued)

Position	δ_H (*multiplicity*, J_{Hz})	δ_C	DEPT	HMBC
25	1.72 (*s*)	17.7	CH_3	C-1, C-9, C-10
26	0.72 (*s*)	107.2	CH_2	C-7, C-8, C-9
27	4.89 (*s*), 4.66 (*s*), 1.81 (*s*)	23.1	CH_3	C-13, C-14, C-15
28	1.80 (*s*)	15.9	CH_3	C-13, C-17, C-18
29	4.83 (*s*), 4.79 (*s*)	114.0	CH_2	C-17, C-22, C-30
30	1.77 (*s*)	22.7	CH_3	C-17, C-22, C-29
31	1.84 (*s*)	29.2	CH_3	C-12, C-13, C-18

Compound 3: 3β-Hydroxyonocera-8(26),14-dien-21-one (Lansionic acid)



Compound **3** is colorless amorphous solid, m.p. 82.6-83.1 °C. The IR spectrum showed a broad absorption bands of a hydroxyl group at 3400-2800 cm^{-1} and a non-chelated conjugated carbonyl group at 1710 cm^{-1}. The ^1H NMR spectrum (**Table 6**) exhibited the resonances of six tertiary methyl groups at δ 0.85 (H-28), 0.86 (H-24), 1.02 (H-25), 1.09 (H-23), 1.75 (H-27) and 1.79 (H-30). The characteristic signals of methylene protons were observed at δ 4.91 (1H, *s*, H_{eq}-26), 4.69 (1H, *s*, H_{ax}-26), 4.89 (1H, *s*, H_{eq}-29) and 4.80 (1H, *s*, H_{ax}-29). ^{13}C NMR spectral data (**Table 6**) showed the signals of a ketone at δ 217.2 (C-3), a carboxyl group at δ 179.7 (C-21), a trisubstituted olefin at δ 135.8

(C-14) and 121.8 (C-15), and two exomethylenes at δ 107.6 (C-26) and 113.4 (C-29). ^{1}H and ^{13}C NMR spectral data as well as melting point were in good agreement with those of 3β-hydroxyonocera-8(26),14-dien-21-one or lansionic acid (Tanaka *et al.*, 2002).

Table 6 NMR spectral data of compound **3**

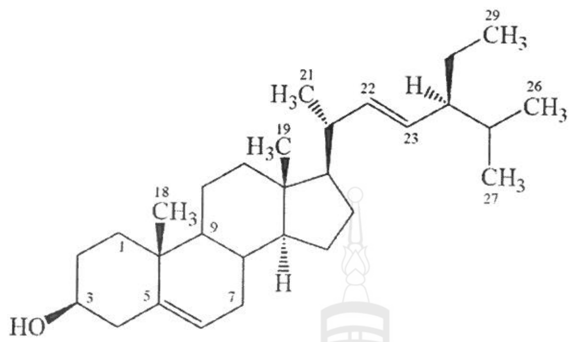
Position	Compound **3**		Lansionic acid	
	δ_H (*multiplicity*, J_{Hz})	δ_C*	δ_H (*multiplicity*, J_{Hz})	δ_C
1	H_{ax} : 1.50 (1H, *m*)	37.5	1.57 (1H, *m*)	37.6
	H_{eq} : 2.03 (1H, *m*)		2.02 (1H, *m*)	
2	H_{ax} : 2.37 (1H, *m*)	34.7	2.38 (1H, *m*)	34.7
	H_{eq} : 2.68 (1H, *m*)		2.61 (1H, *m*)	
3	-	217.2	-	217.2
4	-	47.7	-	47.8
5	1.68 (1H, *m*)	55.0	1.66 (1H, *m*)	55.1
6	H_{ax} : 1.48 (1H, *m*)	25.1	1.48 (1H, *m*)	25.1
	H_{eq} : 1.69 (1H, *m*)		1.69 (1H, *m*)	
7	2.42 (2H, *m*)	37.8	2.43 (2H, *m*)	37.8
8	-	147.5	-	147.5
9	1.61 (1H, *m*)	57.5	1.61 (1H, *m*)	57.5
10	-	39.3	-	39.3
11	H_{ax} : 1.40 (1H, *m*)	26.2	1.39 (1H, *m*)	26.3
	H_{eq} : 1.67 (1H, *m*)		1.67 (1H, *m*)	
12	1.25 (2H, *m*)	27.2	1.21 (2H, *m*)	27.2
13	1.83 (1H, *m*)	48.2	1.82 (1H, *m*)	48.3
14	-	135.8	-	135.8

Table 6 (continued)

Position	Compound **3**		Lansionic acid	
	δ_H (*multiplicity*, J_{Hz})	δ_C*	δ_H (*multiplicity*, J_{Hz})	δ_C
15	5.40 (1H, *br s*)	121.8	5.36 (1H, *br s*)	121.8
16	H_{ax} : 1.87 (1H, *m*)	29.3	1.86 (1H, *m*)	29.4
	H_{eq} : 2.09 (1H, *m*)		2.19 (1H, *m*)	
17	2.20 (1H, *m*)	49.1	2.20 (1H, *m*)	49.2
18	-	38.6	-	38.7
19	1.68 (2H, *m*)	32.6	1.68 (2H, *m*)	32.6
20	H_{ax} : 2.29 (1H, *m*)	28.9	2.27 (1H, *m*)	28.6
	H_{eq} : 2.45 (1H, *m*)		2.43 (1H, *m*)	
21	-	179.7	-	178.0
22	-	147.5	-	147.6
23	1.09 (3H, *s*)	26.0	1.07 (3H, *s*)	25.9
24	0.86 (3H, *s*)	21.7	1.00 (3H, *s*)	21.7
25	1.02 (3H, *s*)	14.1	0.84 (3H, *s*)	14.1
26	H_{ax} : 4.69 (1H, *s*)	107.6	4.89 (1H, *s*)	107.6
	H_{eq} : 4.91 (1H, *s*)		4.61 (1H, *s*)	
27	1.75 (3H, *m*)	22.9	1.72 (3H, *m*)	22.9
28	0.85 (3H, *s*)	16.3	0.81 (3H, *m*)	16.3
29	H_{ax} : 4.80 (1H, *s*)	113.4	4.81 (1H, *s*)	114.0
	H_{eq} : 4.89 (1H, *s*)		4.77 (1H, *s*)	
30	1.79 (3H, *s*)	22.9	1.76 (3H, *s*)	22.8

* Carbon type was deduced from DEPT experiments.

