Phenolic Constituents from *Garcinia intermedia* and Related Species

by

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Abstract

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Antioxidants from plants scavenge free radicals and prevent reactive oxygen species from having damaging effects in common ailments such as inflammation, atherosclerosis, and Alzheimer's disease. As part of our ongoing studies of antioxidants from tropical edible fruits, we have studied *Garcinia intermedia* (Pittier) Hammel [synonym: *Rheedia edulis* (Seem.) Planch. & Triana], native to Central America. In the fruits the following compounds were identified: guttiferone A, guttiferone E, xanthochymol, fukugetin, volkensiflavone and fukugeside. A new compound was tentatively identified in the fruits of this species. The antioxidant activity of guttiferone A in the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was IC_{50} = 46 μM. The antiproliferative effect of guttiferone A against colon cancer cells (HT-29) was IC_{50} = 15.8 μM.

A reversed-phase high-performance liquid chromatography (RP-HPLC) method with diode array detection (DAD) was developed and validated to quantify seven major phenolic compounds in eight *Garcinia* species from different geographic regions*: G. mangostana*, *G. xanthochymus*, *G. spicata, G. livingstonei*, *G. intermedia*, *G. hombroniana*, *G. kola*, and *G. aristata*. *Garcina intermedia* and *G. mangostana* had the highest antioxidant activities.

In memory of my father, Alvaro Muñoz

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Chapter 1
Introduction

Fruits and vegetables are rich in essential antioxidant nutrients, including the pro-vitamin β-carotene, and the vitamins C and E. Additional antioxidants, such as polyphenols ingested through the daily diet increase the antioxidant capacity of plasma[1], and are an important source of protective compounds against the damaging effects of free radicals.[2] The benefits of certain non-nutritional antioxidants such as flavonoids have been evaluated in several epidemiological studies.[3,4,5] In particular, it has been shown that a diet rich in fruits and vegetables significantly reduces the incidence and mortality rate of cardiovascular diseases.[6] A high intake of fruit and vegetables has been shown to have protective effects against cancer.[7, 8]

Additional antioxidants, such as polyphenols present in the daily diet, increases the antioxidant capacity of plasma.[1] Polyphenols are an important source of protective compounds against the damaging effects of free radicals.[2] Free radicals are very reactive in biological systems and are involved in producing reactive oxygen species (ROS) such as $O_2^{\bullet}$, $ROO^{\bullet}$, $RO^{\bullet}$, $HO^{\bullet}$, and $NO^{\bullet}$. ROS are naturally involved in cellular respiration, phagocytosis and synthesis of biologically important compounds. However, they are also damaging to lipids, proteins, and DNA and are formed in ailments such as cataracts, Alzheimer's disease, certain inflammatory conditions, and atherosclerosis.[9]

Plants in the Clusiaceae are a rich source of secondary metabolites with biological activities. The major classes of secondary metabolites have been isolated from these plants are: xanthones, coumarins and benzophenones. The Clusiaceae comprises 36 genera and around 1600 species,[10] which are mainly distributed throughout the tropics, although plants in a few genera are found in temperate regions.[11] The presence of latex is a common character of this family. The Clusiaceae is comprised of three subfamilies: the

Kielmeyeroideae, the Hypericoideae, and the Clusioideae.[11] The genus *Garcinia* includes more than 250 species of dioecious trees and shrubs with pantropical distribution, with its center of diversity in Southeast Asia and Madagascar. The floral characters exhibit a high variability in this genus, thus taxonomical issues arise when studying these plants. A broad concept of the genus, was supported by a recent molecular phylogenetic study, including a geographically, morphologically, and taxonomically comprehensive sampling.[12] The previously segregated genera *Rheedia, Ochrocarpus*, *Pentaphalangium* and *Tripetalum* are now accepted as part of genus *Garcinia* (Table **1.1)**, although in this thesis, names in *Rheedia* are sometimes used as reported in the literature.

Table 1.1. Classification of genera in the Clusiaceae.[11]

I. Subfamily **Kielmeyeroideae** Engler	**II.** Subfamily **Hypericoideae** Engler	**III.** Subfamily **Clusioideae** Engler
A. Tribe Calophylleae Choisy *Neotatea* Maguire *Marila* Swartz *Mahurea* Aublet *Kielmeyera* Martius *Caraipa* Aublet *Haploclathra* Bentham *Poeciloneuron* Beddome *Mesua* L. *Kayea* Wall. *Mammea* L. *Calophyllum* L. **B. Tribe Endodesmieae** Engler *Endodesmia* Bentham *Lebrunia* Staner	**A. Tribe Vismieae** Choisy *Vismia* Vand. *Harungana* Lamarck **B. Tribe Hypericeae** Choisy *Hypericum* L. *Lianthus* N. Robson *Triadenum* Raf. *Thornea* Breedlove & McClintock *Santomasia* N. Robson **C. Tribe Cratoxyleae** Bentham *Cratoxylum* Blume *Eliea* Cambess.	**A. Tribe Clusielleae** (P.F.Stevens) *Clusiella* Planch. & Triana **B. Tribe Clusieae** Choisy *Clusia* L. *Dystovomita* D'Arcy *Tovomita* Aublet *Chrysochlamys* Poepp. *Tovomitidium* Ducke **C. Tribe Garcinieae** Choisy *Garcinia* L. *Pentaphalangium* Warb. *Rheedia* L. *Tripetalum* K. Schum. *Allanblackia* Oliver *Ochrocarpus* Thouars. **D. Tribe Symphonieae** Choisy *Pentadesma* Sabine *Moronobea* Aublet *Platonia* Martius *Montrouziera* Planch. & Triana *Lorostemon* Ducke *Thysanostemon* Maguire *Symphonia* L. f.

The antioxidant capacity of plants is mainly attributed to the phenolic constituents.[13] Phenolic compounds are antioxidants found in plants, and can be found in agricultural crops such as apples, grapes, blackberries, and strawberries.[14] In my research, the phenolic content of edible *Garcinia* fruits (Clusiaceae) has been studied. Products containing *G. mangostana* are commonly sold as dietary supplements in the US. This phytochemical investigation included eight *Garcinia* species from different geographical origins. In this study, the main objectives were; [i] a literature review of the bioactivity of polyisoprenylated benzophenones[15], [ii] identification of new polyisoprenylated benzophenones isolated from *Garcinia intermedia*, [iii] identification and quantification of polyisoprenylated benzophenones and biflavonoids in eight *Garcinia* species, [iv] *in vitro* investigation of the antioxidant and antiproliferative effects against colon cancer cells of guttiferone A.

Chapter 2
Polyisoprenylated Benzophenones from Clusiaceae: Potential Drugs and Lead Compounds
A Review* [15]

*This chapter has been published previously.

2.1. Introduction

Natural products of plants origin are important sources of new chemical compounds leading to the future discovery of new drugs. In the areas of cancer chemotherapies, anti-inflammatory drugs, antibacterial drugs, and antiparasitic drugs, there is demand for new, more effective treatments.[16] Herein, we examine the bioactivity of the polyisoprenylated benzophenones isolated from plants of the Clusiaceae family. The Clusiaceae family is a rich source of secondary metabolites, including the widely used medicinal herb *Hypericum perforatum* L., commonly known as St. John's wort, which has been traditionally used for its antidepressant activity.[17] Four major classes of compounds are found in the Cluisaceae family: xanthones, coumarins, biflavonoids, and benzophenones.

The polyisoprenylated benzophenones are major intermediates in the biosynthetic pathway of xanthones, and have been rarely reported to occur outside the Clusiaceae family.[18] The benzophenones are non-polar phenolic compounds, with increased hydrophobicity as a function of the number of prenyl functional groups attached. Most polyprenylated benzophenones isolated from plants have the bicyclo[3.3.1]-nonane-2,4,9-trione core structure linked to a 13,14-dihydroxy substituted phenyl ring (Fig. **1**).[19] This planar structure, with polar carbonyl and hydroxyl groups, can be simple or substituted by isoprene units.

Benzophenones exhibit cytotoxic,[20] antimicrobial,[21,22] antiviral,[23] and antioxidant[24, 25, 26] activities. The most cited natural benzophenones in the literature are garcinol (**1**) and xanthochymol (**2**) (Tables **1** and **2**). These compounds are cytotoxic in

several cancer cell lines including colon,[25, 27, 28] breast,[28, 29, 30] ovarian,[31, 32, 33] oral, [34, 35] and leukemia.[36, 37]

Polyisoprenylated benzophenones induce apoptosis and arrest the cell-cycle in sub G_1-phase [38]; however, the mechanism of action by which benzophenones in induce apoptosis and exert cytotoxic effects is still under investigation.

Several groups have independently investigated the mechanism of action of polyisoprenylated benzophenones.[34,37,38] Although studies have shown that polyisoprenylated benzophenones bind to microtubules and inhibit the disassembly of tubulin during cell division, our findings show[38] that benzophenones increase the activity of caspase-3, possibly inducing apoptosis.[38] Benzophenone action has the same mechanism as that of paclitaxel, but at a lower potency. Several studies suggest that benzophenones increase the activity of caspase-3, inducing apoptosis.[27, 37, 38] Besides inhibition of microtubule disassembly,[34] other possible mechanisms of action against cancer cells have been presented: inhibition of microtubule disassembly,[34] inhibition of histone acetyl tranferase activity,[39] inhibition of protease activity,[40] inhibition of kinase activity,[36] or directly acting as prooxidant damaging DNA.[41]

We are not aware of any review publications on benzophenones from natural sources. Thus, this work presents, to our knowledge, the first summary of polyisoprenylated benzophenones as secondary metabolites found mainly in Clusiaceae family.

This review is based primarily on Internet research of the literature using different electronic databases such as MEDLINE, Science-Direct, Basic-Biosis, and Chemical Abstracts. The following search terms, without language restrictions, were used during

the research: polyisoprenylated benzophenones, garcinol, xanthochymol, Clusiaceae and benzophenones, *Garcinia* sp. and benzophenones, antioxidant activity, cytotoxicity, antiviral, anti-HIV, antimicrobial, and antiinflammatory properties of benzophenones. Additionally, an advanced search was performed to select only the articles that involve benzophenones isolated from natural sources. Searches were performed over the time period, January through May 2009.

2.2. Chemical structure

The core of a polyisoprenylated benzophenone is composed of a bicyclo[3.3.1]-nonane-2,4,9-trione structure, linked to a 3,4-dihydroxybenzoyl moiety (Fig. **1**).[19] The benzoyl ring is usually hydroxylated, in position *ortho* and *para*. The 3-methyl-2-butenyl groups or prenyl groups are attached at carbon C-1, C-5, and C-7 of the bridged bicyclic structure (Fig. **1**). There are examples, such as isoxanthochymol (**3**) or cambogin (**4**), where the isoprenyl substituting unit at C-5 undergoes cyclization. During cyclization, the oleofinic bond undergoes epoxidation, and subsequently forms a bond with the oxygen on carbon C-4, forming an additional pyran ring.

