PHYTOCHEMICALS AND BIOACTIVITIES OF *Garcinia prainiana* KING AND *G. hombroniana* PIERRE

SHAMSUL ON

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To My Beloved Wife

Najatulhayah Alwi

and My children,

Muhd Nabil AnNajat, Muhd Nabihan AnNajat, Muhd Naqib AnNajat,
Muhd Nazeem AnNajat, Shahmina Nasyamah AnNajat

For Their Love, Support and Best Wishes.

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ABSTRACT

Phytochemical and bioactivity investigations of two *Garcinia* species, namely *Garcinia prainiana* King and *G. hombroniana* Pierre have been carried out. The leaves and barks of *G. prainiana* and the roots of *G. hombroniana* were extracted using *n*-hexane, dichloromethane and methanol, consecutively. Isolation and purification of the crude extracts carried out using several chromatographic techniques have led to the identification of seventeen compounds. The structures of the isolated compounds were elucidated by spectroscopic methods such as UV, IR, 1D (^{1}H, ^{13}C, DEPT) and 2D (COSY, HMQC, HMBC) NMR and MS. Purification of the leaves and barks extracts of *G. prainiana* afforded eleven known compounds. Six triterpenes identified as squalene, friedelin, lupeol, methyl putranjivate, eupha-5,22-diene-3β-ol and eupha-5,22-diene-3β-acetate were isolated from the *n*-hexane extracts of leaves and barks of *G. prainiana* while five biflavonoids characterized as morelloflavone, *O*-methylfukugetin, volkensiflavone, amentoflavone and 4'''-methylamentoflavone were obtained from the methanol extract of barks of *G. prainiana*. Purification of the roots extract of *G. hombroniana* yielded two new compounds, 1,3,7-trihydroxy-4-(1,1-dimethylprop-2-enyl)-8-methoxyxanthone and 3,3',4'',5',5'',6-hexahydroxybenzophenone together with four known compounds, garceduxanthone, cheffouxanthone, norathyriol and 2,3′,4,5′-tetrahydroxy-6-methoxybenzophenone. Total phenolic content, total antioxidant, antibacterial, antityrosinase and toxicity studies were carried out on the crude extracts and selected compounds. Measurement of the total phenolic content using Folin-Ciocalteau reagent indicated that the crude methanol extract of the barks of *G. prainiana* had the highest value of gallic acid and (±)-catechin equivalents. The crude methanol extract also showed the highest value of ascorbic acid and butylated hydroxytoluene equivalents on forming the phosphomolybdenum complex in the total antioxidant assay. The antioxidant assays on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical showed that the crude methanol extract of barks of *G. prainiana* had the highest radical scavenging activity. Morelloflavone revealed a strong free radical scavenging activity with IC_{50} value 15.7 µg/mL. The antibacterial assay was carried out using disc diffusion method, followed by minimum inhibition concentration (MIC) determinations. The crude methanol extract of the barks of *G. prainiana* displayed weak antibacterial activity against Gram-negative bacteria *Pseudomonas aeruginosa* and Gram-positive bacteria *Bacillus subtilis*. The selected compound, morelloflavone, showed moderate antibacterial activity against *B. subtilis* and *P. aeruginosa* with MIC value of 450 µg/mL. The cytotoxic activities of the isolated compounds were evaluated using the MTT assay against H1299 and A549 lung cancer cells. The results showed that eupha-5,22-diene-3β-acetate had strong cytotoxity activity against H1299 and A549 cell lines with IC_{50} values of 18.0 and 36.3 µg/mL, respectively while squalene gave IC_{50} values of 23.2 µg/mL (H1299) and 74.8 µg/mL (A549). Meanwhile, the screening for antityrosinase activity using the mushroom tyrosinase enzyme assay for all crude extracts and isolated compounds showed that the percentage of tyrosinase inhibition at a concentration of 100 µg/mL were higher than 50%.

ABSTRAK

Kajian fitokimia dan bioaktiviti dua spesies *Garcinia* iaitu *Garcinia prainiana* King dan *G. hombroniana* Pierre telah dijalankan. Daun dan kulit batang *G. prainiana* serta akar *G. hombroniana* telah diekstrak menggunakan *n*-heksana, diklorometana dan metanol secara berturut-turut. Pengasingan dan penulenan ekstrak mentah yang dilakukan dengan menggunakan beberapa teknik kromatografi telah membawa kepada pengenalpastian tujuh belas sebatian. Struktur sebatian yang telah diasingkan itu telah dijelaskan dengan kaedah spektroskopi seperti UV, IR, 1D (^{1}H, ^{13}C, DEPT) dan 2D (COSY, HMQC, HMBC) NMR dan MS. Penulenan ekstrak daun dan kulit batang *G. prainiana* memberikan sebelas sebatian yang diketahui. Enam triterpena dikenal pasti sebagai skualena, friedelin, lupeol, metilputranjivat, eufa-5,22-dien-3β-ol dan eufa-5,22-dien-3β-asetat telah diasingkan daripada ekstrak *n*-heksana daun dan kulit batang *G. prainiana* manakala lima biflavonoid telah dicirikan sebagai morelloflavon, *O*-metilfukugetin, volkensiflavon, amentoflavon dan 4'''-metilamentoflavon telah diperoleh daripada ekstrak metanol kulit batang *G. prainiana*. Penulenan ekstrak akar *G. hombroniana* menghasilkan dua sebatian baru, 1,3,7-trihidroksi-4-(1,1-dimetilprop-2-enil)-8-metoksixanton dan 3,3',4'',5',5'',6-heksahidroksibenzofenon serta empat sebatian yang diketahui, garceduxanton, cheffouxanton, noratiriol dan 2,3',4,5'-tetrahidroksi-6-metoksibenzofenon. Kajian kandungan fenolik total, antioksidan total, antibakteria, antitirosinase dan ketoksikan telah dijalankan ke atas ekstrak mentah dan sebatian terpilih. Pengukuran kandungan fenolik total menggunakan reagen Folin-Ciocalteau menunjukkan bahawa ekstrak metanol daripada kulit batang *G. prainiana* mempunyai nilai tertinggi asid galik dan (±)-katekin setara. Ekstrak mentah metanol juga menunjukkan nilai tertinggi asid askobik dan hidroksitoluena terbutil setara apabila membentuk kompleks fosfomolibdenum dalam penilaian antioksidan total. Penilaian antioksidan ke atas radikal 2,2-difenil-1-pikrilhidrazil (DPPH) menunjukkan bahawa ekstrak mentah metanol kulit batang *G. prainiana* mempunyai aktiviti pemerangkapan radikal tertinggi. Morelloflavon menunjukkan aktiviti pemerangkapan radikal bebas yang tinggi dengan nilai IC_{50} 15.7 μg/mL. Penilaian antibakteria dilakukan menggunakan kaedah peresapan cakera, diikuti dengan penentuan kepekatan perencatan minimum (MIC). Ekstrak mentah metanol daripada kulit batang *G. prainiana* memaparkan aktiviti perencatan lemah terhadap bakteria Gram-negatif *Pseudomonas aeruginosa* dan bakteria Gram-positif *Bacillus subtilis*. Sebatian terpilih, morelloflavon, menunjukkan aktiviti perencatan sederhana terhadap bakteria *B. subtilis* dan *P. aeruginosa* dengan nilai MIC 450 μg/mL. Aktiviti ketoksikan sebatian yang diasingkan itu dinilai menggunakan penilaian MTT terhadap sel kanser paru-paru H1299 dan A549. Keputusan menunjukkan eufa-5,22-dien-3β-asetat mempunyai aktiviti ketoksikan yang kuat terhadap sel kanser H1299 dan A549 masing-masing dengan nilai IC_{50} 18.0 μg/mL dan 36.3 μg/mL manakala skualena memberikan nilai IC_{50} 23.0 μg/mL (H1299) dan 74.0 μg/mL (A549). Sementara itu, saringan aktiviti antitirosinase menggunakan penilaian enzim tirosinase cendawan bagi semua ekstrak mentah dan sebatian yang diasingkan menunjukkan peratusan perencatan tirosinase pada kepekatan 100 μg/mL adalah lebih tinggi daripada 50%.