Compound 4: 5,22-Stigmastadien-3*β*-ol (Stigmasterol)

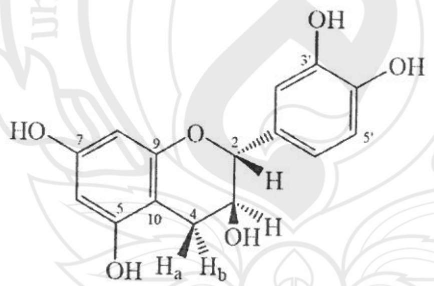


Compound **4** was obtained as a white solid, m.p. 156-157 °C, $[\alpha]_D^{29}$ -55.48 ° (c = 0.02, CH_3OH). In IR spectrum, the absorption bands of C-H stretching (2959 and 2869 cm^{-1}) and O-H stretching (3433 cm^{-1}) were shown. The ^1H NMR spectrum (**Table 7**) contained signals for an oxymethine proton at δ 3.56-3.48, three olefinic protons at δ 5.36-5.33 (*m*), 5.16 (*dd*) and 5.02 (*dd*) and six methyl groups at δ 1.02, 1.05, 0.86, 0.82, 0.80 and 0.69. The ^1H NMR data, optical rotation value and melting point were corresponded to the previous reported data (Forgo and Köver, 2004). Therefore, compound **4** was assigned to be 5,22-stigmastadien-3*β*-ol or stigmasterol.

Table 7 NMR spectral data of compound **4**

Position	δ_H (*multiplicity*, J_{Hz})	
	Compound **4**	Stigmasterol
3	3.56-3.48 (*m*)	3.51 (*m*)
6	5.36-5.33 (*m*)	5.34 (*m*)
18	0.69	0.70 (*s*)
19	1.02	10.1 (*s*)
21	1.05	1.03 (*d*, 6.2)
22	5.16 (*dd*)	5.17 (*dd*, 15.2, 8.6)
23	5.02 (*dd*)	5.04 (*dd*, 15.2, 8.6)
26	0.86	0.85 (*d*, 6.4)
27	0.80	0.80 (*d*, 6.4)
29	0.82	0.81 (*t*, 7.3)

Compound 5: 3,5,7,3',4'-Pentahydroxyflavan ((+) Catechin)



Compound **5** was isolated as a cream solid, m.p. 169.7-170.3 °C. The optical rotation was $[\alpha]_D^{29}$ +20° (c = 0.03 in CH_3OH). The ^1H NMR spectrum (**Table 8**) showed two *doublet* signals with *meta*-coupling (J = 2.5 Hz) at δ 6.03 (H-6) and 5.88 (H-8). The resonances of aromatic protons H-2', H-6' and H-5' were observed at δ 6.90 (1H, *d*, J = 2.0 Hz), 6.76 (1H, *dd*, J = 8.0 and 2.0 Hz) and 6.80 (1H, *d*, J = 8.0

Hz), respectively. In addition, the resonance of H-2 (δ 4.56, *d*, *J* = 7.5 Hz), H-3 (δ 4.00, *m*), H_a-4 (δ 2.90, *dd*, *J* = 16.0 and 5.0 Hz) and H_b-4 (δ 2.53, *dd*, *J* = 16.0 and 8.5 Hz) indicated that the configuration of H-2 and H-3 are *trans*. ^{13}C NMR exhibited the existence of a methylene carbon (δ 28.9), seven methine carbons (δ 120.1 115.7, 115.2, 96.1, 95.4, 82.7 and 68.3) seven quaternary carbons (δ 157.8, 157.2, 157.0, 145.7 and 132.1). The ^{1}H and ^{13}C NMR data, optical rotation value and melting point were corresponded to 3,5,7,3',4'-pentahydroxyflavan (Panthong, 1999). Therefore, **5** was proposed to be (+)catechin.

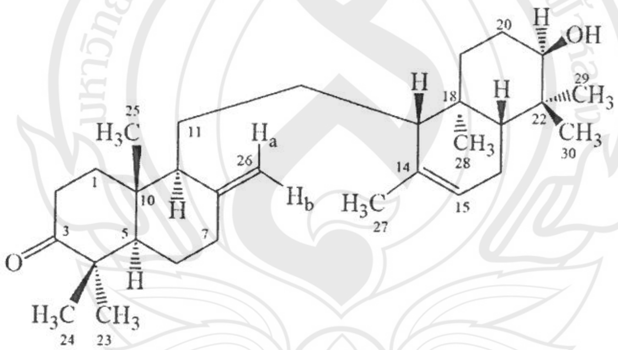
Table 8 NMR spectral data of compound **5**

Position	Compound **5**		(+)Catechin	
	δ_H (*multiplicity*, J_{Hz})	δ_C	δ_H (*multiplicity*, J_{Hz})	δ_C
2	4.56 (1H, *d*, 7.5)	82.7	4.84 (1H, *br s*)	78.1
3	4.00 (1H, *m*)	68.3	4.19 (1H, *br s*)	65.6
4	H_a : 2.90 (1H, *dd*, 16.0, 5.0)	28.9	2.84 (1H, *dd*, 16.5, 4.5) 2.76 (1H, *dd*, 16.5, 2.5)	27.9
5	H_b : 2.53 (1H, *dd*, 16.0, 8.5)	157.8	-	156.4
6		96.1	6.05 (1H, *d*, 2.0)	95.6
7	-	157.2	-	156.2
8	6.03 (1H, *d*, 2.5)	95.4	5.96 (1H, *d*, 2.0)	94.5
9	-	157.0	-	155.5
10	5.88 (1H, *d*, 2.5)	100.6	-	98.3
1'	-	132.1	-	130.4
2'	-	145.7	7.25 (1H, *d*, 2.0)	144.8
3'	-	145.7	-	144.2

Table 8 (continued)

Position	Compound **5**		(+)Catechin	
	δ_H (*multiplicity*, J_{Hz})	δ_C	δ_H (*multiplicity*, J_{Hz})	δ_C
4'	-	145.7	-	144.4
5'	6.80 (1H, *d*, 8.0)	120.1	6.83 (1H, *d*, 8.0)	117.8
6'	6.76 (1H, *dd*, 8.0, 2.0)	115.2	6.80 (1H, *dd*, 8.0, 2.0)	114.1
OH-3	3.03 (1H, *br s*)	-	3.64 (1H, *br s*)	-
OH-5	8.11 (1H, *br s*)	-	8.85 (1H, *br s*)	-
OH-7	8.30 (1H, *br s*)	-	8.74 (1H, *d*, 2.0)	-
OH-3'	8.00 (1H, *br s*)	-	8.40 (1H, *br s*)	-
OH-4'	7.94 (1H, *br s*)	-	8.17 (1H, *br s*)	-