These polyisoprenylated benzophenones commonly occur as tautomeric pairs. That is, the diketone easily forms hydroxyl groups that are formed at C-4 and C-1 in the enolized diketone. It has been suggested that the enolic hydroxyl group in the β-hydroxy-α,β-unsaturated ketone plays an important role concerning bioactivity.[41] Keto-enol tautomerism has been reported for curcumin, whose structure has certain similarities to benzophenones.[42, 43]

The position C-7 constitutes a chiral center forming stereoisomers. Thus the isopentenyl substituent at C-7 is either in an equatorial or in an axial position. The

relative configuration of benzophenone compounds can be deduced from NOE and ROESY spectra and the coupling constant when analyzing these compounds by nuclear magnetic resonance (NMR).[44]

In summary, the planar ring structure of benzophenones is highly conjugated, with delocalized electrons and strongly polarized carbonyl and hydroxyl groups. The UV spectrum shows absorption bands caused by an aromatic chromophore at 282 and at 234 nm, due to a conjugated carbonyl group.

2.3. Clusiaceae genera

Most of the polyisoprenylated benzophenones included in this review were isolated from the genus *Garcinia* L. The genus *Garcinia* (Subfamily: Clusioideae, tribe Garciniae) is distributed throughout tropical regions of the world. The genus *Garcinia* comprises 200 species, and some are used for their latex and resins. The fruits are edible and many have medicinal uses. Benzophenones isolated from seven genera, have been assayed for cytotoxicity: *Garcinia, Rheedia, Hypericum*, *Clusia*, *Ochrocarpus*, *Tovomita*, and *Vismia* (Table **1**). Antibacterial benzophenones have been isolated from the seven following genera: *Garcinia*, *Rheedia*, *Hypericum, Clusia*, *Allanblackia*, *Symphonia*, and *Moronobea* (Table **2**). Polyisoprenylated benzophenones are rarely found outside the Clusiaceae family. However, structurally similar isoprenylated benzophenones with cytotoxic activity have been isolated from *Cudrania* in the Moraceae family[45] as well as from other natural sources.[46]

2.4. Effects on Cancer Cells *in vitro*

2.4.1. Cytotoxicity

Plants are an important source of cytotoxic compounds for anticancer therapies. In total, twenty-six studies reported cytotoxic activity for polyisoprenylated benzophenones isolated from plants in the Clusiaceae family (Table **1**). Most of these studies were performed using human leukemia (HL-60) and oral carcinoma (KB) cells. Although benzophenones show relatively potent cytotoxic effects, paclitaxel showed a hundredfold more effective cytotoxic activity than the tested benzophenones.[20,34,47] Paclitaxel, a cytotoxic compound originally isolated from *Taxus brevifolia* Nutt., was used as a positive control in three studies, comparing effects of polyisoprenylated benzophenones to that of paclitaxel (Table **1**).[20, 34, 47]

In a comprehensive cytotoxic study,[20] both xanthochymol (**2**) and isoxanthochymol (**3**) were tested against three different cancer cell lines, including breast (MCF-7), liver (WRL-68), and colon (COLO-320-DM) cancer cells. The activities of paclitaxel were tested in each of the cell lines: IC_{50}= 0.005 μM (MCF-7), IC_{50}= 0.054 μM (WRL-68), and IC_{50}= 0.01 μM (COLO-320-DM).[20] Xanthochymol (**2**) displayed IC_{50}=0.457 μM (MCF-7), IC_{50}= 2.52 μM (WRL-68), IC_{50}= 0.62 μM (COLO-320-DM).[20] The IC_{50} values for isoxanthochymol (**3**) in each of the above mentioned cell lines were: 2.85 μM (MCF-7), 15.52 μM (WRL-68), and 4.85 μM (COLO-320-DM).[20]

Kumar *et al*. (2007) suggested that xanthochymol (**2**) is more effective than isoxanthochymol (**3**) particularly against breast cancer cells MCF-7 and liver cancer cells WRL-68, although it is a hundredfold less effective than paclitaxel.[20]

Xanthochymol (**2**) was screened for cytotoxic activity in four different studies (Table **1**), and it was more active against breast cancer cells (MCF-7) than against the other tested cell lines (IC_{50}= 0.475 μM), while isoxanthochymol (**3**) showed highest activity (IC_{50}= 0.62 μM) against colon cancer cells (COLO-320-DM).[48]

Guttiferone A (**5**) displayed activity against A2780 human ovarian cancer cell lines, IC_{50} = 6.8 μg/ml.[33] The effect was a thousand times weaker than the activity of the control actinomycin D, IC_{50} = 0.003 μg/ml.[33] Guttiferone I (**6**) and J (**7**) showed inhibitory activity in KB oral carcinoma cells, with an IC_{50} = 4.70 μg/ml and an IC_{50} = 5.0 μg/ml, this in comparison with the control taxotere, IC_{50} = 0.37 ng/ml.[47] Nevertheless, isoprenylated benzophenones from the Clusiaceae family definitely displayed cytotoxic activities, although consistently lower than the positive controls, paclitaxel and actinomycin D.

Xanthochymol **(2)** was the most cytotoxic of all the tested polyisoprenylated benzophenones, particularly against MCF-7 cells, (IC_{50}= 0.475 μM).[20] Nemorosone (**8**) was the second most active, and had a cytotoxic activity of IC_{50}= 1.5 ± 0.08 μg/ml against HEPT-1 larynx cancer cells. In leukemia cells nemorosone (**8**) showed activity in the ranges of IC_{50} = 2.10 μg/ml to IC_{50} = 3.10 μg/ml[36] (Table **1**). Vismiaguianone D (**9**) was the third most active compound reported among the studied benzophenones, particularly against oral carcinoma cells KB, IC_{50} = 2.40 ± 0.9 μg/ml.[35]

Oblongifolin C **(9)** from *Garcinia yunnanensis* Hu showed activity against cervical HeLa-C3 cells, and apoptosis was induced after 72 h of incubation with a concentration of 15 μM.[49] Tovophenone A **(11)** was isolated from *Tovomita*

brevistaminea Engl., and was found to be inactive against KB cells; the effective concentration (EC_{50}) was 10 μg/ml.[50]

2.4.2. Garcinol

The most frequently tested polyisoprenylated compound was garcinol (**1**), which showed cytotoxic effects in five studies (Table **1**). Garcinol is the trivial name of camboginol (**1**),[23] as they have the same stereochemical structure.[23] Camboginol (**1**) was originally isolated from *G. cambogia* Desr. and its stereochemistry has been identified and reported.[51]

Garcinol (**1**) was included in five cytotoxicity studies, and the activity and inhibitory concentration (IC_{50}) ranged from 5 to 21.4 μM [37] (Table **1**). Treatment with garcinol caused the reduction of the mitochondrial transmembrane potential, an important evidence of apoptotic effects.[37] Furthermore, it was shown that garcinol (**1**) increased the activity of caspase-3 during apoptosis.[37] In another study, the activity of caspase-3 increased when tested at 2 and 6 hours.[27]

Garcinol (**1**) was also tested against HL-60 leukemia cells.[27, 37, 52, 53] Treatment of leukemia cells with garcinol (**1**), isoxanthochymol (**3**), and xanthochymol (**2**) was shown to cause apoptosis, mediated by caspase-3.[37] However, no activation of caspase-8 was found in this study.[37] In this study, the effects of garcinol **(1**) on the cell cycle wpould consist in targeting the mitochondria in the early phase of the apoptotic process. Furthermore, another study shows that garcinol induces apoptosis and increases caspase-3 activity in a dose-dependent manner.[43]

To date, there is no clear evidence concerning the mechanisms through which garcinol and other isoprenylated compounds cause apoptosis and cytotoxic effects on cancer cells.

2.4.3. Inhibiton of histone acetyl transferases

In a systematic approach to indentify compounds from plants that have effect on histone acetyl transferases (HAT), it was found that garcinol (**1**), isolated from the fruit rind of *Garcinia indica* Choisy*,* was a potent inhibitor of HAT, specifically p300 and PCAF, IC_{50} = 7 μM and IC_{50} = 5 μM.[39] The inhibiting effects on histone acetyltransferases, p300 and PCAF for garcinol (**1**) led to apoptosis in these HeLa cells, suggesting that HAT inhibition might be the possible mechanism of action through which polyisoprenylated benzophenones from plants exert cytotoxic effects in different cancerous cell lines.

The balance between acetylation and deacetylation of histones is important during cell growth and differentiation. Acetylation of histones plays an important role in DNA and protein interaction during transcription. Notably, garcinol (**1**) has been shown to be a specific inhibitor of acetylation. The concentration range of garcinol tested was between 1 μM and 100 μM.[39] The HAT inhibiting activity of garcinol (**1**) was investigated using HeLa cells and recombinant HAT p300 and PCAF.[39] A microarray analysis showed that transcription was globally repressed. Further evidence showed that garcinol (**1**) competitively inhibited the activity of both p300 and PCAF, competing with histones for the active site of the enzyme. Very few HAT inhibitors have been reported, thus garcinol (**1**), inhibiting HAT and transcription, is a candidate molecule to be tested for future cancer therapies.

2.4.4. Gene expression analysis

In our own studies,[25, 38] xanthochymol (**2**) isolated from *Garcinia xanthochymus* Hook.f. showed cytotoxic activity against colon cancer cells. The reported inhibitory concentration against SW-480 cells for xanthochymol (**2**) was IC_{50} = 8.3 μM and for guttiferone E (**12**) it was IC_{50} = 7.5 μM.[25] Xanthochymol (**2**) showed significant cytotoxicity against cell lines HCT-116 and HT-29 derived from colon cancer tissues, IC_{50} = 10 μM and IC_{50} = 15 μM.[25] In addition to xanthochymol (**2**) and guttiferone E (**12**), guttiferone H (**13**) was isolated by our group and was shown to display an IC_{50} = 12 μM against SW-480 cancer cells and an IC_{50} = 9.0 μM against HCT-116.[25, 38] We found that caspase-3 activation occurred after 24 h.[38] These findings are supported by similar results reported by Matsumoto *et al*.[37] and Hong *et al*.[42] We found no significant activation of caspase-8 and 9 in HCT-116 and HT-29 cells.[38]

The proapoptotic effect of xanthochymol (**2**), guttiferone E (**12**), and guttiferone H (**13**) in HCT116 and HT29 cells was examined more closely by microarray analysis.[38] The effect on gene expression in HCT116 and HT29 cells was studied at IC_{50} concentration for 18, 24, and 48 hours. Poly(A)-RNA was extracted from cells used in these experiments and used as templates to synthesize biotin-labeled cDNA to perform the hybridization and gene array analysis. Gene tree cluster analysis showed that array samples clustered into three main groups, and few genes were induced.