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LIST OF ABBREVIATIONS

α	-	Alpha
AA	-	Ascorbic acid
Abs	-	Absorbance
AChE	-	Acetylcholinesterase
ATR	-	Attenuated Total Reflectance
β	-	Beta
$BaCl_2$	-	Barium chloride
BHT	-	Butylated hydroxytoluene
br	-	broad
c	-	Concentration
^{13}C	-	Carbon-13
CC	-	Column Chromatography
$CDCl_3$	-	Deuterated chloroform
$CHCl_3$	-	Chloroform
cm	-	Centimeter
cm^{-1}	-	Per centimeter
COSY	-	Correlation spectroscopy
COX-2	-	Cyclooxygenase-2
1D	-	1 Dimension
2D	-	2 Dimension
δ	-	chemical shift
d	-	doublet
dd	-	doublet of doublets
DEPT	-	Distortionless Enhancement by Polarization Transfer
DMSO	-	Dimethyl sulfoxide
DPPH	-	2,2-Diphenyl-1-picrylhydrazyl
EIMS	-	Electron Impact Mass Spectrometry
Et_2O	-	Diethyl ether

EtOAc	-	Ethyl acetate
GA	-	Gallic acid
GC	-	Gas Chromatography
GC-MS	-	Gas Chromatography-Mass Spectrometry
Glc	-	Glucose
h	-	Hour(s)
n-Hex	-	Hexane
^{1}H	-	Proton
H_2O	-	Water
H_2SO_4	-	Sulfuric acid
HCl	-	Hydrochloric acid
HMBC	-	Heteronuclear Multiple Bond Correlation
HMQC	-	Heteronuclear Multiple Quantum Coherence
HREIMS	-	High Resolution Electron Impact Mass Spectrometry
Hz	-	Hertz
IR	-	Infrared
IC	-	Inhibition concentration
iNOS	-	Inducible nitric oxide synthase
J	-	Coupling constant
KBr	-	Potassium bromide
KI	-	Kovats Index
L	-	Liter
lit.	-	Literature
LOX	-	Lipoxygenase
λ	-	Lambda
m	-	multiplet
M^+	-	Molecular ion
MBC	-	Minimum Bactericidal Concentration
MeOH	-	Methanol
MHz	-	Megahertz
MFC	-	Minimum Fungicidal Concentration
MIC	-	Minimum Inhibitory Concentration
min	-	Minute(s)
m/z	-	Mass to charge ion
MeOH	-	Methanol
mg	-	milligram

m.p	-	Melting point
$MgSO_4$	-	Magnesium sulphate
mL	-	milliliter
mm	-	millimetre
MS	-	Mass Spectroscopy
NaCl	-	Sodium chloride
NaOH	-	Sodium hydroxide
NA	-	Nutrient agar
NB	-	Nutrient broth
NMR	-	Nuclear Magnetic Resonance
nm	-	nanometer
PTLC	-	Preparative Thin Layer Chromatography
R_f	-	Retention factor
Rha	-	Rhamnose
s	-	singlet
SD	-	Standard deviation
SDA	-	Sabouraud dextrose agar
SDB	-	Sabouraud dextrose broth
SiO_2	-	Silica gel
t	-	triplet
TLC	-	Thin Layer Chromatography
TPC	-	Total phenolic content
μM	-	Micro molar
UV	-	Ultraviolet
VLC	-	Vacuum Liquid Chromatography

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CHAPTER 1

INTRODUCTION

1.1 General Introduction

Medicinal plants have played a key role in world health. Plants are rich sources of fine chemicals, largely unknown and yet they still make an important contribution to health care in spite of the great advances in the field of modern medicine [1]. Various medicinal plants have been used for years in daily life to treat diseases all over the world. In fact, plants produce a diverse range of bioactive molecules, making them rich sources of different types of medicines. Higher plants, which produce medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient time [2]. Over 50 percent of all modern clinical drugs are of natural products origin and natural products play an important role in drug development programs in pharmaceutical industries [3].

Malaysia with its tropical forest is blessed with high biological diversity, which enclosed over 10% of the world's total number of species, with some of them are unique only to Malaysia. Among more than 7,000 species of angiosperms and 600 species of ferns in Malaysia, about 12 to 18% of trees, shrubs and herbs are reported to have medicinal properties [4]. Many biologically active plant-derived compounds were discovered as a result of chemical studies through isolation of active compounds from traditional medicine [5,6]. Our Malaysian flora represents a huge, barely untapped reserve of natural resources which is believed to contain substances with therapeutic potentials that yet to be explored.

Research projects related to natural products carried out in Malaysia included among others the chemistry and technology of palm oil, natural pesticides, natural flavours and pharmacological testing of medicinal plants [7]. Development of organic chemistry has been closely associated with the chemistry of natural products. Many techniques of extraction, separation, structure determination and synthesis have been developed to understand the structural variation among the natural products. Research in this area has certainly led to better understanding of the structural requirements for a variety of physiological activities, leading to the synthesis and modification of several leads compounds and analogues.

The study of bioactive natural products constituents is the first step in drug discovery programs, while the eventual outcomes of blockbuster drugs may not be that easily realized in view to the high cost and research effort [8]. Despite all these, natural products drug discovery programs are developed all over the world, mainly because of the high chemical diversity from natural products as compared to synthetics. The potential of these natural products is largely unknown and endangered plants have added the urgency for more vigorous screening programs.

A number of advances in capability and technology are fostering a renaissance in natural product research and directly or indirectly reducing the historical impediments to development of natural products [9]. The advantage in methodologies for separation technologies such as High Performance Liquid Chromatography (HPLC) and countercurrent partition chromatography have further expanded the capacity for separation of plant chemical constituents [10]. Structure elucidation technology has improved especially with the development of high field NMR allowing rapid and straight forward structure elucidation [11,12]. Gas Chromatography (GC) and High Performance Liquid Chromatography (HPLC) are now being coupled with detectors in what is known as hyphenated techniques (GC-MS, LC-MS, LC-NMR, LC-MS-NMR) enable direct (online) identification of plants constituent prior to their isolation [13].

1.2 Guttiferae (Clusiaceae) Family

Guttiferae synonym as Clusiaceae is composed of about 40 genera and 1000 species [14]. In Malaysia, four genera and 121 species of the Guttiferae are found, namely *Garcinia* (49 species), *Callophyllum* (45 species), *Mesua* (23 species) and *Mammea* (4 species) in different habitat [15]. The majority of these plants are trees, shrubs or herbs which grow from small to medium size woody plants producing resinous white to yellow viscous exudates. Several plants of the Guttiferae family produce important commercial products, edible fruits, seeds oils while timbers of many species are used for building houses and making furniture [16]. Some species of this family are used for traditional medicinal purposes.

1.3 Traditional Medicinal Uses of Guttiferae Family

Garcinia are often used for traditional medicines to treat abdominal pain, dysentery, diarrhoea, infected wound and gonorrhoea [17]. The fruits of *G. xanthochymus* have been widely used for bilious condition, diarrhoea and dysentery in Thailand [18]. In Malaysia and Philippines, a tea made from the rind of *G. mangostana* is used to reduce fever and to treat diarrhea, thrush, dysentery, aphthae, and various disorders of urinary system [19-21]. Meanwhile the fruit hull of *G. mangostana* used for healing skin infections and wound [22]. The bark of *G. cochinchinnensis* is used to cure allergy, itches and skin diseases while the buds are used for the treatment of threatened abortion in Vietnam [23]. In Indonesia, the leaves and seeds of *G. dulcis* have been used for the treatment of lymphatitis, parotitis and struma [24].

Calophyllum is a pan-tropical genus which comprises approximately 180 - 200 species. Generally, they are confined to the tropical rain forest and locally known in Malaysia as '*bintangor*'. This plants are commonly employed as traditionally medicine to treat malaria, bronchitis, gastric and hepatic disturbances, wound infections, inflammation, rheumatism, varicose, hemorrhoids, chronic ulcer and act as diuretic [25-27]. In Brazil, *C. brasiliense*, has been used in folk medicine to treat bronchitis, gastric and hepatic disturbances diarrhea, herpes, rheumatism,

varicose, hemorrhoids and chronic ulcer [28,29]. In Indonesia, the bark of *C. inophyllum* is used after childbirth for vaginal discharge, the passing of blood and also for use in gonorrhoea [30]. In Cambodia, the leaves of *C. inophyllum* are prescribed as an inhalation for migraine and vertigo and the oil for scabies. Meanwhile, in Madagascar, the leaves are applied to sore eyes and the oil from seeds is used against psoriasis and is antirheumatic [31].