Compound 6: 21*R*-Hydroxyonocera-8(26),14-dien-3-one



Compound **6** was a colorless amorphous solid, m.p. 101-102 °C. The IR spectrum showed a broad absorption band at 3400-2800 cm^{-1} and a strong absorption at 1710 cm^{-1}. The ^{1}H NMR spectral data (**Table 9**) indicated that **6** was similar to **3**. Six *singlet* signals of methyl protons H-23 (δ 1.05), H-24 (δ 1.09), H-25 (δ 0.94), H-27 (δ 1.72), H-29 (δ 0.77) and H-30 (δ 1.00) were observed. Two *singlet* signals for terminal methylene protons H_a-26 (δ 4.86) and H_b-26 (δ 4.55) were displayed in the spectrum. The resonances of an olefinic and an oxymethine protons were detected at δ 5.42 (*br s*, H-15) and δ 3.27 (*dd*, J = 7.0, 3.0 Hz, H-21), respectively. The ^{13}C NMR and DEPT experiments indicated the existence of a carbonyl carbon (δ 217.2), seven methyl carbons (δ 28.2, 24.9, 22.2, 22.1, 15.4, 14.6 and 13.3), ten methylene carbons (δ 106.9, 38.1, 38.0, 37.2, 34.7, 27.9, 25.5, 24.8, 24.0 and 29.9), six methine carbons (δ 121.6, 56.5, 54.6, 54.3, 51.5 and 27.9) and six quarternary carbons (δ 148.0, 135.7, 47.5, 39.2, 39.1 and 36.4). The assignment was in agreement with the previous data of 21*R*-hydroxyonocera-8(26),14-dien-3-one (Tanaka *et al.*, 2002).

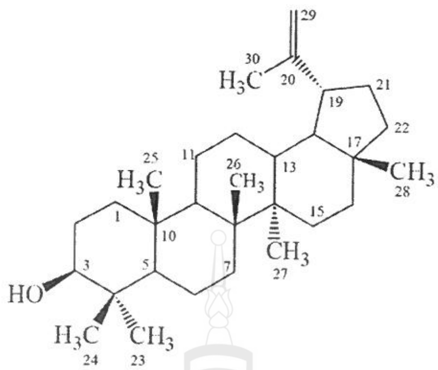
Table 9 NMR spectral data of compound **6**

Position	Compound **6**		21*R*-Hydroxyonocera-8(26),14-dien-3-one	
	δ_H (*multiplicity*, J_{Hz})	δ_C	δ_H (*multiplicity*, J_{Hz})	δ_C
1	2.04 (1H, *m*), 1.94 (1H, *m*)	38.1	2.01 (1H, *m*), 1.93 (1H, *m*)	37.8
2	2.41 (2H, *qd*)	34.7	2.37 (2H, *m*)	34.7
3	-	217.2	-	217.2
4	-	47.5	-	47.8
5	1.59 (2H, *m*)	54.3	1.57 (1H, *m*)	55.2
6	1.38 (2H, *m*)	24.8	1.35 (2H, *m*)	25.7
7	2.40 (2H, *m*)	38.0	2.41 (2H, *m*)	37.9
8	-	148.0	-	147.4
9	1.60 (1H, *m*)	56.5	1.64 (1H, *m*)	56.6
10	-	39.2	-	38.7
11	1.95 (1H, *m*), 1.90 (1H, *m*)	23.9	1.95 (1H, *m*), 1.92 (1H, *m*)	23.5

Table 9 (continued)

Position	Compound **6**		21*R*-Hydroxyonocera-8(26),14-dien-3-one	
	δ_H (*multiplicity*, J_{Hz})	δ_C	δ_H (*multiplicity*, J_{Hz})	δ_C
12	1.69 (1H, *m*), 1.54 (1H, *m*)	24.0	1.69 (1H, *m*), 1.51 (1H, *m*)	25.1
13	1.58 (1H, *m*)	54.6	1.57 (1H, *m*)	55.3
14	-	135.7	-	135.2
15	5.42 (1H, *br s*)	121.6	5.37 (1H, *br s*)	122.1
16	1.40 (2H, *m*)	25.5	1.49 (2H, *m*)	25.8
17	1.13 (1H, *m*)	51.5	1.16 (1H, *m*)	49.6
18	-	36.4	-	36.5
19	1.76 (1H, *m*), 1.06 (1H, *m*)	37.2	1.76 (1H, *m*), 1.06 (1H, *m*)	37.2
20	1.60 (2H, *m*)	27.9	1.60 (2H, *m*)	27.4
21	3.27 (1H, *dd*, 7.0, 3.0)	78.3	3.23 (1H, *dd*, 11.0, 5.0)	79.2
22	-	39.1	-	39.2
23	1.05 (3H, *s*)	28.2	1.08 (3H, *s*)	26.0
24	1.09 (3H, *s*)	22.1	1.01 (3H, *s*)	21.6
25	0.94 (3H, *s*)	14.6	0.83 (3H, *s*)	14.2
26	H_a : 4.86 (1H, *s*), H_b : 4.55 (1H, *s*)	106.9	4.89 (1H, *s*) 4.60 (1H, *s*)	107.6
27	1.72 (3H, *s*)	24.9	1.68 (3H, *s*)	22.3
28	0.67 (3H, *s*)	13.3	0.69 (3H, *s*)	13.6
29	0.77 (3H, *s*)	15.4	0.82 (3H, *s*)	15.1
30	1.00 (3H, *s*)	22.2	0.95 (3H, *s*)	17.9

Compound 7: Lup-20(29)-en-3β-ol (Lupeol)



Compound **7** was isolated as colourless crystal, m.p. 213-215 °C. The IR spectrum exhibited the absorption band of O-H stretching at 3235 cm^{-1}. The ^1H NMR spectrum (**Table 10**) showed the characteristic signals of a terminal olefinic methylene protons at δ 4.68 and 4.56 (1H each, *d*, *J* = 2.4 Hz) for H_a-29 and H_b-29, respectively. In addition, ^1H NMR spectrum showed the resonances of an oxymethine proton (δ 3.39, *dd*, *J* = 5.7 and 1.5 Hz, H-3) and seven methyl groups (δ 0.96 (H-23), 0.84 (H-24), 0.82 (H-25), 1.03 (H-26), 0.93 (H-27), 0.78 (H-28) and 1.68 (H-30)) were observed. ^1H NMR and ^{13}C NMR spectral data as well as melting point were identical with those of lup-20(29)-en-3β-ol which was known as lupeol (Imam *et al.*, 2007)

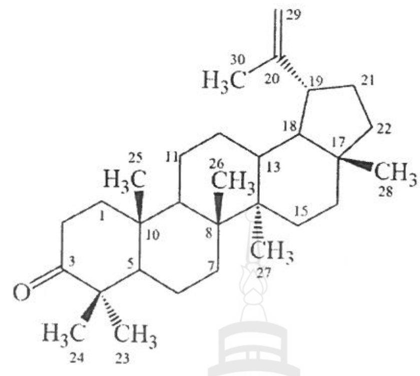
Table 10 NMR spectral data of compound **7**