Genes involved in the stress response of the endoplasmatic reticulum were up-regulated namely: X-box binding protein 1 (XBP1); activating transcription factor 4 (ATF4); and DNA-damage inducible transcript 3 (DDIT3/CHOP), possibly a response to unfolded proteins and possibly affecting the progression through the cell cycle leading to

cell cycle arrest.[38] The cellular energy stress response gene, DNA-damage inducible transcript 4 (DDIT4/REDD1) was highly induced by treatment. DDIT4/REDD1 is a transducer that inhibits the mammalian target of rapamycin (mTOR) pathway. In addition, our results also indicated that the mTOR survival pathway was inhibited in treated cells.[38] The mTOR pathway plays an important role during cellular stress responses, cell growth and survival and it is dysregulated by various oncogenic events. It was found that curcumin also affects the mTOR pathway.[54]

It seems that both the polyisoprenylated benzophenones and curcumin might target phosphatases regulating the phosphoinositide-3 kinase/Akt/mTOR pathway and have cytotoxic effect in cancer cells. Curcumin was found to activate protein phosphatase 2A (PP2A) and calyculin-A in treated cancer cells. This led to dephosphorylation and inhibition of Akt/PKB protein kinase B and mTOR mediators. This was followed by inhibition of down-stream mediators of the pathway such as glycogen synthase kinase (GSK-3). Consequently, expression of proteins essential for cell survival and proliferation of a cancer cell, such as cyclin D1, was suppressed.[54] However, the specific phosphatases activated by curcumin need to be further investigated.[54] It remains to be determined if histone deacetylase (HDAC) inhibitor alters the activity of Akt protein kinase as well, through the protein phosphatase complex (PP1).[55]

2.4.5. Protease inhibition

Guttiferone A has a planar structure with strongly polarized groups which interact with serine proteases.[40] The X-ray crystal structure confirmed that guttiferone A (**5**) bound to the active site of serine proteases. The phenyl ring and the prenyl groups present in guttiferone A (**5**) form van der Waals interactions with the catalytic site of the enzyme:

the hydroxyl moieties donate or accept hydrogen from the amino acids of the protein, thus binding more tightly to the residus in volved in the proteolytic activity of the enzyme. Thus hydroxylation of the bridge carbon in the benzophenone activity tautomere seems to be important for the activity of benzophenones.[40,56] These findings suggest that isoprenylated benzophenones such as garcinol (**1**) might bind to proteases, indicating that both antiviral and cytotoxic effects caused by guttiferone-type compounds take place through binding and inhibiting proteases.[40]

2.4.6. Akt/PKB kinase inhibition

Nemorosone (**8**) from *Clusia rosea* Jacq. was found to have antiproliferative and proapoptotic effects in Jurkat and K-562 human leukemia cells.[36] Apoptosis was induced within 4 hours after treatment. Later on, an increase of sub G_0/G_1 cell population was observed, and it appears that nemorosone (**8**) prevents the progression G_1/S transition in cells, as well as altering the controllers of G_2/M progression. These line of evidence lead to conclude that Akt/PKB kinase appears to be inhibited by nemorosone (**8**), making this enzyme is another likely physiological by causing apoptosis.

Akt/PKB is a serine/threonine protein kinase that plays a fundamental role in carcinogenesis. As a response to damaged DNA, Akt/PKB does not activate GSK-3. Further, the oncogene c-Myb was down-regulated in nemorosone-treated Jurkat and K-562 cells.

It was shown that nemorosone (**8**) disrupted the cell cycle in a dose dependent manner. Western blot analysis showed that cyclin D1 and E were down-regulated.[36] Moreover, the cyclin-dependant kinase 1 (Cdk1/cdc2) was down-regulated and dephosphorylated in cells treated with (**8**). According to these findings and in order to

define more closely which pathways are affected br nemorosone during G_1/S progression leading to apoptosis, it will be necessary to perform further studies on the physiological status of inhibitors of cell cycle regulators.

2.4.7. Prooxidant activity and DNA breakage

Garcinielliptone (**14**) was tested against lines derived from breast cancer cells, such as MCF-7. The effect of treatment in MCF-7 cells was observed using fluorescence microscopy. Nuclear fragmentation were observed to occur in a dose dependent manner. Flow cytometry analysis showed accumulation of sub-G_1 DNA of apoptotic cells. Further, it was shown that garcinielliptone had a prooxidant effect in the presence of Cu(II). Nevertheless, prooxidants such as Cu(II) might not be commonly found unbound in the nucleus. Garcinielliptone-generated ROS, caused the breakage of DNA and, subsequently cell death.[28, 41]

2.4.8. SAR studies

With respect to vismiaguinones, the contrary to what other studies have suggested, the number of prenylated substituents in the structure, did not affect cytotoxicity as significantly as other substituents did. On the other hand, the number of hydroxylations, the presence of an additional phenyl group and π-bonds, significantly increase the cytotoxicity of vismiaguinones.[35] Vismiaguianone A (**15**) through D (**9**) isolated from *Vismia guianensis* (Aubl.) Choisy, were tested against oral carcinoma cells.[35] Vismiaguianones A (**15**), B (**16**), and C (**17**) have displayed a lower cytotoxicity than that of vismiaguianones D (**9**) and E (**18**) in the KB cell line.[35] The vismiaguianones A (**15**) through C (**17**) have three hydroxyl groups, while vismiaguianone D (**9**) and E (**18**) have two.[35] Only vismiguianones D (**9**) and E (**18**) have an additional phenyl ring,

and only D (**9**) and E (**18**) were the most active compounds.[35] Vismiaguianone D (**9**) showed the highest activity and had a four-ring structure, with three additional π-bonds and an ester function, possibly leading to higher cytotoxic activity.[35] Thus the number of hydroxy-groups and double bonds correlated with higher cytotoxic activity. All the tested vismiaguianones had one prenyl substituting group,[35] indicating that the number of hydroxyl groups and double bonds in the isoprenylated benzophenone are more important than the number of prenyl-substituent groups, in this particular case, against KB cell lines.

2.4.9. Synthetic analogues and cytotoxicity

Synthetic benzophenones were modified and tested on P388 leukemia cells and on PC-6 human lung carcinoma cells. Among the synthetic benzophenone derivatives tested for cytotoxicity, the nitrobenzophenones showed a potent cytotoxic activity both *in vitro* and *in vivo*.[57] Whereas the aminobenzophenones showed weak activity. Fluorouracil (5-FU) was used as a control.[57] When functional groups were oxidized the nitroketone of a modified benzophenone was more active than the corresponding nitro-alcohol.[57] Amide derivatives were only weakly active.[57] Among the nitrobenzophenones, the morpholino derivative (**19**) showed higher cytotoxicity and growth inhibition (GI_{50}= 16.8 ng/ml) effects *in vitro* against P388 leukemia cells than those obtained with the positive control 5-FU (GI_{50}= 55 ng/ml).[57] The carbonyl and nitro moieties of the synthetic analogues were considered to be essential for cytotoxic activity *in vitro*; however, *in vivo*, in CDF1 mice inoculated with P388 cells, it was the thiomorpholino derivative which increased the life span (ILS) by 35%, at a dose of 2 x 206 mg/kg, although the effect was not as potent as 5-FU (82% ILS) at 2 x 100 mg/kg. Chlorination of nitrobenzophenones, in particular the

morpholino benzophenones, showed the highest cytotoxic activity. Further studies of animals treated with this compound, however, did not show an increase in the ILS of P388 cells inoculated in mice.[57] In conclusion, given their potent cytotoxic activity and low toxicity the synthetic nitrobenzophenones, are promising cytotoxic compounds.[57]

2.5. Effects on Cancer cells *in vivo*

In an animal study, male F344 rats were fed with garcinol (**1**) to to test the hypothesis that dietary garcinol (**1**) suppresses chemically induced colon carcinogenesis.[58] Oral administration of (**1**) showed no toxic effect on normal animal growth and was well tolerated with no adverse effects reported. Further in the same study it was shown that the dietary administration of (**1**) significantly inhibited and reduced the frequency of the chemically induced aberrant crypt foci (ACF), induced by azoxymethane. These findings suggested that dietary garcinol (**1**) suppresses chemically induced colon carcinogenesis in F344 rats as shown by reduced the frequency of ACF: 72 ± 15 (26% reduction, $P < 0.01$) at a dose of 0.01% and 58 ± 8 (40% reduction, $P < 0.001$) at a dose of 0.05%.[58] The results from this study[58] suggested that the chemopreventive effects of (**1**) on colon tumorogenesis were possibly due to the suppression of the production of NO and O_2^-. The levels of O_2^- and NO were induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) treatment in differentiated human promyelocytic HL-60 cells and lipopolysaccharide (LPS) and interferon (IFN)-γ-treated mouse macrophage RAW 264.7 cells.[58] In the same study, western blot analysis showed the suppression of inducible nitric oxide synthase (iNOS) protein expression as well as cyclooxygenase-2 (COX-2) protein expression. Although the mechanism through which (**1**) reduced the expression of these proteins was not presented in the above mentioned

experiments, the role of iNOS and COX-2 in inflammation and as enhancers during carcinogenesis in colon tissue was discussed and further studies are investigating the chemopreventive potential of (**1**).

2.6. Antimicrobial activity

2.6.1. Antibacterial activity

Antibiotic resistant bacterial strains cause severe infection worldwide. The majority of new potent antibiotics are derived from natural products. Polyisoprenylated benzophenones from plants in the Clusiaceae family have significant antimicrobial activities (Table **2**). In particular, two studies show that polyisoprenylated benzophenones have potent antibacterial activity against antibiotic resistant strains.[22, 59]

Five benzophenone compounds isolated from *G. pupurea* Roxb. and *G. subelliptica* Merr. were tested against methicillin resistant *Staphylococcus aureus* (MRSA).[59] Antibacterial activities of the benzophenone derivatives garcinol (**1**), isogarcinol (**20**), xanthochymol (**2**), isoxanthochymol (**3**), and cycloxanthochymol (**21**) were evaluated, displaying a minimum inhibitory concentration (MIC) range from 3.13 to 12.5 μg/ml for benzophenones mentioned above (Table **2**).[59] Xanthochymol (**2**) was the most active with a MIC = 3.13-12.5 μg/ml (Table **2**). Garcinol (**1**) showed a MIC = 6.25 μg/ml. The antibacterial activity of garcinol (**1**) was nearly equal to that of the positive control vancomycin (MIC = 6.25 μg/ml), which is commonly used against Gram-positive bacteria.[59] In addition, garcinol **(1)** from *G. bacana* Miq. showed antimicrobial effects against *S. aureus*, MIC = 16 μg/ml.[60] No synergistic effect was detected for isoxanthochymol (**3**) and cycloxanthochymol (**21**).[59]

The antimicrobial effects of 7-epiclusianone (**22**), isolated from the root of *Hypericum sampsonii* Hance was tested against MRSA (SA-1199B).[22] This particular bacterial strain overexpressed the NorA efflux protein, considered the major drug pump responsible for antibiotic resistance. It was shown that 7-epiclusianone (**22**) had a MIC = 7.3 μM. The antibacterial activity of 7-epiclusianone (**22**) was compared to the activity of norfloxacin (MIC= 100 μM). These results confirm that 7-epiclusianone (**22**) is a potent antimicrobial agent.