Mesua is a small genus of flowering plants in the family Guttiferae. This genus consists of about 48 species in tropical southern Asia and locally known in Malaysia as '*penaga*'. *M. ferrea* is traditionally being used for its antiseptic, antiinflammatory, blood purifier, anthelmintic, cardiotonic, diuretic, expectorant, antipyretic, purgative, antiasthamatic, antiallergic, and several other effects [32]. The dried flower buds are used for dysentery with mucus, astringent, haemostatic, antiinflammatory, stomachic and used in cough, bleeding haemorrhoids and metrorrhagia [33]. In Malaysia and India, a mixture of pounded kernels and seed oil of *M. ferrea* are used for poulticing wounds. The seed oil is used for treating itch and other skin eruptions, dandruff and against rheumatism. In Java, a decoction of the flowers is drunk by women after childbirth.

The genus *Mammea* consists of about 30 species, found in Southeast Asia, Africa and Western India. Many of this species are used in traditional medicine for the treatment of various diseases such as fever, internal heat, stomach pains, scabies and microbial infections [34]. In Nigeria, the stembark of *M. africana* is used in the treatment of malaria related fever, diabetes, microbial infections, mental disorders, stomach pains, rheumatism pains, scabies, cough and hypertension [35]. In India, the flowers of *M. siamensis* are used as a heart tonic whilst the flower buds of *M. longifolia* are chewed after heavy meals to improve digestion [36]. *M. americana* has been used in traditional folk medicine for problems of scalp infections, diarrhea, digestive and eye infections in Central America, South America and the Caribbean.

1.4 *Garcinia* Genus

Garcinia is the largest genus of Guttiferae family which consists of about 450 species, indigenous to tropical Asia, tropical and southern Africa, Madagascar, North East Australia, West Polynesia and tropical America [16]. It is typically small to medium evergreen fruit trees, occasionally shrubs, usually with hard timber and abundant latex which can be found from seashore to the lowland and up to mountain forest. In the rain forest, *Garcinia* tree is shade-tolerent tree and classified as a canopy and under-storey tree.

1.5 Scientific Classification and Botany of *Garcinia* Genus

Scientific classification of *Garcinia* [37]:

Kingdom	:	Plantae
Order	:	Malpighiales
Family	:	Guttiferae
Subfamily	:	Clusioideae
Tribe	:	Garcinieae
Genus	:	*Garcinia*

The *Garcinia* genus is characterized by evergreen trees and shrubs with opposite, coriaceous, shining, glabrous leaves and extreme branches usually 4-sided. Trees in this genus are either dioecious or polygamous. The flowers may be solitary, fascicled and umbelled or panicled, usually with four to five decussate or imbricate and free sepals, and four to five imbricate petals. The male flowers two or four lobed free or joined stamens. Anthers are straight with horse shoe shape. Female flowers have 2-12 celled ovaries. The stigma is visible and sessile. The berry is covered by tough peel and contains numerous large seeds suspended in a pulpy inner part [16].

1.6 *Garcinia prainiana* King

G. prainiana is indigenous to Peninsular Malaysia and southern Thailand. It can be found in the lowland forests from north to south Peninsular Malaysia and southern part of Thailand. It is also cultivated in villages for it fruits [38]. *G. prainiana*, known as button mangosteen or cherapu is a small evergreen tree growing to 10 m high with a narrow, dense and bushy crown with rough greyish-brown bark. It produces white latex. The leaves are large and elliptic, 10-23 cm long, 4.5-11.5 cm wide, simple pointed or blunt, many ribbed and almost sessile. Five-petalled flowers are in dense clusters on the leafy twigs, pale yellowish to wholly pink in colour. The fruits are round and smooth, rather flattened, small 2.5-4.5 cm wide, ripening to orange-yellow with a blackish button-like stigma attached, and possess, thin rinds and pale orange, subacid but sweet pulp **(Figure 1.1)** [38].

Figure 1.1: The leaves, fruits and flower of *G. prainiana*

1.7 *Garcinia hombroniana* Pierre

G. hombroniana is widely distributed and is native to Peninsular Malaysia where it can be found in the coastal regions, from the lowland forests near the sea to the lower mountain forest and the highlands, upper part of Borneo, Cambodia, Thailand, in Andaman, Nicobar Islands and Vietnam [38]. *G. hombroniana*, a sea-shore mangosteen or manggis hutan is an evergreen fast growing medium sized dioecious tree, reaching to a height of 9-18 m and 1.8 m girth with grey bark, peeling off in small oblong flakes. It produces a white latex. The leaves are opposite, 10-14 cm long and 4-8 cm wide. Male flowers are creamy white to yellowish cream in colour. The fruit is round with thin rind, 5 cm in diameter, brightrose in colour with

the scent of apples, and with the flat, disc-like stigma generally raised on the pointed end of the fruit. Fresh fruit is similar to a small mangosteen (*G. mangostana*) and has a taste like a peach but the pulp is somewhat sour **(Figure 1.2)** [38].

Figure 1.2: The leaves and fruits of *G. hombroniana*

1.8 Problem Statement

The plants of *Garcinia* have great interest due to their variety in phytochemicals and biological properties. Extensive works on the isolation and identification of chemical compounds from *Garcinia* species, as well as their biological activities have been reported, as many species from this genus are easily available and are known for their medicinal values. There are many *Garcinia* species from Malaysia that have not been explored thoroughly, both chemically and biologically to support their uses. Several *Garcinia* species are used in traditional medicine to cure various ailments such as abdominal pain, dysentery, diarrhea, infected wound and gonorrhea [17]. *G. hombroniana* is used to relieve itching and as a protective medicine against infections after childbirth [39]. These medicinal uses highlight the need to study the chemical constituents and bioactivity of this species. Based on the above statements, this work is directed towards the study of two *Garcinia* species namely *G. prainiana* King (leaves and barks) and *G. hombroniana* Pierre (roots) aiming on the isolation, identification of phytochemicals as well as the bioactivity screening. To date the phytochemicals of the roots extract of *G. hombroniana* have never been investigated and only a few studies on *G. prainiana* have been reported in the literature. The current studies will contribute to the database of phytochemicals and biological activities of *G. prainiana* and *G. hombroniana*.

1.9 The Research Objectives

The research objectives are:

1. To isolate the phytochemicals from crude extracts of the leaves and bark of *G. prainiana* and roots of *G. hombroniana*
2. To characterize and identify the isolated phytochemicals using spectroscopic technique such as UV, IR, NMR (1D and 2D) and mass spectrometry.
3. To evaluate the biological activities of the crude extracts and pure phytochemicals using several bioassays including antioxidant, antibacterial, tyrosinase inhibition and toxicity activities.

1.10 Scope of Research

The crude extracts were extracted using soxhlet extractor followed by fractionation and purification by chromatographic techniques (vacuum liquid chromatography, column chromatography, preparative thin layer chromatography and centrifugal preparative thin layer chromatography) and recrystalization to afford the pure phytochemicals. Characterization of the isolated phytochemicals was carried out using the combined spectroscopic methods which included IR, UV, NMR (^{1}H, ^{13}C, COSY, HMQC, HMBC) and MS. The evaluations of the biological activities of the crude extracts and selected characterized compounds were carried out by using more than one technique to cover the bioactivity of interest. The crude extracts and selected isolated phytochemicals were screened for total phenolic content, antioxidant, antimicrobial, tyrosinase inhibition activities and toxicity assays. The total phenolic content was evaluated using Folin-Ciocalteau method. The antioxidant activity was performed by using several methods such as phosphomolybdenum complex and DPPH radical scavenging assays. The antimicrobial activity was tested using disc diffusion method with Gram-positive bacteria strain, *Bacillus subtilis* and *Staphylococcus aureus,* and Gram-negative bacteria strain, *Pseudomonas aeruginosa, Pseudomonas putida* and *Escherichia coli*. The cytotoxic activities of the isolated phytochemicals was evaluated using the MTT assay against H1299 and A549 human lung cancer cells. The tyrosinase inhibition assay was performed using the mushroom tyrosinase enzyme assay.