Position	Compound 7		Lupeol	
	δ_H (*multiplicity*, J_{Hz})	δ_C	δ_H (*multiplicity*, J_{Hz})	δ_C
1	1.50 (2H, *m*)	35.6	1.68 (2H, *d*)	38.6
2	1.70 (*m*)	27.4	1.61 (1H, *d*), 1.54 (1H, *q*)	27.3
3	3.39 (1H, *dd*, 5.7, 1.5)	76.3	3.18 (1H, *dd*)	78.9
4	-	37.6	-	38.8
5	1.40 (*m*)	50.2	0.69 (1H, *d*)	55.2

Table 10 (continued)

Position	Compound **7**		Lupeol	
	δ_H (*multiplicity*, J_{Hz})	δ_C	δ_H (*multiplicity*, J_{Hz})	δ_C
6	1.45 (*m*)	18.3	1.54 (1H, *d*), 1.39 (1H, *q*)	18.2
7	1.20 (*m*)	33.1	1.41 (2H, *m*)	34.2
8	-	41.1	-	40.7
9	1.20 (*m*)	49.0	1.28 (1H, *d*)	50.3
10	-	37.3	-	37.1
11	1.45 (*m*)	20.8	1.42 (1H, *d*), 1.29 (1H, *q*)	20.9
12	1.70 (*m*)	25.1	1.07 (1H, *q*), 1.68 (2H, *d*)	25.0
13	1.65 (*m*)	38.0	1.67 (1H, *t*)	38.0
14	-	42.9	-	42.7
15	1.52 (*m*)	25.4	1.71 (1H, *t*), 1.01 (1H, *d*)	27.4
16	1.40 (*m*)	34.2	1.49 (1H, *d*), 1.38 (1H, *t*)	35.5
17	-	43.0	-	42.9
18	1.35 (*m*)	48.3	1.37 (1H, *t*), 0.91 (1H, *t*)	48.2
19	2.39 (*ddd*, 5.7, 5.7, 5.4)	48.0	2.39 (1H, *m*)	47.9
20	-	151.1	-	150.8
21	1.95 (1H, *m*)	29.9	1.93 (1H, *m*)	29.8
22	1.40 (1H, *m*), 1.20 (*m*)	40.0	1.42 (1H, *m*), 1.20 (1H, *m*)	39.9
23	0.96 (3H, *s*)	28.3	0.98 (3H, *s*)	27.9
24	0.84 (3H, *s*)	22.2	0.79 (3H, *s*)	15.3
25	0.82 (3H, *s*)	16.0	0.27 (3H, *s*)	16.1
26	1.03 (3H, *s*)	15.9	1.04 (3H, *s*)	15.9
27	0.93 (3H, *s*)	14.7	0.97 (3H, *s*)	14.5
28	0.78 (3H, *s*)	18.0	0.84 (3H, *s*)	17.9
29	4.68 (1H, *d*, 2.4), 4.56 (1H, *m*)	109.3	4.69 (1H, *m*), 4.56 (1H, *m*)	109.3
30	1.68 (3H, *s*)	19.3	1.69 (3H, *s*)	19.2

Compound 8: 3-*oxo*-Lupeol (Lupenone)



Compound **8** was a isolated as white solid, m.p. 168-171 °C. The IR spectrum showed the absorption band of C=O stretching at 1701 cm^{-1}. The ^1H NMR spectrum (**Table 11**) indicated that it was a triterpene derivative of **7**. The ^1H NMR spectrum exhibited the resonances of an isoprenyl side chain at δ 1.72 (H-30), 4.70 (*d*, *J* = 2.4 Hz, H_a-29) and 4.58 (*m*, H_b-29). Four *multiplet* signals at δ 1.42, 1.21 and 2.40, 1.90 were in agreement with the α,β-unsaturated ketone. The six methyl groups H-23, H-24, H-25, H-26, H-27 and H-28 resonated at δ 1.08, 1.00, 0.94, 1.07, 0.95 and 0.79, respectively. The ^{13}C NMR signals at δ 19.4, 109.5, 151.0 and 218.3 were attributed to the isopropenyl and carbonyl carbons, respectively. The absence of the methine proton signal at C-3 indicated that the carbonyl group located at C-3. The resulting structure was confirmed by ^{13}C NMR spectral data (**Table 11**). 3-*oxo*-Lupeol was assigned for compound **8**. It was known as lupenone (Boonsri, 2004).

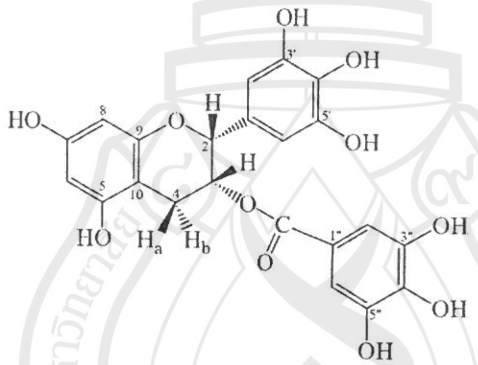
Table 11 NMR spectral data of compound **8**

Position	Compound **8**		Lupenone	
	δ_H (*multiplicity*, J_{Hz})	δ_C	δ_H (*multiplicity*, J_{Hz})	δ_C
1	1.21 (*m*), 1.42 (*m*)	39.5	1.20 (*m*), 1.40 (*m*)	39.6
2	1.90 (*m*), 2.40 (*m*)	33.8	1.89 (*m*), 2.43 (*m*)	34.1
3	-	218.3	-	218.2
4	-	47.5	-	47.3
5	1.32 (*m*)	55.2	1.30 (*m*)	54.9
6	1.43 (*m*)	20.1	1.45 (*m*)	19.7
7	1.43 (*m*)	34.0	1.45 (*m*)	33.6
8	-	41.0	-	40.8
9	1.36 (*m*)	49.7	1.38 (*m*)	49.8
10	-	36.9	-	36.9
11	1.43 (*m*), 1.26 (*m*)	22.0	1.28 (*m*), 1.45 (*m*)	21.5
12	1.71 (*m*)	25.3	1.70 (*m*)	25.2
13	1.66 (*m*)	38.5	1.68 (*m*)	38.2
14	-	43.2	-	42.9
15	1.71 (*m*)	27.8	1.70 (*m*)	47.4
16	1.35 (*m*), 1.48 (*m*)	36.1	1.36 (*m*), 1.48 (*m*)	35.5
17	-	42.9	-	43.0
18	1.36 (*m*)	48.3	1.38 (*m*)	48.2
19	2.35 (*m*)	48.0	2.43 (*m*)	48.0
20	-	151.0	-	105.9
21	1.90 (*m*)	29.9	1.90 (*m*)	29.8
22	1.90 (*m*)	40.0	1.90 (*m*)	40.0
23	1.08 (3H, *s*)	26.5	1.07 (*s*)	26.6
24	1.00 (3H, *s*)	21.0	1.02 (*s*)	21.0
25	0.94 (3H, *s*)	15.7	0.93 (*s*)	15.8
26	1.07 (3H, *s*)	16.0	1.07 (*s*)	16.0

Table 11 (continued)

