UNIVERSITI PUTRA MALAYSIA

CHEMICAL CONSTITUENTS OF *GARCINIA MANGOSTANA*, G. *PARVIFOLIA*, G. *GRIFFITTI* AND G. *DIVERSIFOLIA* (*GUTTIFERAE*) AND THEIR BIOLOGICAL ACTIVITIES

BONNIE TAY YEN PING

FSAS 1996 6

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BY

BONNIE TAY YEN PING

Thesis submitted in Fulfilment of the
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LIST OF ABBREVIATIONS

br	broad
$CDCl_3$	deuterated chloroform
CDOD	deuterated methanol
$CHCl_3$	chloroform
d	doublet
EtOAc	ethyl acetate
g	gram
IR	Infra Red
kg	kilogram
LD	Lethal Dosage
m	medium (in IR data)
m	multiplet (in ^{1}H-NMR data)
m.p.	melting point
Me	methyl
MeOH	methanol
mg	miligram
ml	mililitre
MS	Mass Spectrum
NMR	Nuclear Magnetic Resonance
PE	petroleum ether
PLC	Preparative Thin Layer Chromatography
ppm	parts per million
s	strong
t	triplet
TLC	Thin Layer Chromatography
UV	ultra violet
w	weak

Abstract of the thesis presented to the Senate of
Universiti Pertanian Malaysia in fulfilment of
requirements for the degree of Master of Science.

CHEMICAL CONSTITUENTS OF *GARCINIA MANGOSTANA*, *G. PARVIFOLIA*, *G. GRIFFITTI* AND *G. DIVERSIFOLIA (GUTTIFERAE)* AND THEIR BIOLOGICAL ACTIVITIES.

BY

BONNIE TAY YEN PING

July 1996

Chairman : Associate Professor Dr. Mawardi Rahmani
Faculty : Science and Environmental Studies

Four plants from the *Garcinia* genus were subjected to chemotaxonomic investigations with the isolation of a number of compounds. The structures of the compounds were established by spectroscopic methods such as MS, UV, IR, ^{1}H-NMR and ^{13}C-NMR and by comparison with previous studies.

The investigations of the pericarp of local grown *Garcinia mangostana* afforded known compounds which are mangostin, β-mangostin, γ-mangostin and gartanin.

In investigations of the bark and heartwood of *G. parvifolia* collected from Ayer Hitam Forest Reserve, Puchong two xanthones , GP2 , GH2 and stigmasterol were isolated. From another separate collection of the same species from Johor, three compounds were isolated : stigmasterol, an unidentified compound and an novel compound, GK3.

Study on the leaves and stems of *G. griffitti* collected from Taman Negara , Pahang afforded two known compounds friedelin and β-amyrin. Investigations on leaves and stems of *G. diversifolia* collected from Fraser's Hill also yielded friedelin.

The plant extracts were evaluated for their biological activities against selected fungal and bacterial pathogens. The antifungal activity was performed using the `Poison Food' method. The extracts from the test plant showed different antifungal activity towards the vegetative growth of plant pathogenic fungi ; *Helminthosporium oryzae*, *Alternaria* padwickii, *Colletotrichum capsici*, *Fusarium oxysporum*, *Curvularia* lunata and *Pestalotia theae* The chloroform extract of the heartwood of *G. parvifolia* collected from Ayer Hitam Forest Reserve, Puchong was the most effective in inhibiting the mycelial growth for the test fungi.

The antibacterial activity was tested using the `Agar Diffusion' method. The extracts from the test plant also showed different antibacterial activity against the growth of six test bacteria ; *Bacillus subtilis*, *E. coli*, *Erwinia carotovora*, *Micrococcus sp*, *Proteus vulgaris* and *Pseudomonas solanacearum*. Chloroform extract of *G. mangostana* gave the highest average zone of inhibition, indicating the degree of its sensitivity.

Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia bagi memenuhi keperluan Ijazah Master Sains

KANDUNGAN KIMIA DARI *GARCINIA MANGOSTANA*, *G. PARVIFOLIA*, *G. GRIFFITTI* DAN *G. DIVERSIFOLIA* (GUTTIFERAE) DAN AKTIVITI BIOLOGINYA.

oleh

BONNIE TAY YEN PING

Julai 1996

Pengerusi : Professor Madya Dr. Mawardi Rahmani
Fakulti : Sains dan Pengajian Alam Sekitar

Kajian kimotaksonomi ke atas empat spesies tumbuhan dari genus *Garcinia* telah dijalankan dengan pemencilan beberapa sebatian. Struktur sebatian-sebatian ini telah dikenalpasti dengan menggunakan kaedah spektroskopi seperti MS, UV, IR, ^{1}H-NMR dan ^{13}C-NMR serta perbandingan dengan kajian-kajian lepas.