REFERENCES

1. Cragg, G. M., Newman, D. G. and Snader, K. M. Natural products in drug discovery and development. *Journal of Natural Products*. 1997. 6:52–60.
2. Gurib, F. A. Medicinal Plant: Traditions of Yesterday and Drugs of Tomorrow. *Molecular Aspects of Medicine.* 2006. 27: 1-93.
3. Chin, Y. M., Balunas, M. J., Chai, H. B., Kinghorn, A. D. Drug Discovery from Natural Sources. *American Association of Pharmaceutical Scientist, AAPS Journal*. 2006. 8(2): 239-253.
4. Kumara, K., Dan, Y. M. and Tuan Marina, T. I. *Economic Significance of Medicinal Plants in Peninsular Malaysia*. Kuala Lumpur: Forestry Department, Peninsular Malaysia. 1998.
5. Donald, P. B. Medicinal Plants and Phytomedicines. Linking Plant Biochemistry and Physiology to Human Health. *Plant Physiology*. 2000. 124: 507-514.
6. Itokawa, H., Morris, N. S. L., Akiyama T. and Lee K. H. Plant-Derived Natural Product Research Aimed at New Drug Discovery. *Journal Natural Medicines*. 2008. 62: 263-280.
7. Feng, M. C., Sam, T. W. and Khoo, L. E. *Chemistry and Structural Elucidation of Natural Products: Lectures from UNESCO Regional Workshop*. Penang: Universiti Sains Malaysia. 1984.
8. Rahmani, M., Mahmood, M., Sukari, M. A. H., Ee, C. L., Yap, Y. H. and Ali, D. A. I. Natural Products Research: Prospects and Retrospect. In: Goh, S. H., Xu, Y. J., Wu, J., Ho, S. H. and Imiyabir, Z. *Interdisciplinary Approach in Natural Products Research*. Malaysia: Universiti Putra Malaysia. 2000. 1-4.
9. McChestney, J. D., Venkataraman, S. K. and Henri, J. K. Plant Natural Products: Back To Future Or Into Extinction. *Phytochemistry*. 2007. 68: 2015-2022.

10. Pauli, G. *Countercurrent Chromatography of Natural Products CCC*. Paper presented at the 4th international conference of countercurrent chromatography, Bethesda. MD. 2006. 3-5.
11. Korfmacher, W. A. Principles and Applications of LC-MS in New Drug Discovery. *Drug Discovery Today*. 2005. 10(20): 1357-1367.
12. Phillipson, J. D. Phytochemistry and Medicinal Plant. *Phytochemistry*. 2007. 68: 2960-2972.
13. Hostettmann, K. and Wolfende, J. L. Application of Liquid Chromatography/UV/MS and Liquid Chromatography/NMR for The On-Line Identification Of Plant Metabolites. In: C. Tringali (Eds.) *Bioactive Compounds from Natural Sources Isolation, Characterization and Biological Properties*. e-Library. Taylor and Francis. 2004. 32-68.
14. Bennet, G. J. and Lee, H. H. Xanthones from Guttifereae. *Phytochemistry*. 1989. 28(4): 967-998.
15. Whitmore, T. C. *Tree Flora of Malaya*. Longman Malaysia Sdn. Bhd: Kuala Lumpur. 1978. Vol.3.
16. Li, X., Li J., Norman K. B. R., Peter F. S. *Flora of China*. Missouri Botanical Garden Press, St. Louis, and Science Press Beijing. 1990. Vol 13.
17. Jayaprakasha, G. K., Negi, P. S. and Jena, B. S. Antioxidative and Antimutagenic Activities of the Extracts from the Rinds of *Garcinia pedunculata*. *Innovative Food Science and Emerging Technologies*. 2006. **7**(3): 246–250.
18. Wisinee, C., Yushan, Li., Masayuki, S., Yasukatsu, O., Nijsiri, R., Yasushi, O. Prenylated xanthones with NGF-potentiating activity from *Garcinia xanthochymus*. *Phytochemistry*. 2003. 64: 981–986.
19. Perry, L. and Metzger, J. Medicinal plants of East and Southeast Asia: Attributed Properties and Uses. Cambridge, Massachusetts, MIT Press. 1980. 174.
20. Lim, T. K. Edible Medicinal and Non-Medicinal Plants. Fruits. Springer. Science Business Media BV. 2012. 2:83-103.
21. Williams, C. Medicinal Plants in Australia. Plants, potions and poisons. Rosenberg Publishing. 2012. 3:229.

22. Mahabusarakam, W., Wiriyachitra, P. and Taylor W. C. Chemical Constituents of *Garcinia mangostana*. *Journal of Natural Products*. 1987. 50: 474 - 478.

23. Hiep, D. N., Binh, T. D. T., Lien, H. D. N. Guttiferones Q-S, cytotoxic polyisoprenylated benzophenones from the pericarp of *Garcinia cochinchinensis*. *Phytochemistry Letters*. 2011. 4: 129–133.

24. Kosela, S. L. H., Hu, T. R., Hanafi M. and Sim, K. Y. Dulxanthones F-H, Three New Pyranoxanthones from *Garcinia dulcis*. *Journal of Natural Products*. 2000. 63: 406-407.

25. Burkill, I. H. A Dictionary of the Economic Products of the Malay Peninsula. Kuala Lumpur: Ministry of Agriculture and Cooperatives. 1966. 410-417.

26. Alkhamaiseh, S. I., Taher, M. and Ahmad, F. The phytochemical Contents and Antimicrobial Activities of Malaysian *Calophyllum rubiginosum*. *American Journal Applied Sciences.* 2011. 8:201-205.

27. Su, X. H., Zhang, M. L., Huo, C. H., Gu, Y. C. and Shi, Q. W. Chemical Constituents of the Plants of the Genus *Calophyllum. Chemistry Biodiversity.* 2008. 5:2579-2608.

28. Sartori, N. T., Canepelle D., Souza, Jr. P. T. and Martins, D. T. O. Gastroprotective effect from *Calophyllum brasiliense* Camb. bark on experimental gastric lesions in rats and mice. *Journal Ethnopharmacology*. 1999. 67:149-156.

29. Da Silva, K. L., Santos, A. R. S., Mattos, P. E. O., Yunes, R. A., Delle-Monache, F. and CechinelFilho, V. Chemical composition and analgesic activity of *Calophyllum brasiliense* leaves. *Therapie*. 2001. 56:431-434.

30. Quisumbing, E. A. Medicinal Plants of the Philippines. Department of Agriculture and Natural Resources, Katha Publishing Company, Manila. 1978.

31. Burkill, H. M. The Useful Plants of West Tropical Africa, Families E-I. Royal Botanic Gardens Kew. 2(2). 1994.

32. Chahar M. K., Kumar D. S. S., Geetha L., Lokesh T. and Manohara K. P. *Mesua ferrea*: A review of the evidence for its phytochemistry and pharmacological actions. *African Journal Pharmacy Pharmcology*. 2013. 7(6):211-219.

33. Chopra R. N., Nayar S. L. and Chopra I. C. Glossary of Indian medicinal plants. Council Scientific Industrial Research, New Delhi. 1986. 330.

34. Chapius, J., Sordat, B. and Hostettmann, K. Screening for cytotoxic activity of plant used in traditional medicine. *Journal Ethnopharmacology*. 1988. 23:273.

35. Adjanohoun, J. E., Aboubakar, N., Dramane, K., Ebot, M. E., Ekpere, J. A., Enoworock, E. G., Foncho, D., Gbile, Z. O., Kamanyi, A., Kamoukom, K. A., Mbenkum, T., Mbi, C. M., Mbielle, A. L., Mbome, I. L., Mubiru, N. K., Naney, W. L., Nkongmeneck, B., Satabie, B., Sofowora, A., Tanze, V. and Wirmum, C. K. *Traditional Medicine and Pharmacopeia Contribution to Ethnobotanical and Floristic Studies in Cameroon.* Porto-Novo, Benin: CNPMS. 1996. 15.