Position	Compound **8**		Lupenone	
	δ_H (*multiplicity*, J_{Hz})	δ_C	δ_H (*multiplicity*, J_{Hz})	δ_C
27	0.95 (3H, *s*)	14.5	0.95 (*s*)	14.5
28	0.79 (3H, *s*)	17.9	0.79 (*s*)	18.0
29	H_a : 4.70 (1H, *d*, 2.4)	109.5	4.68 (*d*, 2.1)	109.4
	H_b : 4.58 (1H, *m*)		4.57 (*m*)	
30	1.72 (*m*)	19.4	1.70 (*s*)	19.3

Compound 9: (-)-Epigallocatechin gallate

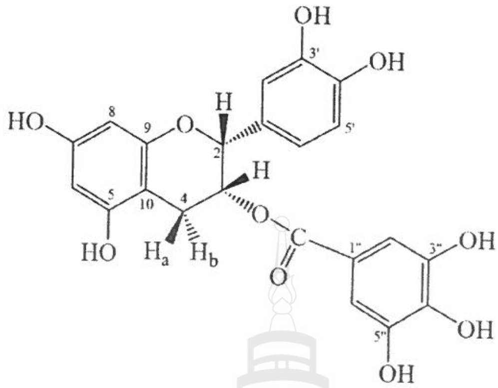


Compound **9** is a white powder, m.p. 254-255 °C. The optical rotation was $[\alpha]_D^{29}$ -181° (c = 0.3 in CH_3OH). The ^1H NMR spectrum (**Table 12**) showed two aromatic *singlets* at δ 6.62 (2H, H-2',6') and 7.02 (2H, H-2'',6'') which could be assigned for the symmetric protons in catechin B-ring and the galloyl moiety, respectively. Two *meta*-coupled protons, originated from catechin A-ring, were observed at δ 6.02 (1H, *d*, *J* = 2.5 Hz, H-6) and 6.04 (1H, *d*, *J* = 2.5 Hz, H-8). In addition, the signals at δ 5.05 (*br s*), 5.54 (*m*), 3.03 (*dd*, *J* = 17.5, 4.5, H_a-4) and 2.90 (*dd*, *J* = 17.1, 2.0, H_b-4) showed the typical resonances of H-2, H-3 and H-4 of catechin skeleton. Considering the coupling constant of H-2 (*broad singlet*), the relative stereochemistry of H-2 and H-3 should be a *cis*-configuration. A carbonyl carbon (δ 165.9) and three sp^3 carbons (δ 79.2, 70.0 and 26.1) were detected in ^{13}C NMR spectrum (**Table 12**). (-)Epigallocatechin gallate (Kim *et al*., 2001) then was proposed for **9**.

Table 12 NMR spectral data of compound **9**

Position	Compound **9**		(-)Epigallocatechin gallate	
	δ_H (*multiplicity*, J_{Hz})	δ_C	δ_H (*multiplicity*, J_{Hz})	δ_C
2	5.05 (1H, *br s*)	79.2	5.05 (1H, *br s*)	78.5
3	5.54 (1H, *m*)	70.0	5.53 (1H, *m*)	69.7
4	H_a : 3.03 (1H, *dd*, 17.5, 4.5)	26.1	3.02 (1H, *dd*, 17.5, 4.6)	25.2
	H_b : 2.90 (1H, *dd*, 17.1, 2.0)		2.89 (1H, *dd*, 17.4, 2.2)	
5	-	156.2	-	157.8
6	6.02 (1H, *d*, 2.5)	96.9	6.02 (1H, *d*, 2.4)	96.8
7	-	157.3	-	158.2
8	6.04 (1H, *d*, 2.5)	96.4	6.04 (1H, *d*, 2.0)	96.2
9	-	157.7	-	157.5
10	-	100.2	-	99.4
1'	-	107.2	-	107.1
2'	6.62 (1H, *s*)	147.3	6.61 (1H, *s*)	146.6
3'	-	133.5	-	133.5
4'	-	146.3	-	146.6
5'	-	108.1	-	107.1
6'	6.62 (1H, *s*)	131.8	6.61 (1H, *s*)	131.1
1''	-	123.2	-	122.2
2'', 6''	7.02 (1H, *s*)	110.0	7.02 (2H, *s*)	110.3
3'', 5''	-	147.2	-	146.3
4''	-	138.7	-	139.2
C=O	-	165.9	-	166.5

Compound 10: (-)Epicatechin gallate

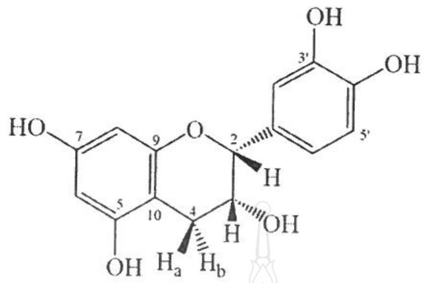


Compound **10** is a white powder, m.p. 257-258 °C. The optical rotation was $[\alpha]_D^{29}$ −181° (c = 0.3 in ethanol). The ^{1}H NMR spectral data (**Table 13**) was very similar to those of **9** except the resonances at δ 7.06 (*d*, *J* = 1.5 Hz, H-2'), 6.76 (*d*, *J* = 8.5 Hz, H-5') and 6.89 (*dd*, *J* = 8.5, 1.5 Hz, H-6') suggesting that B-ring of **10** was substituted with a catechol moiety instead of a pyrogallol. In ^{13}C NMR spectrum, a carbonyl (δ 170.2), three *sp*^{3} (δ 75.5, 68.0 and 26.2), six methine (δ 118.0, 114.5, 113.2, 109.2, 95.1 and 94.8) and ten quartenary (δ 156.1, 156.0, 155.2, 144.8, 144.0, 143.8, 137.5, 130.3, 121.1 and 97.9) carbon signals were detected. The coupling constant between H-2 and H-3 was almost zero as in the case of **9**. The final structure was identified as (–) epicatechin gallate by referring reported data (Kim *et al.*, 2001).