Kajian terhadap kulit buah tempatan, *G. mangostana* telah menghasilkan empat sebatian yang telah dikenali : gartanin, β-mangostin, mangostin dan γ-mangostin.

Kajian terhadap kulit dan kayu batang spesies yang lain, *G. parvifolia* yang dikumpulkan dari kawasan Hutan Simpanan Ayer Hitam, Puchong dua sebatian dari jenis xanthon, GP2 dan GH2 dan stigmasterol telah dipencilkan.

Dari suatu pengumpulan berasingan spesies yang sama dari Johor, tiga kompaun berjaya diasingkan iaitu stigmasterol, sebatian yang tidak dikenali, dan satu sebatian baru, GK3.

Kajian ke atas daun dan ranting pokok *G. griffitti* yang dikumpulkan dari Taman Negara, Pahang menghasilkan dua sebatian yang sudah dikenali, friedelin dan β-amirin. Kajian ke atas ranting dan daun *G. diversifolia* yang dikumpulkan dari Bukit Fraser juga menghasilkan friedelin.

Ekstrak tumbuhan tersebut telah diuji untuk aktiviti biologinya terhadap beberapa patogen kulat dan bakteria. Aktiviti antikulat dijalankan dengan menggunakan teknik 'Makanan Beracun". Ekstrak -ekstrak dari tumbuhan yang dikaji menunjukkan aktiviti antikulat yang berbeza terhadap pertumbuhan kulat patogen tumbuhan ; *Helminthosporium oryzae*, *Alternaria padwickii*, *Colletotrichum capsici*, *Fusarium oxysporum*, *Curvularia lunata* dan *Pestalotia theae*. Ekstrak kloroform dari kayu batang spesies *G. parvifolia* yang dikumpul dari Kawasan Hutan Simpanan Ayer Hitam, Puchong adalah paling berkesan terhadap perencatan pertumbuhan miselium kulat yang dikaji.

Aktiviti antibakteria dijalankan menggunakan kaedah " Resapan Agar".
Ekstrak dari tumbuhan yang dikaji juga menunjukkan aktiviti anti bakteria yang berbeza terhadap pertumbuhan enam bakteria ; *Bacillus subtilis, E. coli*, *Erwinia carotovora*, *Micrococcus sp*, *Proteus vulgaris* dan *Pseudomonas solanacearum*. Ekstrak kloroform dari *G. mangostana* memberikan purata zon perencatan pertumbuhan yang tertinggi menunjukkan keamatan kepekaanya.

PART I

EXTRACTION AND ISOLATION OF CHEMICAL COMPONENTS FROM *GARCINIA MANGOSTANA*, *G. PARVIFOLIA*, *G. GRIFFITTI* AND *G. DIVERSIFOLIA* (GUTTIFERAE)

INTRODUCTION

The family Guttiferae numbers over 1000 species and is mainly confine to the tropics. Xanthones or the related benzophenones have been found in all the major and several minor genera of the Guttiferae. *Garcinia* is a rather large genus of trees or shrubs of this family. There are about 100 species, in tropical Asia, Africa and Polynesia and 24 species are found in Malaya (Whitmore, 1983)

In Malaysia, *Garcinia mangostana L.* is locally known as "Manggis", "Setar" or "Seta". It is a small tree with a dense, domed, glossy green crown, about 20 to 30 feet high. It bears fruits usually after 15 years. It is extensively grown in association with the durian and rambutan in the states of Johore, Negeri Sembilan, Pahang and Perak. The Malays make a conserve, called `Halwa manggis' by boiling the young fruits, with the rind removed, with sugar. The firm fruit rind contains tannin and is used for tanneries. The rind is used medicinally as an astringent, cholera, dysentery and diarrhoea. The Malays make a decoction that they administer for dysentery. Burkill (1966) recorded that an infusion of the leaves with unripe bananas and a little benzoin is applied to the wound of circumcision and other wounds.