The antimicrobial activity of 7-epiclusianone (**22**) was further confirmed by another study, where the source of the phytochemical was obtained the peel and seed extracts of *Rheedia brasiliensis* (Mart.) Planch. & Triana (synonym: *Garcinia brasiliensis*) and was tested against *Streptococcus mutans*.[61] In this study, 7-epiclusianone (**22**) showed an MIC of 1.25-2.5 μg/ml,[61] and the control clorhexidine had an MIC = 1-2 μg/ml.

Two guttiferone-type compounds, guttiferone I (**6**) and isoxanthochymol (**3**) from *Garcinia smeathmannii* (Planch. & Triana) Oliv. were tested against Gram-negative and Gram-positive bacteria.[21] Guttiferone I (**6**) showed a potent activity against Gram-negative bacterial strains, while isoxanthochymol (**3**), with a cyclic ring, was the most active against Gram-positive strains, mainly *Bacillus cereus* and *B. stearothermophilus*. Guttiferone I (**6**) showed potent activity against *B. megatorium*, a Gram-positive strain (IC_{50}= 0.61 μg/ml). It was noted that guttiferone I (**6**) showed higher activity than the control, genthamycin (IC_{50}= 2.44 μg/ml) against all tested strains in this particular study (Table **2**).

The extracts from Clusiaceae plants had a more pronounced activity against the tested Gram-positive bacterial strains, than against the Gram-negative bacteria. The extracts of *Garcinia cowa* Roxb. and *G. pedunculata* Roxb. were tested against *Bacillus cereus, B. coagulans, B. subtilis*, *S. aureus* and *Escherichia coli.*[62] The chloroform extract of the fruit rinds of *G. cowa* were active against *B. cereus* (MIC= 15 μg/ml)*, B. coagulans* (MIC=25 μg/ml)*, B. subtilis* (MIC= 25 μg/ml), and *S. aureus* (MIC= 30 μg/ml).

In another study both chamone I (**23**) and nemorosone (**8**) isolated from the tropical species *Clusia grandiflora* Splitg. showed potent antibacterial activity against honey-bee pathogens. Nemorosone (**8**) showed an activity against *Paenibacillus larvae* (total inhibition 19.5 ± 1.6 mm) and *Paenibacillus alvei* (total inhibition 2.3 ± 9 mm). Chamone I (**23**) also had activity against the above mentioned pathogens, (total inhibition 19.7 ± 0.3 mm) and (total inhibition 10 ± 0.6 mm).[63] Plants generally produce secondary metabolites as a defense mechanism; however it is possible that plants in the Clusiaceae family produce isoprenylated benzophenones as pollination rewards and that pollinating bees use the latex with these antibacterial substances for protection against pathogens. Nemorosone (**8**) has been identified and described as one of the major constituents of the Cuban propolis, coming from the floral resin of *Clusia rosea* Jacq.[64, 65]

Potent antibacterial benzophenones have been isolated from natural sources other than plants, most notably from fungi.[46, 66, 67] Pestalone (**24**) was obtained from a mixed fermentation of a marine fungus, a marine deuteromycetes (*Pestalotia* sp. strain CNL-365,) and an unidentified antibiotic resistant marine bacterium.[66] Pestalone **(24)** showed potent activity against MRSA (MIC = 37 ng/ml) as well as against vancomycin-resistant

Enterococcus faecium (MIC = 78 ng/ml).[66] Benzophenone dimer, microsphaerin D **(25)** was obtained from the anamorphic fungus *Microsphaeropsis* strains F2076 and F2078.[67] Microsphaerin D **(25)** showed activity against MRSA, IC_{90} = 1 μM.[67] Pestalachloride A, **(26)** obtained from a plant endophytic fungus, showed activity against plant pathogenic fungi, specifically against *F. culmorum* (IC_{50} = 0.89 μM).[46]

2.6.2. Antiviral activity

Activity against the human immunodeficiency virus-1 (HIV-1) was first reported for the polyisoprenylated benzophenone guttiferone A (**5**), subsequently, new lead molecules for antiviral drug treatment have been isolated.[68] The identification of guttiferone A (**5**) was followed by the isolation and characterization of guttiferone F (**27**), which exhibited partial cytoprotection against HIV-1 *in vitro* (EC_{50} = 23 μg/ml) as well as cytotoxicity (IC_{50} = 82 μg/ml). Additional polyisoprenylated benzophenones from natural sources such as, garciosaphenone A (**28**) showed anti-HIV-1 activities (IC_{50} < 7.8 μg/ml).[69] In addition, vismiaphenone D (**29**) showed HIV-inhibitory activity.[70] Garciosaphenone A, a new digeranylbenzophenone from *G. speciosa* Wall. showed activity against HIV-1 reverse transcriptase, IC_{50} = 23.9 μg/ml.[69]

Since these findings in natural products research, several synthetic benzophenone analogues have been developed and are currently important lead molecules for future antiviral therapies. These analogues are drug candidates for new non-nucleotide reverse transcriptase inhibitors (NNRTIs) have been reported to display a broad spectrum of antiviral activity against HIV-1 and drug resistant mutants.[71] The benzophenone analogue GW678248 (**30**) showed potent antiviral activity *in vitro* and thus was selected for further preclinical studies.

Several synthetic benzophenone analogues showed potent activity *in vitro* against clinically relevant drug resistant mutants of HIV-1. Effective synthetic analogues were obtained by comparing antiviral activity to the NNRTIs nevirapine and efavirens.

Several drug resistant strains were included in a HeLa-CD4 MAGI screening assay. The nevirapine resistant strain Y181C was used to test the activity of **30**. The results showed an IC_{50} = 0.7 ± 0.5 nM for **30** and an IC_{50} = 10,300 ± 4,100 nM for nevirapine.[71] Another strain, K103N, was included in the panel. The analogue (**30)** showed an IC_{50} = 1.0 ± 0.9 nM while efavirens showed an IC_{50} = 25 ± 13 nM, against this particular strain.[71]

Structure-activity studies have shown that the lead structure of **30** binds to the non-nucleoside binding site of the enzyme, thus forming an enzyme complex.[72] The part of the ring structure of **30** that reached the hydrophobic pocket was ring A, while the modified C-ring of **30** counters the scaffold of the benzophenone structure, displaying hydrophilic interactions. This synthetic analogue has a *p*-chloro substituent in the B-ring, which is important for the activity in particular against the resistant mutants of the virus.[72] The additional C-ring is attached to the benzophenone core structure through a linker region composed of an amide group and a secondary amine. It was found that the carbonyl group in the benzophenone structure is important for activity.[72] In addition, the carbonyl group shows an intramolecular hydrogen interaction with the amide NH in the linker region of this analogue (**30**).[72] The *para* position of the C-ring was substituted with a sulfonamide to increase activity.[72] The hydrophobic A-ring of the benzophenone was modified by adding a *m*-CN group, which showed the most potent activity of the tested analogues.[72] Lastly, the remaining *meta* position of the A-ring was substituted with a

chlorine group[72] to give this analogue a longer half-life and a lower clearance. This analogue (**30**) was selected for further preclinical evaluation.

Pharmacokinetic studies were performed for this benzophenone analogue in rats, dogs, and monkeys.[72] The administration of an intravenous bolus dose of 1 mg/kg in 2 ml injection (0.5 mg/ml) was followed by a clearance of 11, 7 and 10 ml/min/kg for rat, dog, and monkey, respectively. The oral bioavailability was higher for a solution than a suspension 27-58% for solution and 3-20% for a suspension, respectively.[73] The prodrug of this analogue (**30**) showed higher oral bioavailability and is currently undergoing clinical studies. This drug candidate is currently in phase 2 of clinical trials and the future drug is expected to provide benefit to HIV-1 infected patients with strains resistant to the current NNRTIs.

2.6.3. Antiparasitic activities

Polyisoprenylated benzophenones showed potent activities as trypanocidal and leishmanicidal agents (Table **2**).[74] [75] Guttiferone A (**5**) was isolated from the leaves of *Garcinia intermedia* (Pittier) Hammel and showed activity against *Trypanosoma cruzi*. The flagellate protista *T. cruzi* is the etiologic agent of Chagas disease.[74] The infectious form is called trypanomastigotes is and the uninfectious form is designated epimastigotes. Guttiferone A (**5**) showed higher activity against the trypomastigotes at MC_{100} = 50 μg/ml, than against the epimastigotes at MC_{100} = 60 μg/ml. These results represent the concentration at which both the epimastigotes and trypomastigotes were terminated after 48h. Gossypol was used as a reference compound and showed trypanosomal activity at MC_{100} = 50 μg/ml. *In vitro* activity was found for 7-epiclusianone from *Rheedia*

gardneriana Planch. & Triana against trypomastigotes of *T. cruzi* (LC_{50} = 260 μg/ml), although this was not confirmed *in vivo.*[76]

As for guttiferone A (**5**) and F (**27**), both displayed activities against *Leishmannia donovani,* more potent than miltefosine, the reference compound in this study. Leishmanicidal activity *in vitro* for guttiferone A (**5**) and F (**27**) were, IC_{50} = 0.2 and IC_{50} = 0.16 μM, respectively. This was in comparison with miltefosine, IC_{50} = 0.47 μM. Given the strong leishmanicidal activity displayed by guttiferone A (**5**) and F (**27**) against axenic amastigotes, these compounds are regarded as lead compounds in the synthesis of novel potent and efficient leishmanicidal agents.