Table 13 NMR spectral data of compound **10**

Position	Compound **10**		(–)Epicatechin gallate	
	δ_H (*multiplicity*, J_{Hz})	δ_C	δ_H (*multiplicity*, J_{Hz})	δ_C
2	5.54 (1H, *br s*)	75.5	5.55 (1H, *br s*)	76.7
3	5.12 (1H, *m*)	68.0	5.14 (1H, *m*)	68.0
4	3.04 (1H, *dd*, 17.5, 5.0)	26.2	3.06 (1H, *dd*, 17.5, 5.0)	25.9
	2.91 (1H, *dd*, 17.5, 2.5)		2.93 (1H, *dd*, 17.5, 2.5)	
5	-	156.0	-	156.1
6	6.05 (1H, *d*, 2.0)	94.8	6.04 (1H, *d*, 2.0)	95.1
7	-	156.1	-	156.4
8	6.03 (1H, *d*, 2.0)	95.1	6.07 (1H, *d*, 2.0)	94.4
9	-	155.2	-	155.7
10	-	97.9	-	97.6
1'	-	130.3	-	130.0
2'	7.06 (1H, *d*, 1.5)	113.2	7.08 (1H, *d*, 2.0)	113.5
3'	-	143.8	-	144.1
4'	-	144.0	-	144.2
5'	6.76 (1H, *d*, 8.5)	114.5	6.78 (1H, *d*, 8.5)	114.2
6'	6.89 (1H, *dd*, 8.5, 1.5)	118.0	6.91 (1H, *dd*, 8.5, 2.0)	117.8
1"	-	121.1	-	120.4
2", 6"	7.02 (2H, *s*)	109.2	6.94 (2H, *s*)	108.5
3", 5"	-	144.8	-	144.6
4"	-	137.5	-	137.5
C=O	-	170.2	-	169.6

Compound 11: 3, 5, 7, 3', 4'-Pentahydroxyflavan ((–)Epicatechin)



Compound **11** was obtained as white powder, m.p. 239.4-240.5 °C. The optical rotation was $[\alpha]_D^{29}$ –70° (c = 0.03 in CH_3OH). The UV spectrum showed maxima absorptions at 282 and 233 nm. The IR spectrum exhibited the absorption band of O-H stretching (3323 cm^{-1}) and C=C stretching (1519 cm^{-1}). The ^1H NMR spectrum (**Table 14**) exhibited the resonances of five aromatic protons, two methine protons and a methylene proton. Two *doublet* resonances (J = 2.5 Hz) at δ 6.02 (1H) and 5.92 (1H) were corresponded to the *meta*-coupling of aromatic protons H-6 and H-8, respectively. A *doublet* at δ 7.06 (J = 1.5 Hz), a *doublet of doublet* at δ 6.84 (J = 8.0, 1.5 Hz) and a *doublet* at δ 6.79 (J = 8.0 Hz) were assigned for the resonances of aromatic protons H-2', H-6' and H-5', respectively. The spectra further showed the resonances of H-2 (δ 4.88, *s*), H-3 (δ 4.20, *m*), H_a-4 (δ 2.87, *dd*, J = 12.0, 4.5 Hz) and H_b-4 (δ 2.74, *dd*, J = 12.0, 4.5 Hz). The chemical shift of H-2 and H-3 indicated that these two protons were next to oxygen-bearing carbons. In addition, the resonances of H-2 and H-3 suggested that the configuration of H-2 and H-3 are *cis* (Sethi *et al.*, 1984). The ^{13}C NMR spectral data corresponded to the assigned structure. Therefore, **11** was proposed to be (–) epicatechin of which the (–) isomer was indicated from optical rotation ($[\alpha]_D^{29}$ –70°). The assignment was in agreement with the previous data of (–) epicatechin (Panthong, 1999).

Table 14 NMR spectral data of compound **11**

Position	Compound **11**		(-)Epicatechin	
	δ_H (*multiplicity*, J_{Hz})	δ_C	δ_H (*multiplicity*, J_{Hz})	δ_C
2	4.88 (1H, *s*)	79.4	4.81 (1H, *br s*)	79.8
3	4.20 (1H, *m*)	66.9	4.17 (1H, *m*)	67.4
4	H_a : 2.87 (1H, *dd,* 12.0, 4.5)	29.0	2.85 (1H, *dd*, 16.8, 4.8)	29.2
	H_b : 2.74 (1H, *dd,* 12.0, 4.5)		2.72 (1H, *dd*, 16.8, 2.8)	
5	-	157.6	-	157.9
6	6.02 (1H, *d*, 2.5)	95.7	5.93 (1H, *d*, 2.0)	95.8
7	-	157.5	-	157.6
8	5.92 (1H, *d*, 2.5)	96.1	5.90 (1H, *d*, 2.0)	96.3
9	-	157.2	-	157.3
10	-	99.8	-	100.0
1'	-	132.3	-	132.2
2'	7.06 (1H, *d*, 1.5)	115.3	6.96 (1H, *d*, 1.6)	115.2
3'	-	145.4	-	145.9
4'	-	145.3	-	145.7
5'	6.79 (1H, *d*, 8.0)	115.5	6.74 (1H, *d*, 8.0)	115.8
6'	6.84 (1H, *dd*, 8.0, 1.5)	119.4	6.79 (1H, *dd*, 8.4, 2.0)	119.3

4.3 Evaluation of antioxidative activity

Estimation of antioxidative effects has been carried out by various methods. The DPPH assay is one of the method used for antioxidant testing on free radical terminator because its odd electron can be used as a convenient tool for the antioxidant assay. The DPPH free radical is a dark violet solid, its solubility is not great, alcoholic solutions having concentrations of approximately 5 x 10^{-4} are nevertheless densely colored. Its solution shows a strong absorption band at λ 517 nm (in ethanol), when DPPH radical accepts an electron or a hydrogen radical, a more stable compound will be formed and consequently its characteristic absorption at 517 nm vanishes (deep violet turns colorless). The capacity of the substances to donate electrons can be estimated from the degree of loss of color (Blois, 1958). Coexistance of an antioxidant compound (AH) and a free radical (DPPH•) leads to the disappearance of DPPH free radical and the appearance the free radical (A•) as shown in **Figure 5**.

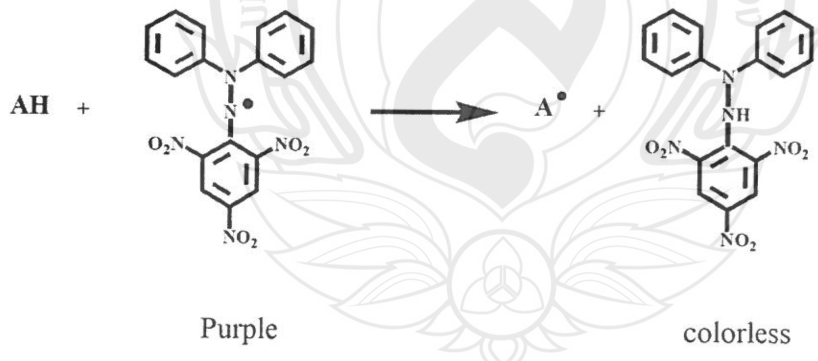


Figure 5 DPPH free radical and the appearance the free radical

4.3.1 Free radical scavenging activity of crude extracts

To determine the scavenging activity, the crude extracts of *C. sinensis* var. *assamica* leaves were tested for scavenging activity at the final concentration of 100 µg/mL. The activity was monitored by following the decrease of absorbance of the solution at 517 nm for 30 min. The results were expressed as % inhibition (**Figure 6**). The activity was exhibited by the dichloromethane as well as by the methanolic extracts.