36. Joshi, B. S., Kamat, V. N., Govindachari, T. R. and Ganguly, A. K. Isolation and structure of surangin A and surangin B, two new coumarins from *Mammea longifolia* (wight) Planch and Triana. *Tetrahedron*. 1969. 25:1453-1458.

37. Corner, E. J. H. *Wayside Trees of Malaya*. Malayan Nature Society: Kuala Lumpur. 1965. Vol. 1.

38. Lim, T. K. Edible Medicinal and Non-Medicinal Plants. Fruits. 2012. p. 56-57, p. 120-121.

39. Nazre, M. Historical review and notes on the correct scientific name for seashore mangosteen. *Genetic Resources Crop Evolution.* 2010. 57:1249-1259.

40. Peres, V. and Nagem, T. J. Trioxygenated naturally occurring xanthones. *Phytochemistry*. 1996. 44: 191-214.

41. Negi, J. S., Bisht, V. K., Singh, P., Rawat, M. S. M. and Joshi, G. P. Naturally occurring xanthones: chemistry and biology. *Journal of Applied Chemistry*. 2013.

42. Mandal, S., Das, P. C. and Joshi, P. C. Naturally occurring xanthones from terrestrial flora. *Journal Indian Chemical Society.* 1992. 69: 611-636.

43. El-Seedi, H. R., El-Barbary, M. A., El-Ghorab, D. M. H., Bohlin, L., Borg-Karlson, A. K., Goeransson, U. and Verpoorte, R. Recent insights into the biosynthesis and biological activities of natural xanthones. *Current Medicinal Chemistry.* 2010. 17: 854-901.

44. Gupta, P., Lewis, J. R. Biogenesis of xanthones in *Gentiana lutea*. *Journal Chemical Society C.* 1971. 4: 629-631.

45. Fujita, M. and Inoue, T. Biosynthesis of mangiferin in *Anemarrhena asphodeloides* bunge. I. The origin of the xanthone nucleus. *Chemical Pharmaceutical Bulletin.* 1980. 28: 2476-2481.

46. Beerhues, L., Barillas, W., Peters, S. and Schmidt, W. *Biosynthesis of plant xanthones. Bioorganic chemistry. Highlights and New Aspects.* Weinheim, Germany. Wiley-VCH. 1999. p. 322-328.

47. Schmidt, W. and Beerhues, L. Alternative pathways of xanthone biosynthesis in cell cultures of *Hypericum androsaemum* L. *FEBS Lett.* 1997. 420: 143-146.

48. Kitanov, G. M. and Nedialkov, P. T. Benzophenone *O*-glucoside, a biogenic precursor of 1,3,7-trioxygenated xanthones in *Hypericum annulatum*. *Phytochemistry*. 2001. 57: 1237-1243.

49. Holker, J. S. E., Lapper, R. D. and Simpson T. J. The biosynthesis of fungal metabolites. Part IV. Tajixanthone: ^{13}C-nuclear magnetic resonance spectrum and feedings with [1-^{13}C] and [2-^{13}C]-acetate. *Journal Chemical Society Perkin I*. 1974. 2135-2140.

50. Mohamed, G. A., Ibrahim, S. R., Shaaban, M. I., Ross, S. A. Mangostanaxanthones I and II, new xanthones from the pericarp of *Garcinia mangostana*. *Fitoterapia*. 2014. 98: 215-21.

51. Hau, T. V., Ngo, T. N., Gerhard, M., Udo, R. W., Hung, D. P., Lien, D. N. Xanthones from the bark of *Garcinia pedunculata*. *Phytochemistry Letters*. 2012. 5: 766–769.

52. Yu, W. H., Wei Z. Z. X., Xue, G., Guang, H. K., Xiu, P. L. Three new xanthones from the stems of *Garcinia oligantha* and their anti-TMV activity. *Phytochemistry Letters*. 2013. 6:629–632.

53. Kanda, P., Wipapan, P., S, P., Walter, C. T. Tetraoxygenated xanthones from the fruits of *Garcinia cowa*. *Phytochemistry*. 2006. 67: 999–1004.

54. Hau, T. V., Ngoc, Tuyet, T. N., Hieu, T. N., Khoa, Q. D., Joseph, D. C., Gerhard, M., Jorg, H., Udo, R. W., Hung, D. P., Lien, H. D. N. Cytotoxic tetraoxygenated xanthones from the bark of *Garcinia schomburgkiana*. *Phytochemistry Letters*. 2012. 5: 553–557.

55. Mbwambo, Z. H., Kapingu, M. C., Moshi, M. J., Machumi, F., Apers, S, Cos, P., Ferreira, D., Marais, J. P., Vanden, B. D., Maes, L., Vlietinck, A., Pieters, L. Antiparasitic activity of some xanthones and biflavonoids from the root bark of *Garcinia livingstonei. Journal Natural Products.* 2006. 69(3): 369-372.

56. Lien, H. D. N., Leslie, J. H. Xanthones and triterpenoids from the bark of *Garcinia vilersiana*. *Phytochemistry*. 2000. 53: 111-114.

57. Vatcharin, R., Thunwadee, R., Athipol, P., Prakart, S., Walter, C. T. Xanthones from the stem bark of *Garcinia nigrolineata*. *Phytochemistry*. 2003. 64: 1149–1156.

58. Beerhues, L., Liu, B. Biosynthesis of biphenyls and benzophenones--evolution of benzoic acid-specific type III polyketide synthases in plants. *Phytochemistry*. 2009. 70(15-16): 1719-1727.

59. Wu, C. C., Lu, Y. H., Wei, B. L., Yang, S. C., Won, S. J. and Lin, C. N. Phloroglucinols with prooxidant activity from *Garcinia subelliptica. Journal Natural Products.* 2008. 71: 246-250.

60. Ibrahim, J., Fadlina, C. S., Benzophenones and xanthones from *Garcinia cantleyana* var. cantleyana and their inhibitory activities on human low-density lipoprotein oxidationand platelet aggregation. *Phytochemistry*. 2012. 80: 58–63.

61. Hiep, D. N., Binh, T. D. T., Lien, H. D. N. Guttiferones Q-S, cytotoxic polyisoprenylated benzophenones from the pericarp of *Garcinia cochinchinensis*. *Phytochemistry Letters*. 2011. 4: 129–133.

62. Ali, S., Goundar, R., Sotheeswaran, S., Beaulieu, C., Spino, C. Benzophenones of *Garcinia pseudoguttifera* (Clusiaceae). *Phytochemistry*. 2000. 53(2): 281-284.

63. Claude, S., Jitendra, L., Subramaniam, S., William, A. Three prenylated phenolic benzophenones from *Garcinia myrtifolia*. *Phytochemistry*. 1995. 38(1): 233-236.

64. Xue, M., Gao, T. Y., Fanny, S. F. L., Jian, X. P., Chun, F. Q., Yan, Z., Xin, L., Jing, Z. S., Kathy, Q. L., Hong, X. X. Novel polyisoprenylated benzophenone derivatives from *Garcinia paucinervis*. *Tetrahedron Letters*. 2010. 51: 2442–2446.

65. Joseph, J. M., Modest, C. K., Merhatibeb, B., Berhanu, M. A. Polyisoprenylated benzophenones from *Garcinia semseii* (Clusiaceae). *Phytochemistry Letters*. 2008. 1: 215–218.

66. Sahu, A., Das, B., Chatterjee, A. Polyisoprenylated benzophenones from *Garcinia pedunculata*. *Phytochemistry*. 1989. 28(4): 1233-1235.

67. Chattopadhyay, S. K., Kumar, S. Liquid Chromatography-tandem mass spectroscopy method for identification and quantification of two biologically active polyisoprenylated benzophenones, isoxanthochymol and camboginol in *Garcinia* species. *Biomedical Chromatography.* 2011. 11: 1159-1165.