Isogarcinol (**20**) and cycloxanthochymol (**21**) were isolated from *Moronobea coccinea* Aubl., and displayed antiplasmodial activity against *Plasmodium falciparum*, particularly against a chloroquine resistant strain, FcB1. The antiplasmodial activity reported for isogarcinol (**20**) and cycloxanthochymol (**21**) was IC_{50}= 3.5 μM and IC_{50}= 2.1 μM. Chloroquine showed an IC_{50}= 0.078 μM. The tetrahydropyrane ring present in these compounds was reported to be responsible for activity against *P. falciparum*. In addition it has been postulated that hydroxylation on the benzoyl moiety of the benzophenone structure is important for the activity displayed by the tested compounds.[77] Antiplasmodial activity for guttiferone A was IC_{50} = 3.17 μM.[78]

2.7. Antioxidant activities

The antioxidant activity of garcinol (**1**) was investigated in four different systems, using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, superoxide anion scavenging assay, hydroxyl radical scavenging assay, and the H_2O_2/NaOH/DMSO system.[26, 41, 79] The latter was particularly designed to evaluate both hydrophilic and hydrophobic free radical

scavengers.[26] It was shown that garcinol (**1**) has high antioxidant activity in comparison with ascorbic acid and α-tocopherol. In addition, garcinol (**1**) showed significant chelating activity and it was noted that garcinol (**1**) showed high scavenging activity against hydroxyl radicals in comparison with α-tocopherol.[26] This was further investigated *in vivo*, and it was shown that garcinol (**1**) efficiently prevented the development of gastric injury and ulcer, in comparison with a positive control[26] These findings are confirmed by ethnobotanical practice and traditional use of *G. brasiliensis*, which has been reported in Brazilian folk medicine to treat peptic ulcer.[80]

Free radicals and reactive oxygen species (ROS) are involved during carcinogenesis, neurodegenerative diseases, and inflammatory processes. The formation of ROS in brain tissue and subsequent oxidative damage is involved in various neurodegerenative diseases. Antioxidants are suggested to have neuroprotective effects. The neuroprotective effect of garcinol (**1**) from *G. indica* was evaluated. It was found that garcinol (**1**) protects against neuronal injury by reducing the generation of free radicals.

The production of ROS occurs through several intracellular pathways, which involve mainly the activities of cyclooxygenase (COX), lipooxygenase, and inducible nitric oxide synthase (iNOS). During oxidative stress, astrocytes in the central nervous system (CNS) produce nitric oxide. It is specifically the iNOS in astrocytes that produces nitric oxide, which leads to neuronal death in the CNS.[24] It has been shown that garcinol (**1**), reduced the expression of iNOS in a dose dependent manner and, subsequently, the production of nitric oxide in astrocytes, and more importantly, the effects of garcinol (**1**) enhanced neuronal survival.[24] These findings suggest that polyphenols with antioxidant

activity from medicinal plants protect against the formation of ROS preventing neuronal cell death of neurons.

2.8. Antiinflammatory Activities

Results from several studies[81, 82] have suggested that garcinol (**1**) possesses anti-inflammatory and anti-carcinogenic activities. Changes in arachidonic acid (AA) metabolism and increased production of nitric oxide (NO) play an important role in the process of inflammation. Cytosolic phospholipase A_2 ($_cPLA_2$), cyclooxigenases (COX), lypooxigenases (LOX) and NO synthases are involved in the arachidonic acid synthesis and NO production.[81] Therefore, modulation of the arachidonic acid metabolism or NO synthesis, by suppressing the enzymes that are involved in their production is an effective way to suppress inflammation.

Hong *et al.,*[81] found that garcinol (**1**) at 1 μM modulates arachidonic acid metabolism in lipopolysaccharide (LPS)-stimulated murine macrophages, mainly blocking the activation of $_cPLA_2$ by inhibition of protein ERK1/2 phosphorylation. In addition, garcinol (**1**) suppressed iNOS expression and NO formation by modulation of the JAK/SAT-1 signaling pathway. Moreover, the same study [81] showed that garcinol (**1**) added to macrophages before the LPS treatment can interact with LPS receptors in these cells and suppress COX-2 expression. Koeberle *et al.*[82] has shown that garcinol (**1**) exerts antiinflammatory and anticarcinogenic activities *in vitro* and *in vivo* by interacting with 5-lipoxygenase (5-LO) and microsomal prostaglandin E_2 synthase-1 (mPGES-1), two enzymes that are involved in inflammation and carcinogenesis. Human 5-LO and mPGES-1 are also molecular targets for garcinol (**1**), which can be used as a template for development and production of new drugs with antiinflammatory properties.

Furthermore, Neves *et al.* showed that 7-epiclusianone (**22**) isolated from *G. brasiliensis* fruit pericarp has anti-anaphylactic properties.[80] *In vitro* experiments, using a model of anaphylactic histamine release from ileum tissue, have shown that 7-epiclusianone (**22**) exhibited mast cells stabilizing properties by inhibiting allergen evoked histamine release in a dose-dependent manner with IC_{50} = 3.8 ± 1.8 nM. In comparison, the standard anti-allergic compound azelastine used as a control showed IC_{50}= 6.6±2.8 nM. It was also found that 7-epiclusianone and azelastine inhibited allergen induced guinea pig ileum contractions with IC_{50} = 2.3 ± 1.1 μM and IC_{50} = 3.3 ± 1.2 μM, respectively. Results from this study suggest that 7-epiclusianone (**22**) can be used as a model for future drug development with anti-anaphylactic properties.

It was suggested that 7-epiclusianone **(22)** had putative mast cell stabilizing properties; however the antihistamine effect *ex-vivo* of 7-epiclusianone **(22)** was not as potent when compared to azelastine, a histamine H1 receptor antagonist. The inhibitory concentration was IC_{50} = 3.7 ± 0.1 μM for 7-epiclusianone (**22**), and IC_{50} = 6.3 ± 0.2 nM for azelastine.[80] Thus the pharmacological effect of 7-epiclusianone (**22**) when tested in *ex vivo* guinea pig ileum experiments indicated that it inhibited acetylcholine induced ileum contractions. It was suggested that 7-epiclusianone (**22**) exerts its effect by blocking the influx of Ca^{2+}.[80]

In a more recent study, benzophenone compounds isolated from *Hypericum thasium* Griseb. namely; 3′,4,5′,6-tetrahydroxy-*2-O-β*-D-xylosylbenzophenone (**31**) and 3,′4,5′,6,-tetrahydroxy-*2-O*-(*4-O*-acetyl-*β*-D-xylosyl)benzophenone (**32**), suppressed the oxidative burst of neutrophils; however quercetin and isoquercetin showed higher activity in this study than the tested benzophenones.[83]

2.9. Toxicology

Although no toxicological studies on natural isopolyisoprenylated benzophenones have been reported, synthetic benzophenones on the other hand are widely used as UV-screens and cosmetics and have been the subject of a number of toxicological studies. UV-screens are topically used and thus may be transcutaneously absorbed. Benzophenone-2 (**33**) binds to both the estrogenic receptors, ERα and ERβ. In addition, (**33**) had powerful estrogenic effects, showing estrogenic effects on uterine tissues.[84]

The highest concentration of (**33**) and its conjugates, BP2-sulfate and BP2-glucuronide, was observed in urine after 120 minutes of administration (298 μg/ml, 1973 μg/ml and 822 μg/ml).[85] For orally administered **33** it has been shown that the first-pass metabolism of the drug is likely to take place in the gastrointestinal lumen, and not in the liver, and this would possibly explain the rapid elimination from plasma.[85] The administration of an intravenous bolus dose of 100 mg/kg of benzophenone (**34**) to rats was followed by the detection of peak plasma concentration after 4 hours. The peak plasma concentration was $C_{max} = 2.06 \pm 0.46$ μg/ml, after which the concentration of (**34**) slowly declined. The elimination half-life was $t_{1/2}$= 19 hours and the area under curve (AUC) was 47.17 ± 5.52 μg/ml h. Hence, apparent clearance was 2.20 ± 0.22 ml/h/kg.[86] This kinetic study and metabolic profile of (**34**) in rats was performed using gas chromatography and mass spectrometry.[86] Only the free, unconjugated forms of the metabolites were detected by this method. These included the parent compound, 4-hydroxybenzophenone and benzohydrol. Even though conjugated benzophenones metabolites could not be detected by the reported method, the kinetic behavior of **34** suggested that it is rapidly absorbed through the gastrointestinal tract. The high

absorption rate is mainly due to the degree of lipophilicity of the two aromatic rings. The compound (**34**) rapidly disappears from plasma and the main route of elimination appears to be urine; however, rapid elimination from plasma might be due to redistribution to other compartment of the body or binding to plasma proteins.[86]

Kinetic studies suggest that the synthetic benzophenones are metabolized to glucuronide- and sulfate-conjugates before being eliminated. These findings are confirmed for garcinol (**1**) since its oral administration in rats showed an increased activity of phase II enzymes, particularly glutathione *S*-transferase and quinone reductase.[81] Studies in rat hepatocytes, however suggests that benzophenone compounds from *Hypericum annulatum* Moris are metabolized by the cytochrome P450 enzyme CYP2A6.[87] Oral administration of 10 mg of garcinol (**1**) was studied in CD-1 female mice.[81] The peak plasma level was 12 μM and urine level was 2.7 μM, suggesting that the polyisoprenylated benzophenones are likely to be eliminated through urine.[81]

Further toxicological studies and pharmacokinetic studies remain to be conducted for garcinol to establish the toxicological and kinetic profile of these compounds and metabolites formed during elimination.

2.10. Conclusions and future prospects

Polyisoprenylated benzophenones isolated from the genus *Garcinia* in the Clusiaceae family have been widely studied. These secondary metabolites have diverse pharmacological properties including antiinflammatory, antimicrobial, and cytotoxic effects. Evidence presented in this review suggests that polyisoprenylated benzophenones induce apoptosis and arrest the cell cycle early in G_1–phase. These compounds prevent the transcription and translation of important cell cycle regulators such as cyclins, which

are involved in cell progression. Thus they arrest the cell cycle and interrupt cell division and inhibit the growth of cancer cells. The mechanism of action involves the inhibition of mediators in the Akt/mTOR stress pathway. Although the specific target still remains unknown, natural benzophenones might reveal new targets for future therapies. Benzophenone compounds have low toxicity and can be synthetically modified to produce new active molecules for future anticancer drugs and treatments for other diseases.