Garcinia parvifolia is a small to medium tree, rather rough barked and can reach to about 33 m tall. The small yellow fruits are eaten as a condiment with Spanish pepper on fish. However, no medicinal use was ever reported. It is found at

low undulating land and to 600 m in the hills throughout Malaya. It is common in primary and secondary forest and peat swamp forest as in Ayer Hitam, Johore.

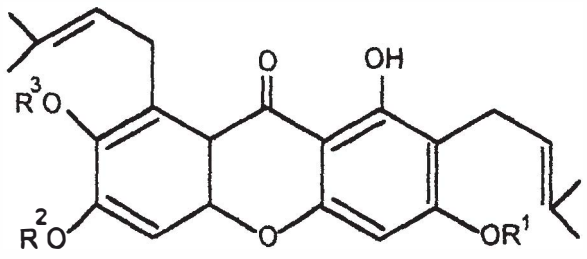
Garcinia griffitti or locally known as "Kandis Gajah" (elephant or big kandis) is a smooth barked tree reaching 23 m tall. The inner barks contain opaque yellow exudate. It is common in woods at Malacca and Perak. The apple like fruit is very acidic and is sometimes cooked and eaten.

Garcinia diversifolia is a rare slender tree reaching 24 m tall, and is found at mountains at 300 to 900 m at Main Range, Gunong Batu Putih and Genting Highland, Johore (G. Belumut, G. Arang , G. Bubu) and Perak.

LITERATURE REVIEW

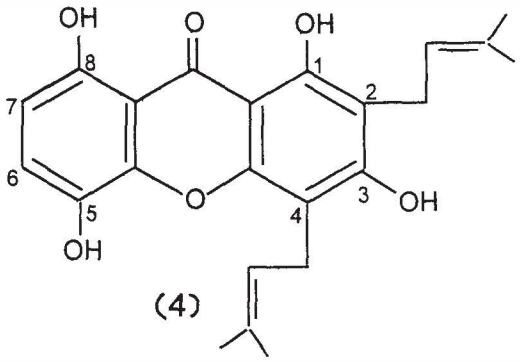
In previous literatures, a number of *Garcinia* species have been investigated, however only a few have been extensively studied. Throughout the investigations, various compounds belonging to the xanthone, benzophenone and biflavanoid groups have been observed to occur in this genus.

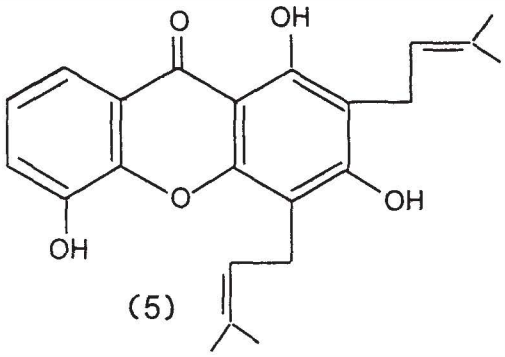
G. mangostana is among the earliest species to be investigated. Yates and Stout (1958) reported the isolation of mangostin (1), $C_{24}H_{26}O_6$, m.p. 182-183°C and β-mangostin (2) from the fruit hulls, bark and dried latex. He assigned mangostin as 1,3,6-trihydroxy-7-methoxy-2,8-di-(3-methyl-2-butenyl)-xanthone through various chemical evidence. Structure of (1) was confirmed by NMR analysis carried out by Wan (1973). Jefferson et al. (1970) reported the isolation of γ-mangostin (3) $C_{23}H_{24}O_6$ m.p. 207 - 208 °C with structure 1,3,6,7-tetrahydroxy-2,8-di(3-methyl but-2-enyl)xanthone.



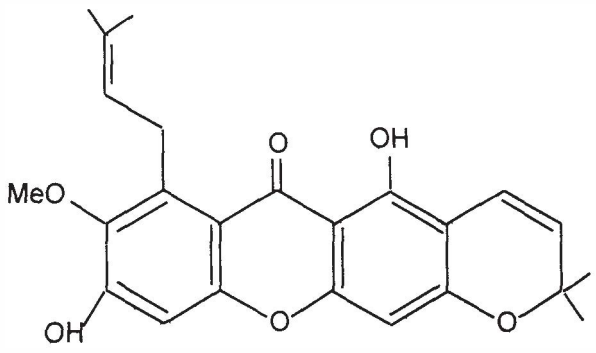
	R^1	R^2	R^3
(1)	H	H	Me
(2)	Me	H	Me
(3)	H	H	H

Govindachari et al. (1971) embarked on another investigation on the fruit hulls of *G. mangostana*. The well ripened fruits yielded gartanin (4), m.p. 167°C $C_{23}H_{24}O_6$; 8-desodygartanin (5), m.p. 165.5° together with mangostin (1) and γ-mangostin (3). Gartanin was the first xanthone with 1,3,5,8-oxygenation pattern observed in the Guttiferae plants then. Interestingly, the less ripe fruits yielded only mangostin (1) and β-mangostin (2).