68. Ito, C., Itoigawa, M., Miyamoto, Y., Onoda, S., Rao, K. S., Mukainaka, T., Tokuda, H., Nishino, H., Furukawa, H. Polyprenylated benzophenones from *Garcinia assigu* and their potential cancer chemopreventive activities. *Journal Natural Products.* 2003. 66(2): 206-209.

69. Geiger, H. and Quinn, C. *Biflavonoids. In: The flavonoids*. Academic Press, New York, 1975. p. 692-742.

70. Dewick, P. M. *Medicinal natural products: a biosynthesis approach,* 3rd edition. John Wiley & Son, Inc., Hoboken, New Jersey. 2009.

71. Mercader, A. G. and Pomilio, A. B. *Biflavonoids: occurrence, structural features and bioactivity.* Nova Science Publishers, Inc. 2012.

72. Yang, G., Liao, Z., Xu, Z., Zhang, H. and Chen, D. Antimitotic and antifungal C-3/C3"-biflavonones from *Stellera chamaejasme*. *Chemical Pharmaceutical Bulletin.* 2005. 53: 776-779.

73. Si, D., Zhong, D., Sha, Y. and Li, W. (2001): Biflavonoids from the aerial part of *Stephania tetranda*. *Phytochemistry*. 2001. 58: 563-566.

74. Parveen, M., Ilyas, M., Mushfiq, M., Busudan, O. A. and Muhaisen, H. M. H. A new biflavonoid from the leaves of *Garcinia nervosa*. *Natural Product Research.* 2004. 18: 269-275.

75. Lin, L. C. and Chou, C. J. Three new biflavonoids from *Selaginella delicatula*. *Chinese Pharmaceutical Journal.* 2000. 52: 211-218.

76. Muhaisen, H. M. H., Ilyas, M., Mushfig, M., Parveen, M. and Basudan, O. A. Flavonoid from *Viburnum continifolium*. *Journal Chemical Research.* 2002. 480-481.

77. Reddy, B. A. K., Reddy, N. P., Gunasekar, D., Blond, A. and Bodo, B. Biflavonoids from *Ochna lanceolata*. *Phytochemistry Letters.* 2008. 1: 27-30.

78. Ofman, D. J., Markham, K. R., Vilain, C. and Molloy, B. P. J. Flavonoid profiles of New Zealand kauri and other species of *Agathis*. *Phytochemistry*. 1995. 38: 1223-1228.

79. Mercader, A. G. and Pomilio, A. B. *Biflavonoids: occurrence, structural features and bioactivity*. Nova Science Publishers, Inc. 2012.

80. Baker, W. and Simmonds, W. H. C. Derivatives of 5,6,4'- and 5,8,4'-trihydroxyflavones and a note on the structure of ginkgetin. *Journal Chemical Society.* 1940.1370-1375.

81. Kongkiat, T., Vatcharin R., Souwalak, P., Nongporn, H. T. Tetraoxygenated xanthones and biflavonoids from the twigs of *Garcinia merguensis*. *Phytochemistry Letters*. 2013. 6(4): 511–513.

82. Zakaria, H., Mbwambo, M. C., Kapingu, M, J., Moshi, F. M., Sandra, A., Paul, C., Daneel, F., Jannie, P. J. M., Dirk, V., Louis, M., Arnold, V., Luc, Pi. Antiparasitic Activity of Some Xanthones and Biflavonoids from the Root Bark of *Garcinia livingstonei. Journal Natural Products.* 2006. 69: 369-372.

83. Vanessa, S. G., Thiago, C. S., Isael, A. R., Marisi, G. S., Marcelo, A. S., Wagner, V., Cláudio, V. J., Marcelo, H. S. Isolation and evaluation of the antioxidant activity of phenolic constituents of the *Garcinia brasiliensis* epicarp. *Food Chemistry*. 2012. 132: 1230–1235.

84. Quan, B. H., Song, F. L., Chun, F. Q., Zhen, D. H., Jing, Z. S., Han, D. S., Hong, X. X. Complete NMR Assignments of the Antibacterial Biflavonoids GB1 from *Garcinia kola*. *Chemical Pharmaceutical Bulletin.* 2005. 53(8): 1034-1036.

85. Chappell, J. The biochemistry and molecular biology of isoprenoid metabolism. *Plant Physiology*. 1995. 107:1-6.

86. Ran, X., Gia, C. F. and Seiichi, P. T. M. On the origins of triterpenoid skeletal diversity. *Phytochemistry*. 2004. 65:261-291.

87. Vatcharin, R., Ajaman, A., Pimchit, D., Walter, C. T., Peter, C. T. Lanostanes and friedolanostanes from the pericarp of *Garcinia hombroniana*. *Phytochemistr*y. 2000. 55: 183-188.

88. Vatcharin, R., Somsak, S., Pueksa, K. and Souwalak, P. Friedolanostanes and Lanostanes from the Leaves of *Garcinia hombroniana. Journal Natural Products.* 2005. 68: 1222-1225.

89. Saranyoo, K., Yaowapa, S., Vatcharin, R., Souwalak, P. Friedolanostanes and xanthones from the twigs of *Garcinia hombroniana*. *Phytochemistry*. 2013. 85: 161–166.

90. Hiep, D. N., Binh, T. D. T., Quyen, N. T., Hoan, D. N., Hung, D. P., Poul, E. H., Fritz, D., Joseph, D. C., Lien, H. D. N. Friedolanostane, friedocycloartane and benzophenone constituents of the bark and leaves of *Garcinia benthami*. *Phytochemistry*. 2011. 72: 290–295.

91. Luis, M. M., Vieira, A. K., Artur, M. S. S., Ing, O. M., Surapong, K., Luis, G., Ana, M. D., Werner, H. Lanostanes and friedolanostanes from the bark of *Garcinia speciose. Phytochemistry*. 2004. 65: 393–398.

92. Saranyoo, K., Yaowapa, S., Vatcharin, R., Nongporn, H. T., and Kanokphorn, C. Flavanone glucuronides from the leaves of *Garcinia prainiana*. *Canadian Journal Chemistry.* 2011. 89: 461–464.

93. Shukranul, M., Ikram, M. S. Chemical Constituents of *Garcinia prainiana. Sains Malaysiana*. 2012. 41(5): 585–590.

94. Elfita, E., Muharni, M., Madyawati, L., Darwati, D., Ari, W., Supriyatna, S., Husein, H. B., Dachriyanus, D., Paul, C., Kenne, F., Sandra, A., Luc, P. Antiplasmodial and other constituents from four Indonesian *Garcinia* spp. *Phytochemistry*. 2009. 70: 907–912.

95. Anne, E. H., Joumaa, M., Anne, L., Marc, L., Fabrice, P., Patrice, L. P., Pascal, R., Antileishmanial polyphenols from *Garcinia vieillardii*. *Fitoterapia*. 2008. 79: 42–46.

96. Panthong, K. N., Hutadilok, T. and Panthong, A. Cowaxanthone F, a new tetraoxygenated xanthone and other anti-inflammatory and antioxidant compounds from *Garcinia cowa*. *Canadian Journal Chemistry.* 2009. 87: 1636-1640.

97. Pereira, I. O., Marques, M. J., Pavan, A. L., Codonho, B. S., Barbiéri, C. L., Beijo, L. A., Doriguetto, A. C., D'Martin, E. C., Santos, M. H. Leishmanicidal activity of benzophenones and extracts from *Garcinia brasiliensis* Mart. Fruits. *Phytomedicine.* 2010. 17(5): 339-345.

98. Lih, G. C., Ling, L. Y., Ching, C. W. Anti-inflammatory activity of mangostins from *Garcinia mangostana*. *Food and Chemical Toxicology*. 2008. 46: 688–693.

99. Chen, S. X., Wan, M., Loh, B. N. Active constituents against HIV-1 protease from *Garcinia mangostana*. *Planta Medica*. 1996. 62: 381-382.

100. Kirk, R., Gustafson, J. W. B., Murray, H. G., Munro, R. W. F., Tawnya, C. M., John, H. C., James, B. M., Gordon, M. C., Michael, R. B. The guttiferones, HIV-inhibitory benzophenones from *Symphonia globulifera*, *Garcinia livingstonei*, *Garcinia ovalifolia* and Clusiarosea. *Tetrahedron*. 1992. 48(46): 10093-10102.