Table 2.1. Cytotoxic activities of benzophenone compounds isolated from natural sources

Compounds	Source	Cell Line	Results	Comments
Aristophenone A	*Garcinia xanthochymus*	SW-480 colon cancer cell line	IC_{50} =33.3 (24-33) µM	Aristophenone A exhibited cytotoxicity against SW-480 colon cancer cells by inducing apoptosis. [25]
Cudraphenone A	*Cudrania cochinchinensis*	HSC-2 oral squamous carcinoma cell line HGF normal human gingival fibroblasts	CC_{50} = 0.17 mM CC_{50} = 0.43 mM	Cudrophenones A-D exhibited stronger cytotoxic activity against HSC-2 human oral squamous carcinoma cells compared to HGF normal human gingival fibroblasts. [45] Positive control : 6-prenyl-4',5,7-trihydroxy-isoflavone CC_{50} = 0.12 mM (HSC-2) CC_{50} = 0.25 mM (HGF)
Cudraphenone B			CC_{50} = 0.036 mM CC_{50} = 0.090 mM	
Cudraphenone C			CC_{50} = 0.092 mM CC_{50} = 0.19 mM	
Cudraphenone D			CC_{50} = 0.052mM CC_{50} = 0.19 mM	
Garcinol	*Garcinia assigu*	Raji cells	IC_{50} = 10.2 ±1.4 µM	Cancer chemo-preventive effects could be associated with the free radical scavenging properties of garcinol. [52] Positive control: Vitamin E IC_{50} =22.8±02 µM
	Garcinia indica	Colon cancer cell lines: HT-29 HCT-116 Normal immortalized intestinal cells: IEC-6 INT-407	IC_{50} =11.4 µM IC_{50} =12.0 µM IC_{50} =21.4 µM IC_{50} =19.4 µM	Garcinol exhibited cytotoxicity against colon cancer cells by inducing apoptosis, mainly increasing caspase-3 activity. Low concentrations <1 µM stimulated the growth of cancer and normal cells ranging from 10 to 100%. [42]
	Garcinia indica	HL-60 leukemia cell line	IC_{50} = 9.42 µM	Garcinol exhibited cytotoxicity against human leukemia cells by affecting caspase-3 and caspase-2 activity. Garcinol-induced apoptosis was related to the release of mitochondrial cytochrome C and loss of mitochondrial intermembrane potential. [43]
	Garcinia indica	HL-60 leukemia cell line	EC_{50} > 10 µM	Garcinol exhibited cytotoxicity against human leukemia cells by inducing apoptosis. It caused inhibition of NO generation and H_2O_2 production. [53]
	Garcinia purpurea	Leukemia cell lines: NB4 K562 U937 HL60	Garcinol was shown to be cytotoxic at a dose ranging from 5 to 20 µM	Garcinol exhibited cytotoxicity against leukemia cells by activation of caspase 3 and decrease in mitochondrial transmembrane potential, subsequently leading to apoptosis. [37]
Guttiferone A	*Garcinia macrophylla*	A2780 ovarian cancer cell line	IC_{50} = 6.8 µg/ml	Guttiferone A exhibited cytotoxicity against human ovarian cancer cells. Positive control: Actinomycin D IC_{50} = 0.003 µg/ml. [33]
Guttiferone E	*Garcinia pyrifera*	KB- oral carcinoma cell line	IC_{50} =10 µM [a]	Mixture of guttiferone E and xanthochymol exhibited cytotoxicity against KB cell lines. Guttiferone E was associated with inhibition of the disassembly of microtubules into tubulin with IC_{50} =1.5 µM. Positive control: Paclitaxel IC_{50} =0.006 µM. [34]

Guttiferone E	*Garcinia xanthochymus*	Human colon cancer cell lines : HCT 116 HT 29 SW-480	IC_{50} =9 (8-12) µM IC_{50} =14 (10-19) µM IC_{50} =17 (14-21) µM	Guttiferone E caused growth inhibition of colon cancer cells by activation of endoplasmic reticulum stress response, subsequently affecting the cell cycle. [37]
		SW-480 colon cancer cell line	IC_{50} =7.5(6.1-7.8) µM	Guttiferone E exhibited cytotoxicity against SW-480 colon cancer cells by inducing apoptosis. [25]
Guttiferone G	*Garcinia macrophylla*	A2780 ovarian cancer cell line	IC_{50} = 8.0 µg/ml	Guttiferone G exhibited cytotoxicity against human ovarian cancer cells. [33] Positive control: Actinomycin D. IC_{50} = 0.003 µg/ml.
Guttiferone H	*Garcinia xanthochymus*	Human colon cancer cell lines : HCT 116 HT 29 SW 480	IC_{50} =9 (8-12) µM IC_{50} =13 (10-17) µM IC_{50} =16 (12-20) µM	Guttiferone E caused growth inhibition of colon cancer cells by activation of endoplasmic reticulum stress response, subsequently affecting the cell cycle. [38]
		SW-480 colon cancer cell line	IC_{50} =12.4 (10.5-12.0) µM	Guttiferone H exhibited cytotoxicity against SW-480 colon cancer cells by inducing apoptosis. [25]
Guttiferone I	*Garcinia virgata*	KB- oral carcinoma cell line	IC_{50} = 4.70 µg/ml	Both guttiferone I and guttiferone J exhibited cytotoxicity against KB cell lines which was not associated with inhibition of tubulin polymerization in the cell. [47] Positive control: Taxotere IC_{50} = 0.37 ng/ml
Guttiferone J			IC_{50} = 5.0 µg/ml	
Guttiferone K	*Garcinia yunnanensis*	HeLa-C3 cervical cancer cell line	Guttiferone K was shown to be cytotoxic at a dose of 20 µg/ml	It was shown that guttiferone K at a concentration of 20 µg/ml can initiate apoptosis in HeLa-C3 cervical cancer cells. Concentration of 10 µg/ml was not active. [49]
	Rheedia calcicola	A2780 ovarian cancer cell line	IC_{50} = 3.6 µg/ml	Guttiferone K exhibited cytotoxicity against A2780 human ovarian cancer cells. [32]
Guttiferone L	*Rheedia calcicola*	A2780 ovarian cancer cell line	IC_{50} = 3 µg/ml	Guttiferone L exhibited cytotoxicity against A2780 human ovarian cancer cells. [32]
Hyperibone K	*Hypericum scabrum*	A549- lung cancer cell line MCF-7 -breast cancer cell line	IC_{50} =13.7 mcg/ml IC_{50} =10.0 mcg/ml	Both hyperibone K and hyperibone L exhibited cytotoxicity against lung and breast cancer cells. [30]
Hyperibone L			IC_{50} =9.2 mcg/ml IC_{50} =15.0 mcg/ml	
Isogarcinol	*Garcinia assigu*	Raji cells	IC_{50} = 13.3 ±1.3 µM	Cancer chemo-preventive effects could be associated with the free radical scavenging properties of isogarcinol. [52] Positive control : Vitamin E IC_{50} =22.8±02 µM

Isogarcinol	*Garcinia purpurea*	Leukemia cell lines: NB4 K562 U937 HL60	Isogarcinol was shown to be cytotoxic at a dose ranging from 5 to 20 µM	Isogarcinol exhibited cytotoxicity against leukemia cells by activation of caspase 3 and decrease in mitochondrial transmembrane potential, subsequently leading to apoptosis. [37]
Isoxanthochymol	*Garcinia indica*	MCF-7 breast cancer cell line WRL-68 liver cancer cell line COLO-320-DM colon cancer cell line	IC_{50} =2.85 µM IC_{50} =15.52 µM IC_{50} =4.85 µM	Isoxanthochymol exhibited growth inhibition in MCF-7, WRL-68 and COLO-320 cancer cell lines. The same study suggested that isoxanthochymol/ xanthochymol mixture (2:1) is more cytotoxic than isoxanthochymol alone. [20] Positive control: Paclitaxel IC_{50} =0.005 µM (MCF-7) IC_{50} =0.054 µM (WRL-68) IC_{50} =0.01 µM(COLO-320)
	Garcinia pyrifera	KB- oral carcinoma cell line	IC_{50} =5.8 µM[b]	Mixture of isoxanthochymol and cycloxanthochymol exhibited cytotoxicity against KB cancer cells. [34] Positive control:Paclitaxel IC_{50} =0.006 µM
Nemorosone	*Clusia rosea*	Leukemia cell lines: HL60 WT HL60 ADR MDR1+ CEM WT CEM VBL MDR1+ Jurkat WT Tanoue WT Kasumi WT K-562 WT	IC_{50} = 2.66±0.66 µM IC_{50} = 2.56±0.05 µM IC_{50} = 3.10±0.11 µM IC_{50} = 3.00±0.06 µM IC_{50} = 2.30±0.08 µM IC_{50} = 2.10±0.05 µM IC_{50} = 2.60±0.25 µM IC_{50} = 2.10±0.06 µM	Nemorosone exhibited cytotoxicity against different lines of leukemia cells mainly by downregulation of the protein levels of cyclins involved in the cell cycle control. These effects are mainly attributed to the negative modification of the Akt/PKB pathway. [36]
	Cuban propolis *Clusia* sp.	HeLa-cell line Hep-2 cell line PC-3 cell line U251- cell line	IC_{50} = 1.6±0.08 µg/ml IC_{50} = 1.5±0.08 µg/ml IC_{50} = 3.6±0.65 µg/ml IC_{50} = 1.9±0.70 µg/ml	Nemorosone exhibited strong cytotoxicity against cervix carcinoma, larynx cancer, prostate cancer, and nervous system cancer cell lines. [65]
Ochrocarpinone A	*Ochrocarpos punctatus*	A2780 ovarian cancer cell line	IC_{50} = 6.9 ±0.3µg/ml	Ochrocarpinones A-C exhibited cytotoxicity against A2780 ovarian cancer cells. [31]
Ochrocarpinone B			IC_{50} = 7.4 ±0.2µg/ml	
Ochrocarpinone C			IC_{50} = 8.2 ±0.3µg/ml	
Tovophenone A	*Tovomita brevistaminea*	KB- oral carcinoma cell line	EC_{50} =10.0 µg/ml^{-1}	The authors considered tovophenones A-C inactive against KB cancer cells because EC_{50} values were bigger than 5 µg/ml^{-1}. [50]
Tovophenone B			EC_{50} =9.0 µg/ml^{-1}	
Tovophenone C			EC_{50} =8.2 µg/ml^{-1}	
Vismiaguianone A	*Vismia guianensis*		EC_{50} > 20 µg/ml^{-1}	Vismiaguianones D-E exhibited moderate cytotoxic activity against KB oral cancer cells, while vismiaguianones A-C were inactive. [35]
Vismiaguianone B			EC_{50} > 20 µg/ml^{-1}	
Vismiaguianone C			EC_{50} > 20 µg/ml^{-1}	