In another investigation of fully ripe fruits of *G. mangostana*, Sen et al. (1980) discovered the presence of 1,3,6,7-tetraoxygenated xanthone (6), $C_{24}H_{24}O_6$ m.p. 156-157°C, in the petrol extract. A year later, he found another two new 1,3,5- and 1,3,7-trioxygenated xanthones with structures (7) and (8), respectively in the same extract.



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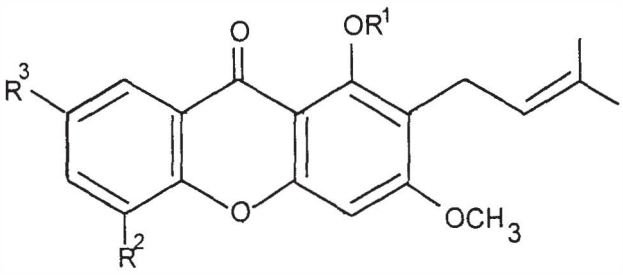
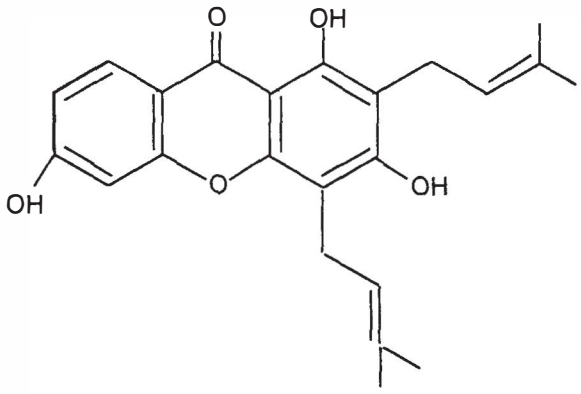


	R^1	R^2	R^3
(7)	H	OH	H
(8)	H	H	OH

Subsequent investigation of the $CHCl_3$ extract of the plant by Sen et al. (1982) afforded three new tetraoxygenated xanthones garcinones A (9), $C_{23}H_{24}O_5$, m.p. 224-225°C, garcinone B (10), $C_{23}H_{22}O_6$, m.p. 190-192°C and garcinone C (11), $C_{23}H_{26}O_7$, m.p. 216-218°C .



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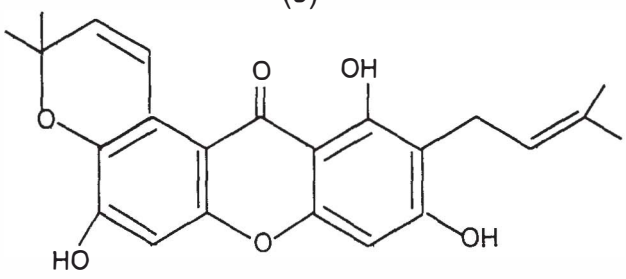

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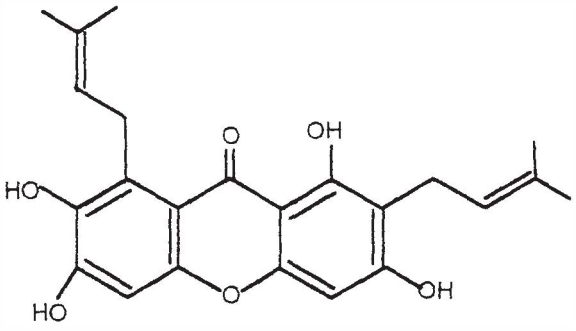
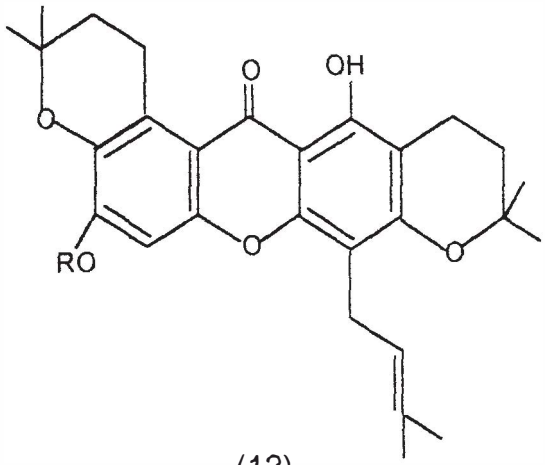