101. Joseph, N., Turibio, K. T., Muhammad, S. A. Antimicrobial and immunomodulatory properties of prenylated xanthones from twigs of *Garcinia staudtii*. *Bioorganic & Medicinal Chemistry*. 2009. 17: 5688–5695.

102. Justin, K., Meli, A. L., Manfouo, R. N., David, L., Ngounou, F. N., Kuete, V., Hippolyte, W. K., Pierre, T.,, Bonaventure, T. N., Beiban, L. S., Joseph, D. C. Xanthones from *Garcinia smeathmannii* (Oliver) and their antimicrobial activity. *Phytochemistry*. 2005. 66: 1713–1717.

103. Muhammad, T., Deny, S., Mohamad, F. R., Farah, S. A. Z., Solachuddin, J. A., Suhaib, I. A., Farediah, A. Apoptosis, antimicrobial and antioxidant activities of phytochemicals from *Garcinia malaccensis* Hk. *Asian Pacific Journal of Tropical Medicine*. 2012. 136-141.

104. Panthong, K., Pongcharoen, W., Phongpaichit, S. and Taylor, W. C. Tetraoxygenated xanthones from the fruits of *Garcinia cowa*. *Phytochemistry.* 2006. 67: 999-1004.

105. Siridechakorn, I., Phakhodee, W., Ritthiwigrom, T., Promgool, T., Deachathai, S., Cheenpracha, S., Prawat, U., Laphookhieo, S. Antibacterial dihydrobenzopyran and xanthone derivatives from *Garcinia cowa* stem barks. *Fitoterapia*. 2012. 83(8): 1430-1434.

106. Sakunpak, A. and Panichayupakaranant, P. Antibacterial activity of Thai edible plants against gastrointestinal pathogenic bacteria and isolation of a new broad spectrum antibacterial polyisoprenylated benzophenone, chamuangone. *Food Chemistry*. 2012. 130: 826–831.

107. Deachathai, S., Mahabusarakam, W., Phongpaichit, S., Taylor, W. C., Zhang, Y. J., Yang, C.R. Phenolic compounds from the flowers of *Garcinia dulcis*. *Phytochemistry*. 2006. 67: 464–469.

108. Su, B. N., Keller, W. J., Mehta, R. G., Kinghorn, A. D. Antioxidant xanthones from the pericarp of *Garcinia mangostana* (Mangosteen). *Journal Agriculture Food Chemistry*. 2006. 54(6): 2077-2082.

109. Joumaa, M., Marie, C. A., David, R., Vincent, D., Anne, M. L. R., Denis, S., Pascal, R. Prenylated xanthones and tocotrienols from *Garcinia virgata*. *Phytochemistry*. 2004. 65: 2915–2920.

110. Lannang, A. M., Komguem, J., Ngninzeko, F. N., Gustave, J. T., D. Lontsi, D. A., Asma, A., Iqbal, C. M., Rosa, R., Krishna, P. D., B. Luc, S. Bangangxanthone A and B, two xanthones from the stem bark of *Garcinia polyantha* Oliv. *Phytochemistry*. 2005. 66: 2351–2355.

111. Vatcharin, R., Wanpen, N., Souwalak, P., Nongporn, H. T., Katesarin, M. Phloroglucinols, depsidones and xanthones from the twigs of *Garcinia parvifolia*. *Tetrahedron*. 2006. 62: 8578–8585.

112. Vanessa, S. G., Thiago, C. S., Isael, A. R., Marisi, G. S., Marcelo, A. S., Wagner, V., Cláudio, V. J., Marcelo, H. S. Isolation and evaluation of the antioxidant activity of phenolic constituents of the *Garcinia brasiliensis* epicarp. *Food Chemistry*. 2012. 132: 1230-1235.

113. Joseph, J. M. A bioactive isoprenylated xanthone and other constituents of *Garcinia edulis*. *Fitoterapia.* 2010. 81: 420–423.

114. Berna, E., Hong, P. H., Soleh, K., Muhammad, H., Xiao, J. H. A new cytotoxic xanthone from *Garcinia rigida*. *Fitoterapia*. 2008. 79: 182–184.

115. Hau, T. V., Ngoc, T. T. N., Hieu, T. N., Khoa, Q. D., Joseph, D. C., Gerhard, M., Jorg, H., Udo, R. W., Hung, D. P., Lien, H. D. N. Cytotoxic tetraoxygenated xanthones from the bark of *Garcinia schomburgkiana*. *Phytochemistry Letters*. 2012. 5: 553-537.

116. Li, L. W., Zhan, L. L., Yong, P. X., Xiao, Q. L., Yue, H. P., Yong, K. J., Hui, M. H., A new cytotoxic caged polyprenylated xanthone from the resin of *Garcinia hanburyi*. *Chinese Chemical Letters*. 2008. 19: 1221–1223.

117. Khalid, A. S., Khozirah, S., Faridah, A., Daud, A. I., Ahmad, S. H., Normawati, S., Khoushik, S., Nordin, H. L. Cytotoxic caged-polyprenylatedxanthonoids and a xanthone from *Garcinia cantleyana*. *Phytochemistry*. 2007. 68: 2537–2544.

118. Xue, M. G., Ting, Y., Fanny, S. F. L., Jian, X. P., Chun, F. Q., Yan, Z., Jing, Z. S., Kathy, Q. L., Hong, X. X., Xin, L. Novel polyisoprenylated benzophenone derivatives from *Garcinia paucinervis*. *Tetrahedron Letters*. 2010. 51: 2442–2446.

119. Pereira, I.O., Marques, M. J., Pavan, A. L. R., Codonho, B. S. Barbi, C. L., Beijo, L. A., D'Martin, E. C., Santos, M. H., Doriguetto A. C. Leishmanicidal activity of benzophenones and extracts from *Garcinia brasiliensis* Mart. Fruits. *Phytomedicine*. 2010. 17: 339–345.

120. Alembert, T. T., Ayele, T., Ermias, D., Norbert, A. and Ludger, A. W. Squalene and Amentoflavone from *Antidesma lacianatum. Bulletin Chemical Society Ethiopia.* 2006. 20(2): 325-328.

121. Alam, A. H. M. K., Rahman, M. A. A., Baki, M. A., Rashid, M. H. and Sadik, G. Chemical constituents of *Hemigraphis hirta* T. Anders (Acantahceae). *Pakistan Journal of Biological Sciences*. 2002. 5(11): 1264-1266.

122. Carmen, L. Q., Guilherme, F. S., Patricia, C. D., Ana, P., Joao, E. C. Evaluation of the antiulcerogenic activity of friedelan-3β-ol and friedelin isolated from *Maytenus ilicifolia* (Celastraceae). *Journal of Ethnopharmacology*. 2000. 72: 465-468.

123. Grasiely, F. S., Lucienir, P. D., Antônio, F. C. A., Grácia, D. F. S., Sidney, A. V. F., Roqueline, R. S., Djalma, M. O. and Jacqueline, A. T. New Triterpenes from *Maytenus robusta*: Structural Elucidation Based on NMR Experimental Data and Theoretical Calculations. *Molecules*. 2012. 17: 13439-13456.

124. Jamal, A. K., Yaacob, W. A. and Laily, B. D. A Chemical Study on *Phyllanthus reticulates*. *Journal of Physical Science*. 2008. 19(2): 45–50.

125. Darcy, B., William, F. R., Greg, B., Paul, B. R. and Raul, G. E. Assignment of ^{1}H and ^{13}C spectra and investigation of hindered side-chain rotation in lupeol derivatives. *Magnetic Resonance Chemistry.* 2000. 38: 488–493.

126. Chopra, G. R. J., Sheshadri, A. C. T. R. Constitution of the triterpenicseco-acid putranjivic acid & methyl putranjivate. *Indian Journal of Chemistry*. 1969. 7(12): 1179-1181.

127. Mradu, G., A review of pharmacological properties, pharmacognosy and therapeutic actions of *Putranjiva roxburghii* Wall. (Putranjiva). *International Journal of Herbal Medicine*. 2016. 4(6): 104-108.