Vismiaguianone D		KB- oral carcinoma cell line	EC_{50} = 2.4±0.9µg/ml^{-1}	Vismiaguianones D-E exhibited moderate cytotoxic activity against KB oral cancer cells, while vismiaguianones A-C were inactive. [35]
Vismiaguianone E			EC_{50} = 3.3±1.5µg/ml^{-1}	
Xanthochymol	*Garcinia indica*	MCF-7 breast cancer cell line WRL-68 liver cancer cell line COLO-320-DM colon cancer cell line	IC_{50} =0.475 µM IC_{50} =2.52 µM IC_{50} =0.62 µM	Xanthochymol exhibited growth inhibition in MCF-7, WRL-68 and COLO-320 cancer cell lines. The same study suggested that isoxanthochymol/ xanthochymol mixture (2:1) is more cytotoxic than xanthochymol alone. [20] Positive control: Paclitaxel IC_{50} =0.005 µM (MCF-7) IC_{50} =0.054 µM (WRL-68) IC_{50} =0.01 µM(COLO-320)
	Garcinia purpurea	Leukemia cell lines: NB4 K562 U937 HL60	Xanthochymol was shown to be cytotoxic at a dose ranging from 5 to 20 µM	Xanthochymol exhibited cytotoxicity against leukemia cells by activation of caspase 3, subsequently leading to apoptosis. [37]
	Garcinia pyrifera	KB- oral carcinoma cell line	IC_{50} =10 µM [a]	Mixture of xanthochymol and guttiferone E exhibited cytotoxicity against KB cancer cells. Xanthochymol was associated with inhibition of the disassembly of microtubules into tubulin with IC_{50} =2 µM. [34] Positive control: Paclitaxel IC_{50} =0.006 µM
	Garcinia xanthochymus	Human colon cancer cell lines : HCT 116 HT 29 SW 480	IC_{50} =10 (7-13) µM IC_{50} =15 (11-19) µM IC_{50} =17(14-21) µM	Xanthochymol caused growth inhibition of colon cancer cells by activation of endoplasmic reticulum stress response, subsequently affecting the cell cycle. [37]
		SW 480- colon cancer cell line	IC_{50} =8.3(7.0-8.2) µM	Xanthochymol exhibited cytotoxicity against SW 480- colon cancer cells by inducing apoptosis. [25]

[a] (Mixture xanthochymol /guttiferone E), [b] (Mixture of isoxanthochymol/cycloxanthochymol)

Table 2.2. Antibacterial, antiviral, and antiparasitic activities of benzophenone compounds isolated from natural sources

Compounds	Source	Strain	Results	Comments and Reference
7-Epiclusianone	*Rheedia gardneriana*	*Trypanosoma cruzi*	LC_{50} =260 µg/ml	7-Epiclusianone exhibited tripanocidal activity *in vitro.* It was inactive tested *in vivo* in mice. [76] Control: Gentian violet LC_{50} =7.5 µg/ml
	Rheedia brasiliensis	*Streptococcus mutans*	MIC= 1.25-2.5 µg/ml MBC=10-20 µg/ml	7-Epiclusianone exhibited antibacterial activity. [61] Positive control: Chlorhexidine MIC= 1-2 µg/ml MBC=8 µg/ml
	Hypericum sampsonii	*Staphylococcus aureus*	MIC= 4 µg/ml (7.3 µM)	7-Epiclusianone exhibited antibacterial activity against *S. aureus* SA-1199B strain. [22] Positive control: Norfloxacin MIC= 32µg/ml (100 µM)
7-Epi-garcinol	*Moronobea coccinea*	*Plasmodium falciparum*	IC_{50} = 10.1 ± 4.6 µM	7-epi-garcinol exhibited moderate antiplasmodial activity against chloroquine-resistant strain of *P. falciparum* FcB1. [77] Control: Chloroquine IC_{50} = 0.078 ± 0.006 7µM
7-Epi-isogarcinol			IC_{50} = 5.1 ± 1.3 µM	7-epi-isogarcinol exhibited strong antiplasmodial activity against chloroquine-resistant strain of *P. falciparum* FcB1 strain. [77]
Chamone I	*Clusia grandiflora*	*Paenibacillus larvae*	19.7±0.3mm [a] (35h)	Chamone I exhibited strong antibacterial activity against honeybee pathogens. [63]
		Paenibacillus alvei	10±0.6mm [a] (35h)	
Coccinone A	*Moronobea coccinea*	*Plasmodium falciparum*	IC_{50} = 4.3 ± 0.5 µM	Coccinones A-E along with cycloxanthochymol exhibited strong antiplasmodial activity, while coccinones F-H exhibited moderate activity against chloroquine-resistant strain of *P. falciparum* FcB1. [77] Control: Chloroquine IC_{50} = 0.078 ± 0.006 7µM
Coccinone B			IC_{50} = 5.5 ± 0.4µM	
Coccinone C			IC_{50} = 9.0 ± 1.2 µM	
Coccinone D			IC_{50} = 7.0 ± 0.9µM	
Coccinone E			IC_{50} = 4.9 ± 0.7µM	
Coccinone F			IC_{50} = 17.0 ± 9.4 µM	
Coccinone G			IC_{50} = 19.2 ± 5.9µM	
Coccinone H			IC_{50} = 16.6 ± 6.1µM	
Cycloxanthochymol			IC_{50} = 2.1 ± 0.5 µM	
Garcinol (Camboginol)	*Allanblackia monticola*	*Leishmania donovani*	IC_{50} =0.82 µM	Garcinol exhibited leishmanicidal activity *in vitro*. [75] Standard: Miltefosine- IC_{50} =0.47 µM
			IC_{50} =0.33 µM	Camboginol exhibited leishmanicidal activity. [75] Standard: Miltefosine- IC_{50} =0.47 µM
	Garcinia bancana	(MRSA)-Methicillin-resistant *Staphylococcus aureus*	MIC=16 µg/ml	Garcinol exhibited antibacterial activity against methicilin-resistant *S.aureus.* [60]
	Garcinia purpurea	(MRSA)-Methicillin-resistant *Staphylococcus aureus*	MIC= 6.25-12.5 µg/ml	Garcinol exhibited strong antibacterial activity against MRSA. [59]Control: Vancomycin MIC= 6.25 µg/ml

	Moronobea coccinea	*Plasmodium falciparum*	IC_{50} = 12.6 ± 4.8 µM	Garcinol exhibited moderate antiplasmodial activity against chloroquine-resistant *P. falciparum* FcB1 strain. [77] Control: Chloroquine IC_{50} = 0.078 ± 0.006 7µM
Garciosaphenone A	*Garcinia speciosa*	HIV-1	IC_{50} =23.9 µg/ml	Garciosaphenone A exhibited HIV-1 inhibitory activities. [69]
Guttiferone A	*Garcinia intermedia*	*Trypanosoma cruzi*	MC_{100}=100 µM (Epimastigotes) MC_{100}=83µM (Trypomastigotes)	Guttiferone A exhibited trypanocidal activity. [74]
	Symphonia globulifera	*Leishmania donovani*	IC_{50} =0.16 µM	Guttiferone A exhibited strong leishmanicidal activity. [75] Standard: Miltefosine- IC_{50} =0.47 µM
	Symphonia globulifera	*Plasmodium falciparum*	IC_{50} = 3.17 µM	Guttiferone A exhibited antiplasmodial activity. [78]
Guttiferone F	*Allanblackia monticola*	*Leishmania donovani*	IC_{50} =0.20 µM	Guttiferone F exhibited strong leishmanicidal activity.[75] Standard: Miltefosine- IC_{50} =0.47 µM
	Allanblackia stuhlmannii	HIV-1	EC_{50} =23µg/ml	Gutifferone F exhibited HIV-1 inhibitory activity *in vitro*. [22]
Guttiferone I	*Garcinia smeathmannii*	*Citrobacter freundii* *Enterobacter cloaclae* *Proteus vulgaris* *Bacillus megaterium* *Streptococcus faecalis*	MIC= 1.22 µg/ml MIC= 1.22 µg/ml MIC =1.22 µg/ml MIC =0.61 µg/ml MIC =0.61 µg/ml	Guttiferone I exhibited antibacterial activities against both gram-positive and gram-negative bacteria. [21] Control: Gentamycin MIC= 4.88 µg/ml MIC= 4.88 µg/ml MIC= 1.22 µg/ml MIC= 2.44 µg/ml MIC= 2.44 µg/ml
Isogarcinol	*Garcinia purpurea*	(MRSA)-Methicillin-resistant *Staphylococcus aureus*	MIC= 12.5 µg/ml (MRSA)	Isogarcinol exhibited antibacterial activity against MRSA. [59] Control: Vancomycin MIC= 6.25 µg/ml
	Moronobea coccinea	*Plasmodium falciparum*	IC_{50} = 3.5 ± 1.1 µM	Isogarcinol exhibited strong antiplasmodial activity against chloroquine-resistant strain of *P. falciparum* FcB1. [77] Control: Chloroquine IC_{50} = 0.078 ± 0.006 7µM
Isoxanthochymol	*Garcinia smeathmannii*	*Bacillus cereus* *Bacillus stearothermophylus*	MIC= 9.76 µg/ml MIC= 4.88 µg/ml	Isoxanthochymol exhibited antibacterial activities against gram-positive bacteria. [21] Control: Gentamycin MIC= 1.22 µg/ml MIC= 4.88 µg/ml
	Garcinia subelliptica	(MRSA)-Methicillin-resistant *Staphylococcus aureus*	MIC >25 µg/ml*	Mixture of isoxanthochymol/cycloxanthochymol exhibited weak antibacterial activity. [59] Control: Vancomycin MIC= 6.25 µg/ml
Microsphaerin A ***Microsphaerin B*** ***Microsphaerin C***		(MRSA)-Methicillin-resistant *Staphylococcus aureus*	IC_{90} =3.0 µM IC_{90} =3.0 µM IC_{90} =5.0 µM	Microsphaerins A-C exhibited strong antibacterial activity against MRSA. [67]