(11)

In another investigation, two novel xanthones, BR-xanthone A (12) , $C_{23}H_{24}O_6$, m. p. 181-182°C and BR-xanthone B (13), $C_{15}H_{10}O_6$, m. p. 308-310°C were isolated by Balasubramanian and Rajagopalan (1988) from the fruit hulls of *G. mangostana*.



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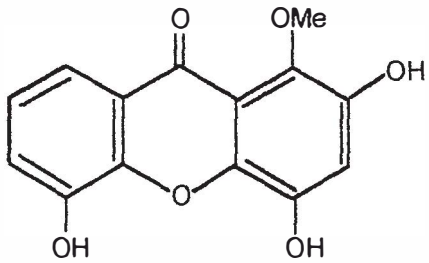
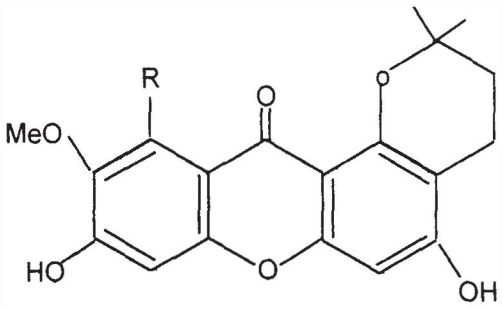


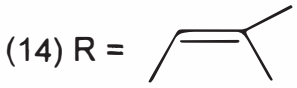
(13)

Recently, Mahabusarakam and Wiriyachitra (1987) embarked on another investigation of the pericarps and arils of *G. mangostana* fruits grown in Thailand. The pericarp afforded the known mangostin (1), gartanin (4), mangostin (2), 1-*iso*mangostin (14), m.p. 245-249°C, 3-*iso*mangostin (15), m.p. 180-182°C, 3-*iso*mangostin-hydrate (16) and 1-*iso*mangostin hydrate (17), m.p. 255-257°C. The fresh arils afforded calabaxanthone (18) mp 170-172°C, mangostin (1), 2-(γ,γ-dimethylallyl)-1,7-dihydroxy-3-methoxyxanthone (19) ,a novel compound

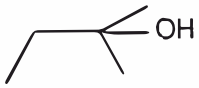
demethylcalabaxanthone (20), $C_{23}H_{24}O_5$, m.p. 184-190°C, and 2,8-*bis*-(γ,γ-dimethylally)-1,3,7-trihydroxyxanthone (21), m.p. 160-170°C.

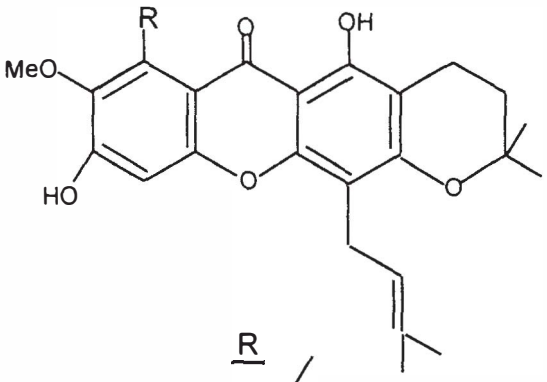


(14) R =



(17)R =





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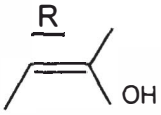

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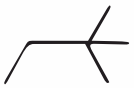