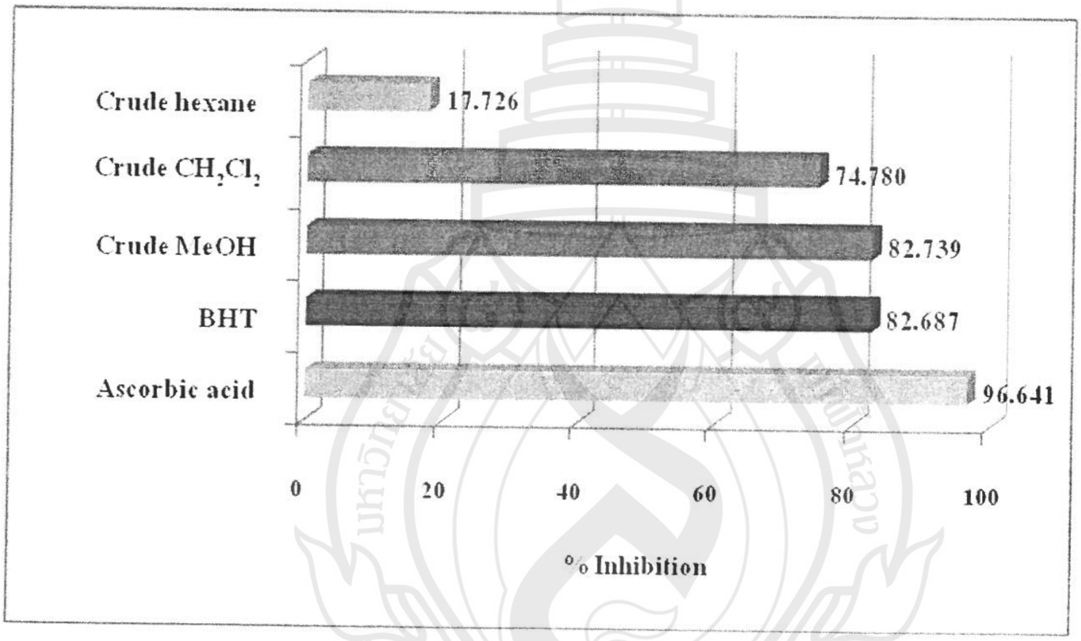


Figure 6 Radical scavenging activity of the crude extracts

The assessment of the antioxidation activity of the crude material was extended. In comparable to the standard antioxidants (BHT and ascorbic acid) and crude dichloromethane and methanolic extracts were evaluated for IC_{50}. The oxidation effect was evaluated as the concentration required to scavenge 50% DPPH free radical. Their IC_{50} were exhibited at 1.75 and 0.30 mM, respectively whereas IC_{50} of ascorbic acid and BHT were shown at 0.10 and 0.70 mM (**Table 15**).

Table 15 IC_{50} values of tested crude extracts and standard antioxidants

Sample	IC_{50} ± S.D. (mM, 30 min)
Crude CH_2Cl_2	1.75 ± 0.020
Crude MeOH	0.30 ± 0.028
BHT	0.71 ± 0.013
Ascorbic acid	0.10 ± 0.004

4.3.2 Free radical scavenging activity of pure compounds

Radical scavenging activities of pure compounds were examined. The samples were tested at the final concentration of 10 μM. The absorption of the solutions were measured at 517 nm (30 min). Ascorbic acid and BHT were used as reference compounds. The activity was expressed in the % inhibition. The results showed that compounds **5**, **9**, **10** and **11** exhibited higher activity than that of ascorbic acid and BHT (**Table 16**). The other compounds showed moderate to weak activity.

The assessment of the activity was extended for compounds **5**, **9**, **10** and **11**. The oxidation effect was evaluated as the concentration required to scavenge 50% DPPH free radical (IC_{50}). Their IC_{50} were exhibited at 0.60, 0.23, 0.27 and 0.07 mM, respectively wheares IC_{50} of ascorbic acid and BHT were shown at 1.75 and 3.03 mM, respectively (**Table 17**). Morever the results indicated that compounds **5, 9, 10** and **11** acted as a radical scavenger more effective than ascorbic acid.

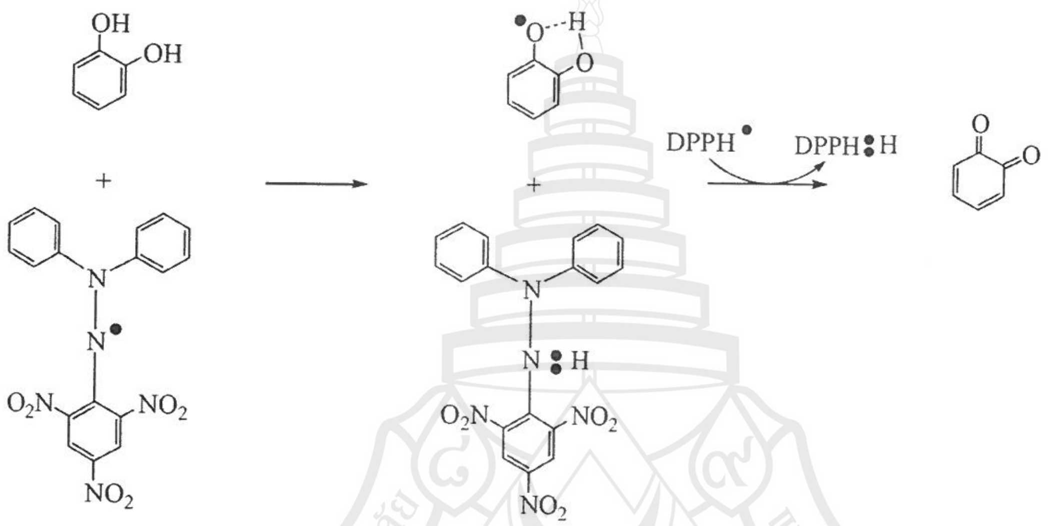
Table 16 %Inhibition of tested compounds and standard antioxidants (10 μM)

Sample	% Inhibition ± S.D. (10 μM, 30 min)
Control (0.05 mM DPPH)	-
Compound **1**	0.458 ± 0.004
Compound **2**	0.305 ± 0.005
Compound **3**	0.305 ± 0.002
Compound **4**	0.153 ± 0.001
Compound **5**	50.992 ± 0.078
Compound **6**	0.763 ± 0.002
Compound **7**	1.069 ± 0.001
Compound **8**	1.069 ± 0.003
Compound **9**	83.664 ± 0.004
Compound **10**	81.985 ± 0.001
Compound **11**	86.565 ± 0.002
BHT	13.130 ± 0.025
Ascorbic acid	31.450 ± 0.014

Table 17 IC_{50} values of tested compounds and standard antioxidants

Sample	IC_{50} ± S.D. (mM, 30 min)
Compound **5**	0.60 ± 0.003
Compound **9**	0.23 ± 0.001
Compound **10**	0.27 ± 0.003
Compound **11**	0.07 ± 0.002
BHT	3.03 ± 0.002
Ascorbic acid	1.75 ± 0.005

The mechanism of trapping by compounds **5**, **9**, **10** and **11** exhibited by a phenolic characteristic which was proposed to donate hydrogen radical to DPPH. The phenoxy radical which formed was stabilized through an intramolecular hydrogen bonding. A subsequent interaction with a second DPPH radical afforded the dehydro form of these compounds as a final product (Sahidi *et al.*, 1992).