128. Xing, C. L., Alpana, S. J., Bo, T., Hala, N. E., Larry, A. W., Jordan, K. Z., Naneel, F. Absolute configuration, conformation and chiral properties of flavanone-(3-8)-flavone biflavonoids from *Rheedia acuminata*. *Tetrahedron*. 2002. 58: 8709-8717.

129. Mabry, T. J., Markham K. R. and Thomas, M. B. *The Systematic Identification of Flavonoids*. Springer-Verlag New York Inc. 1970.

130. Peter, G. W. and Elizabeth, G. C. Xanthone and Biflavanoids from *Garcinia densivenia* stem bark. *Phytochemistry*. 1980. 19: 2723-2726.

131. Herbin, G. A., Jackson, B., Locksley, H. D., Scheinmann, F., Wolstenholme, W. A. The biflavonoids of *Garcinia volkensii* (Guttiferae). *Phytochemistry*. 1970. 9(1): 221-226.

132. Klaiklay, S., Sukpondma, Y., Rukachaisirikul, V., Phongpaichit, S. Friedolanostanes and xanthones from the twigs of *Garcinia hombroniana*. *Phytochemistry*. 2013. 85: 161-166.

133. Yuan, W. L., Leslie, J. H. (20R,23E)-Eupha-8,23-diene-3β,25-diol from *Tripetalum cymosum*. *Phytochemistry*. 1999. 50: 849-857.

134. Kaikabo, A. A., Samuel, B. B., Eloff, J. N. Isolation and activity of two antibacterial biflavonoids from *Garcinia livingstonei* leaf extract. *Natural Products Communications*. 2009. 4: 1–4.

135. Kenneth, M., Carolyn, S., Hans, G. ^{13}C NMR studies of some naturally occurring amentoflavone and hinokiflavone biflovonoids. *Phytochemistry*. 1987. 26(12): 3335-3337.

136. Joseph, J. M. A bioactive isoprenylated xanthone and other constituents of *Garcinia edulis*. *Fitoterapia*. 2010. 81: 420–423.

137. Alain, M. L., Justin, K., Fernande, N. N., Jean, G. T., David, L., Asma, A., Muhammad, I. C., Beiban, L. S. and Atta, U. R. Antioxidant benzophenones and xanthones from the root bark of *Garcinia smeathmannii*. *Bulletin Chemical Society Ethiopia.* 2006. 20(2): 247-252.

138. Vatcharin, R., Wanpen, N., Nongporn, H. T., Souwalak, P. and Katesarin, M. Phloroglucinols, depsidones and xanthones from the twigs of *Garcinia parvifolia*. *Tetrahedron*. 2006. 62: 8578–8585.

139. Ahmed, A. S., Adonis, B. A., Osmany, C., Luca, R. Biflavonoids, Main Constituents From *Garcinia bakeriana* Leaves. *Natural Product Commun*ica*tions.* 2013. 8(9): 1237-1240.

140. Eva, M., Gomotsang, B. M., Runner, R.T. M. Benzophenone derivatives from *Garcinia livingstonei* and their antioxidant activities. *Phytochemistry Letters*. 2016. 18: 29–34.

141. Hemshekhar, M., Sunitha, K., Santhosh, M. S., Devaraja, S., Kemparaju, K., Vishwanath, B. S., Niranjana, S. R. and Girish, K. S. : An overview on genus *Garcinia*: phytochemical and therapeutical aspects. In: *Phytochemistry Reviews*. 2011. 10: 325-351.

142. Williams G. M., Iatropoulos, M. J. and Whysner, J. Safety Assessment of Butylated Hydroxyanisole and Butylated Hydroxytoluene as Antioxidant Food Additives. *Food and Chemical Toxicology*. 1999. 37(9-10): 1027-1038.

143. Ling, S. K., Fukumori, S., Tomii, K., T. Tanaka, T., Kouno, I. Isolation, Purification and Identification of Chemical Constituents from *Elateriospermum tapos*. *Journal of Tropical Forest Science*. 2006.18(1): 81-85.

144. Ozen, T. Antioxidant Activity of Wild Edible Plants in the Black Sea Region of Turkey. *Grasas Y Aceites*. 2010. 61: 86-94.

145. Ali, S., Kasoju, N., Luthra, A., Singh, A., Sharanabasava, H., Sahu, A. and Bora, U, Indian Medicinal Herbs as Sources of Antioxidants. *Food Research International*. 2008. 4: 1-15.

146. Prieto, P., Pineda, M. and Angiular, M. Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. *Analytical Biochemistry*. 1999. 269: 337-341.

147. Rahim, A. A., Rocca, E., Steinmetz, J., Jain, K., Sani, I. M. and Osman, H. Antioxidant activities of mangrove *Rhizophora apiculata* bark extracts. *Food Chemistry*. 2008. 107(1): 200-207.

148. Shimizu, K. R., Sakai, K. Inhibition of Tyrosinase by Flavanoids, Stilbenes and Related 4-Substituted Recorcinols: Structure-Activity Investivigations. *Planta Medica*. 2000. 66: 11-15.

149. Sousa, R. M. F., Morais, S. A. L. D., Viera, R. B. K., Napolitano, D. R., Guzman, V. B., Moraes, T. S., Cunha, L. C. S., Nascimento, E. A. D. and Oliviera, A. D. Chemical Composition, Cytotoxic and Antibacterial Activity of the Essential Oil from *Eugena calycina* Cambess. Leaves Against Oral Bacteria. *Industrial Crops and Products*. 2015. 65: 71-78.

150. Saewan, N., Koysomboon, S., Chantrapromm, K. Anti-tyrosinase and anti-cancer activities of flavonoids from *Blumea balsamifera* DC. *Journal of Medicinal Plant Research*. 2011. 5: 1018-1025.

151. Wibowo, A., Ahmat, N., Hamzah, A. S., Sufian, A. S., Ismail, N. H., Ahmad, R., Jaafar, F. M. and Takayama, H. Malaysianol A, A New Trimer Resveratrol Oligomer from the Stem Bark of *Dryobalonops aromatica*. *Fitoterapia*. 2011. 82: 676-681.

152. Taher, M., Susanti, D., Rezali, M. F., Zohri, F. S., Ichwan, S. J., Alkhamaiseh, S. I., Ahmad, F. Apoptosis, antimicrobial and antioxidant activities of phytochemicals from *Garcinia malaccensis* Hk. *Asian Pasific Journal Tropical Medicine.* 2012. 5: 136.

153. Reddy, L. H., Renoir, J. M., Marsaud, V., Lepetre-Mouelhi, S., Desmaele, D., Couvreur, P. Anticancer efficacy of squalenoyl gemcitabine nanomedicine on 60 human tumor cell panel and on experimental tumor. *Molecular Pharmaceutics*. 2009. 6: 1526-35.

154. Loo, A. Y., Jain, K. and Darah, I. Antioxidant Activity of Compounds Isolated from the Pyroligneus Acid, *Rhizopora apiculata*. *Food Chemistry.* 2008. 107: 1151-1160.

155. Murray, P. R., Baron, E. J., Pfaller, M. A., Tenover, F. C., Yolken, R. H. *Manual of clinical microbiology*, 7th ed. ASM, Washington, DC, USA. 1999.

156. Gulluce, M., Sokmen, M., Sahin, M., Sokmen, A., Adiguzel, A., Oze,l H. Biological activities of essential oil and methanolic extract of *Micromeria fruticosa* (L.) Druce ssp. *serpyllifolia* (Bieb) plants from the eastern Anatolia region of Turkey. *Journal of Science Food Agriculture*. 2004. 84: 735-741.

157. Kobo, I., Kinst-Hori, I., Chaudri, S. K., Kubo, Y., Sanchez, T., Ogura, T. Flavanols from *Heterotheca inuloide* : tyrosinase inhibitory activity and structural criteria. *Bioorganic Medicinal Chemistry.* 2000. 8: 1749-1755.

158. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxic assays. *Journal Immunological Methods*. 1983. 65: 55-63.

159. Nottola S. A., Makabe, S., Stallone, T., Familiari, G., Correr, S., Macchiarelli, G. Surface morphology of the zona pellucida surrounding human blastocysts obtained after in vitro fertilization. *Archives Histology Cytology.* 2005. 68: 133-141.