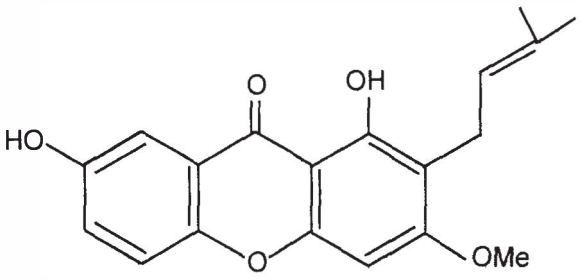

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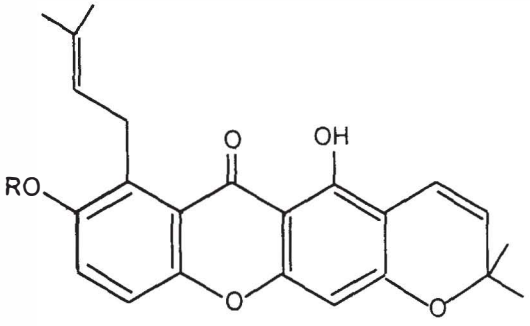
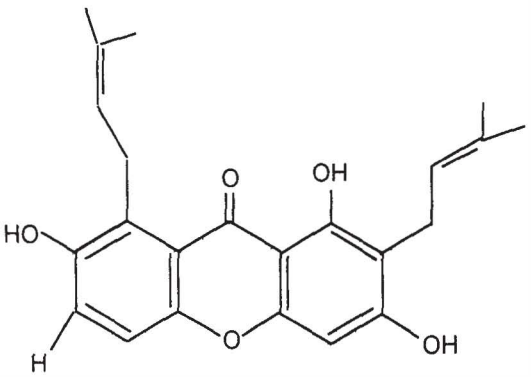


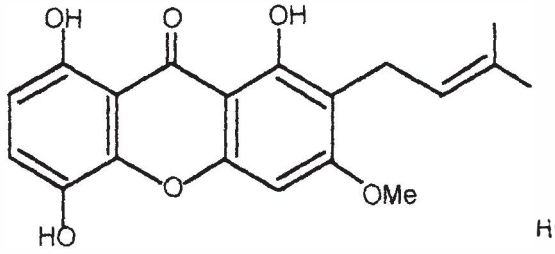
(20) R = H

(18) R = Me



(21)

Work on the leaves of *G. mangostana* by Parveen and Khan (1988) yielded two new xanthones, 1,5,8-trihydroxy-3-methoxy-2[3-methyl-2-butenyl]xanthone (22), m.p. 193-195^{o}C and 1,6-dihydroy-3-methoxy-2[3-methyl-2-butenyl]xanthone (23), m.p. 162-164^{o}C along with the known xanthone gartanin (4). A new triterpene, 3β-hydroxy-26-nor-9,19-cyclolanost-23-en-25-one (24),
by Parveen et al. (1991).



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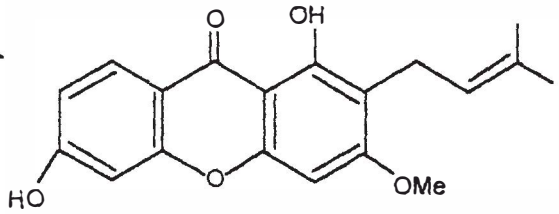

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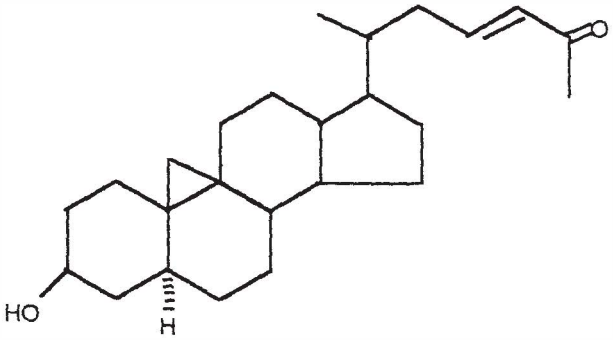

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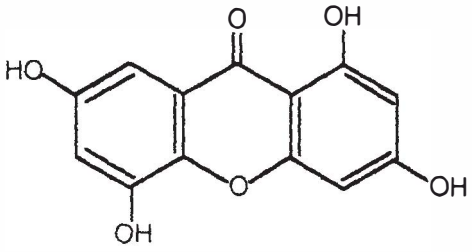

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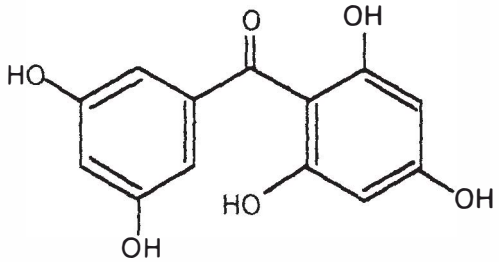
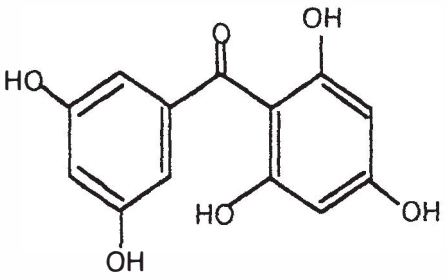