4.4 Evaluation of Antibacterial activity

4.4.1 Antibactrial activity of crude extracts

Dried leaves of *C. sinensis* var. *assamica* were extracted with hexane, CH_2Cl_2 and MeOH to give crude hexane extract (HC), dichloromethane extract (DC) and methanolic extract (MC). Each extract was tested for antibacterial activity on *Escherichia coli* (EC), *Bacillus cereus* (BC), methicillin-resistant strain MRSA SK1, *Pseudomonas fluorescens* (PF), *Pseudomonas aeruginosa* (PA), *Staphylococcus aureus* (SA) and *Salmonella typhimurium* (ST). It was found that the extracts showed no activity (**Table 18**).

Table 18 Antibacterial activity of crude extracts

Sample	Antibacterial activity (MIC, μg/mL)						
	Gram Negative				Gram Positive		
	EC	PF	PA	ST	BC	MRSA SK1	SA
HC	>1280	1280	>1280	1280	1280	>1280	>1280
DC	>1280	640	>1280	1280	1280	>1280	1280
MC	>1280	1280	>1280	1280	640	1280	1280
Gentamycin	0.5	0.125	0.5	0.5	-	-	-
Vancomycin	-	-	-	-	0.5	0.5	0.5

4.4.2 Antibactrial activity of pure compounds

Some of the pure compounds obtained from each extract were evaluated for their antibacterial activity against *Escherichia coli* (EC), *Pseudomonas fluorescens* (PF), *Salmonella typhimurium* (ST), *Bacillus cereus* (BC) and *Staphylococcus aureus* (SA). Flavans **5**, **9**, **10** and **11** (MIC 16-128 μg/mL) were more active than the methanolic extract (**Table 19**), however it was less active than gentamycin and vancomycin, the standard antibiotic. The other compounds were not tested due to insufficient quantities.

Table 19 Antibacterial activity of pure compounds

Sample	Antibacterial activity (MIC, μg/mL)				
	Gram Negative			Gram Positive	
	EC	PF	ST	BC	SA
Compound **1**	>200	64	32	64	>200
Compound **4**	>200	>200	>200	>200	>200
Compound **5**	64	64	128	16	64
Compound **7**	>200	>200	>200	>200	>200
Compound **9**	32	64	16	64	128
Compound **10**	16	32	32	32	128
Compound **11**	16	32	64	32	64
Gentamycin	0.5	0.125	0.5	-	-
Vancomycin	-	-	-	0.5	0.5

CHAPTER 5

CONCLUSION

In conclusion, the search on the chemical constituents from the leaves of *Camellia sinensis* var. *assamica* resulted in the isolation of a new compound: 13-methyl lansic acid (**2**) together ten known compounds: *β*-sitosterol (**1**), lansionic acid (**3**), stigmasterol (**4**), (+) catechin (**5**), 21*R*-hydroxyonocera-8(26),14-dien-3-one (**6**), lupeol (**7**), lupenone (**8**), (-) epigallocatechin gallate (**9**), (-) epicatechin gallate (**10**) and (-) epicatechin (**11**).

Compounds **5**, **9**, **10** and **11** showed the moderate activity to inhibit the growth of *Escherichia coli*, *Pseudomonas fluorescens*, *Salmonella typhimurium*, *Bacillus cereus* and *Staphylococcus aureus* with MIC 16-128 μg/mL. Moreover flavans **5**, **9**, **10** and **11** exhibited stronger antioxidant activity (IC_{50} 0.60, 0.23, 0.27 and 0.07 mM, respectively) than that of ascorbic acid (IC_{50} 1.75 mM) and BHT (IC_{50} 3.03 mM).

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Educational Attainment

1995-1999 Bachelor of Science in Chemistry. Prince of Songkla University.

1999-2001 Master of Science in Organic Chemistry. Prince of Songkla University.
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Publications

1. W. Mahabusarakam, **S. Deachathai**, S. Phongpaichit, C. Jansakul, W.C. Taylor. "A benzil and isoflavone derivatives from *Derris scandens* Benth." *Phytochemistry*, 65, 1185-1191, 2004.

2. **S. Deachathai**, W. Mahabusarakam, S. Phongpaichit, W.C. Taylor. "Phenolic Compounds from the Fruit of *Garcinia dulcis*" *Phytochemistry*, 66, 2368-2375, 2005.

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Presentations

1. **S. Deachathai**, T. Promgool, M. Treesub, R. Yulianthi and A. Somsri, Antioxidant Activity of the Phenolic Compounds from Three Thai Medicinal Plants, The 1st SFRR-Thai Meeting and Workshop on Advance of Free Radicals, Oxidative Stress and Their Evaluation Methods, 15-16 December, 2008. (**Poster presentation**)
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3. Achjanee Somsri and **Suwanna Deachathai**, Chemical Constituents from the Root of *Mucuna macrocarpa* Wall., International Conference on Mathematics and Natural Sciences 2008, 28-30 October, 2008. (**Oral presentation**)
4. Rolly Yulianthi and **Suwanna Deachathai**, Chemical constituents from dried leaves of *Camellia sinensis* var. *assamica* in Chiang Rai, Thailand, International Conference on Mathematics and Natural Sciences 2008, 28-30 October, 2008. (**Oral presentation**)
5. **Suwanna Deachathai,** Chemical constituents from the roots of *Garcinia cowa* Roxb., International Conference on Mathematics and Natural Sciences 2008, 28-30 October, 2008. (**Oral presentation**)
6. Achjanee Somsri and **Suwanna Deachathai** (2007) Chemical Constituents from the Root of *Mucuna macrocarpa* Wall., Antimicrobial and Antioxidation Properties. TSB 19th Annual Meeting, Bangkok, Thailand. (Poster Presentation)

7. Achjanee Somsri and **Suwanna Deachathai** (2007) Chemical Constituents from the Root of *Mucuna macrocarpa* Wall., Antimicrobial and Antioxidation Properties. STT 33, Walailak University, Nakorn Sri Thummarat, Thailand. **(Poster Presentation)**

8. P. Thongbai, P. Damrongkul Eungwanichayapant, **S. Dechathai,** S. Popluechai, S. Unto, Ratana Kalapa and Patanapong Sengkeaw (2007) Improving Jatropha Plant to be more Suitable Biodiesel Crop. Proceedings of the 6th Asian Crop Science Association Conference and BioAsia 2007, Bangkok, Thailand. **(Poster Presentation)**