(26)

Holloway and Scheinmann (1975) discovered that the twigs and branches of *G. mangostana* were devoid of isoprenylxanthones, instead 1,3,6,7 tetrahydroxyxanthone (25) m.p. 290°C,
crude extract. In addition the pentahydroxybenzophenone, maclurin (26) was present and isolated as pentamethyl-maclurin with m.p. 165°C. These two compounds were believed to be the precursors of mangostin (1).

In 1973, Rao et al. reported the isolation of 2,4,6,3',5'-pentahydroxybenzophenone (27), $C_{13}H_{10}O_6$, m.p. 258-260°C with it's corresponding xanthone, 1,3,5,7-tetrahydroxyxanthone (28) from the heartwood of *G. pedunculata*. This is the first natural xanthone to have the 1,3,5,7-orientation of hydroxyl groups. In addition, they isolated known compounds, biflavonone GB-1a (29) and talbatoflavone (30).



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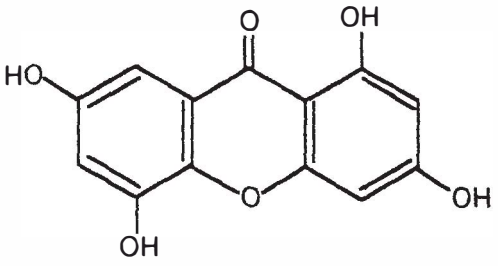
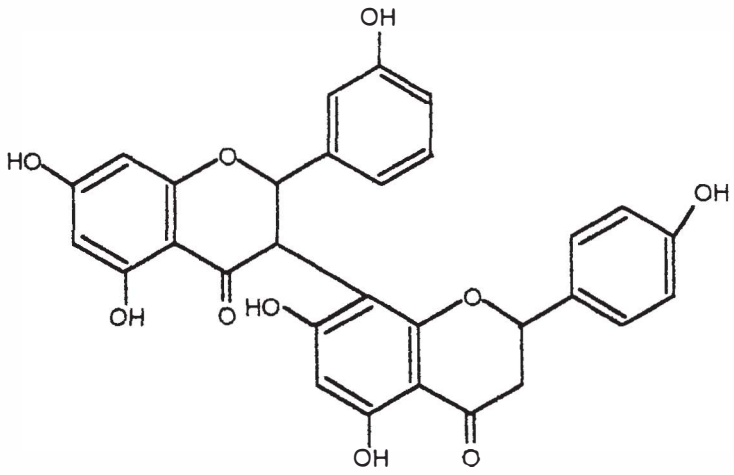
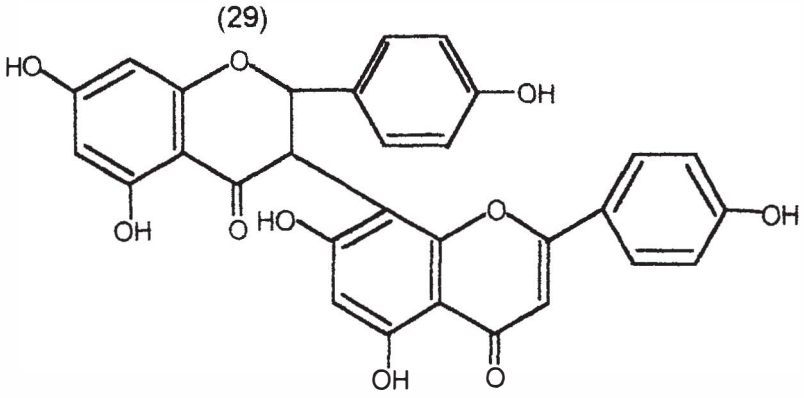


(28)



(29)



(30)

Sahu et al. (1989) undertook the extraction of the pericarp from the fruits of *G. pedunculata* and reported the isolation of a new polyisoprenylated benzophenone derivative pedunculol (31) m.p.125°C, $C_{38}H_{52}O_6$ and two known polyisoprenylated , garcinol (32), m.p. 120°C and cambogin (33) , m.p. 240°C.