Microsphaerin D	*Microsphaeropsis* sp. [b] *(F2076) and (2078)*	*Staphylococcus aureus* *MRSA* *Enterococcus faecalis* *Streptococcus pneumonia* *Bacillus subtilis* *Escherichia coli* *Klebsiella pneumoniae* *Moroxella catarrhalis* *Haemophilus influenzae* *Pseudomonas aurogenosa*	IC_{90} =1.3 µM IC_{90} =1.0 µM IC_{90} =1.3 µM IC_{90} =3.6 µM IC_{90} =3.0 µM IC_{90} >50 µM IC_{90} >50 µM IC_{90} =1.3 µM IC_{90} >50 µM IC_{90} >50 µM	Microsphaerin D exhibited strong antibacterial activity against gram-positive bacteria; but it was not active against gram-negative bacteria with one exception (*K. pneumoniae).* [67]
Nemorosone II	*Clusia grandiflora*	*Paenibacillus larvae* *Paenibacillus alvei*	19.5±1.6 mm [a] (35h) 2.3 ± 2.3 mm [a] (35h)	Nemorosone II exhibited strong antibacterial activity against honeybee pathogens. [63]
Pestalachloride A	*Pestalotiopsis adusta*[c]	*Fusarium culmorum* *Gibberrella zeae* *Verticillium aibo-atrum*	IC_{50} =0.89±0.10µM / MIC=7.2 µM IC_{50} =54.4±0.8µM / MIC=114.4µM IC_{50} =58.3±1.4µM / MIC=114.4µM	Pestalachlorides A-B exhibited strong antifungal activities against plant pathogens. Pestalachloride C did not exhibit significant activity against the same plant pathogens. [43]
Pestalachloride B		*Fusarium culmorum* *Gibberrella zeae* *Verticillium aibo-atrum*	IC_{50} =4.7±0.2µM / MIC=49µM IC_{50} =1.1±0.08µM / MIC=12.2µM IC_{50} =7.9±0.2µM / MIC=12.2µM	
Pestalachloride C		*Fusarium culmorum* *Gibberrella zeae* *Verticillium aibo-atrum*	IC_{50} > 118.5µM / MIC >236.9µM IC_{50} > 118.5µM / MIC >236.9µM IC_{50} > 118.5µM / MIC >236.9µM	
Pestalone	*Pestalotia* sp.[d]	(MRSA)-Methicillin-resistant *Staphylococcus aureus* Vancomycin –resistant *Enterococcus faecium*	MIC= 37 ng/ml MIC= 78 ng/ml	Pestalone exhibited strong antibacterial activity against two lines of antibiotic resistant bacteria. [66]
Vismiaphenone D	*Vismia cayennensis*	HIV	EC_{50} =11 µg/ml	Only vismiaphenone D exhibited HIV inhibitory activity, while vismiaphenones E-G were inactive. [70]
Vismiaphenone E			Inactive	
Vismiaphenone F			Inactive	
Vismiaphenone G			Inactive	
Xanthochymol	*Garcinia subelliptica*	(MRSA)-Methicillin-resistant *Staphylococcus aureus*	MIC= 3.13-12.5 µg/ml (MRSA)	Xanthochymol exhibited strong antibacterial activity against MRSA. [59] Control: Vancomycin MIC= 6.25 µg/ml

[a] Total inhibition, [b] Anamorphic fungus , [c] Plant endophytic fungus, [d] Marine fungus, * Mixture of isoxanthochymol/cycloxanthochymol

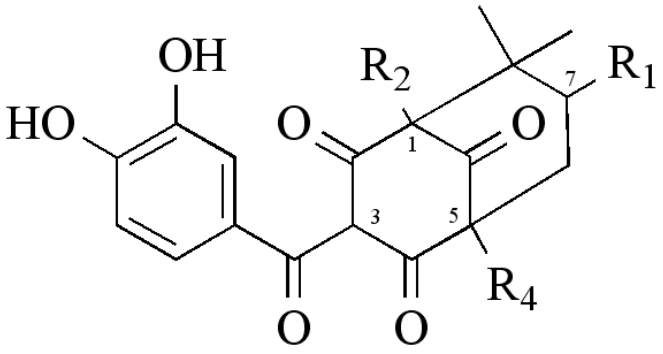


Figure 2.1. General structure of polyisoprenylated benzophenone

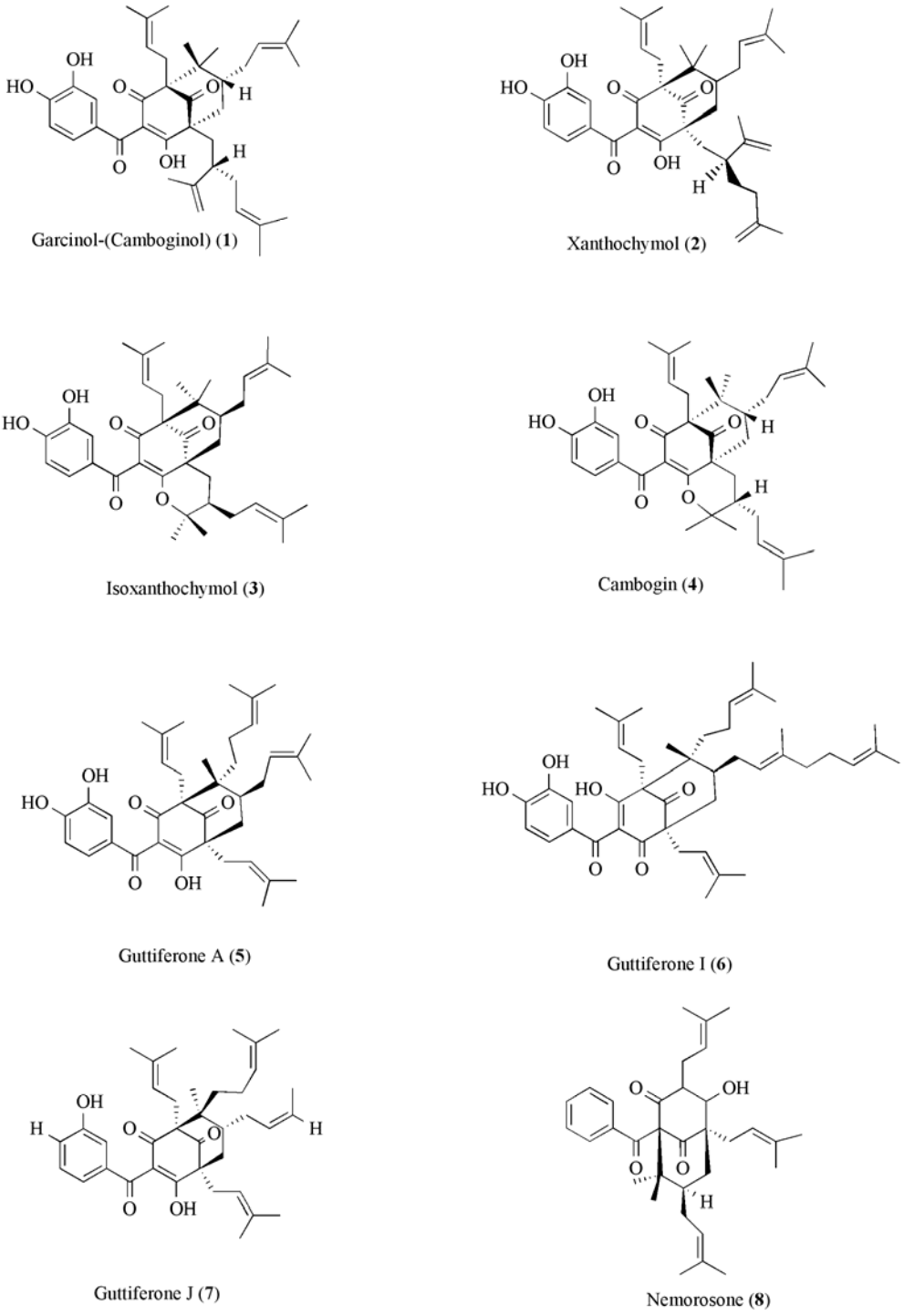


Figure 2.2. Polyisoprenylated benzophenones from natural sources and synthetic analogues (compounds 1-34)

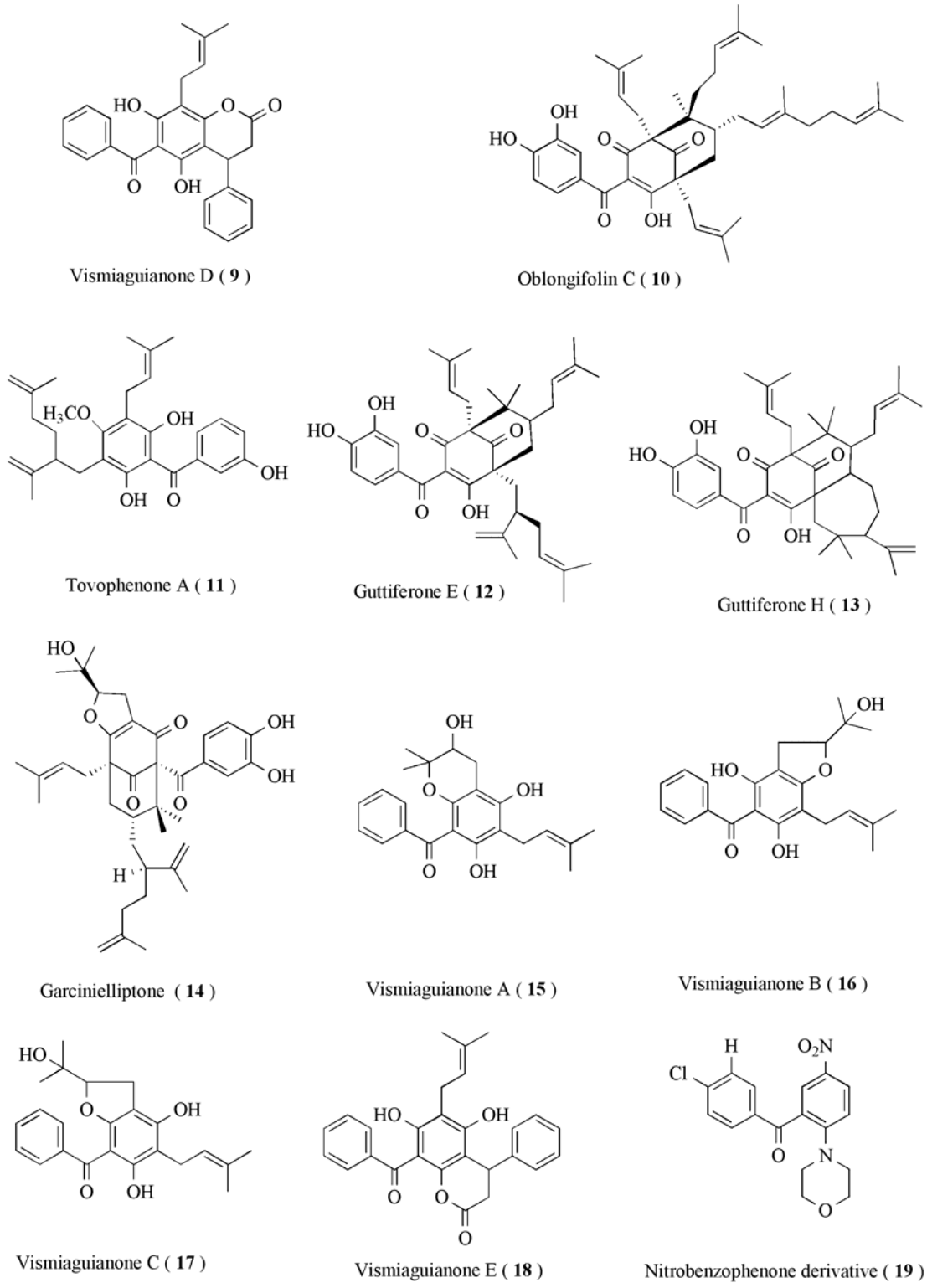


Figure 2.2. (Continued) Polyisoprenylated benzophenones from natural sources and synthetic analogues (compounds 1-34)

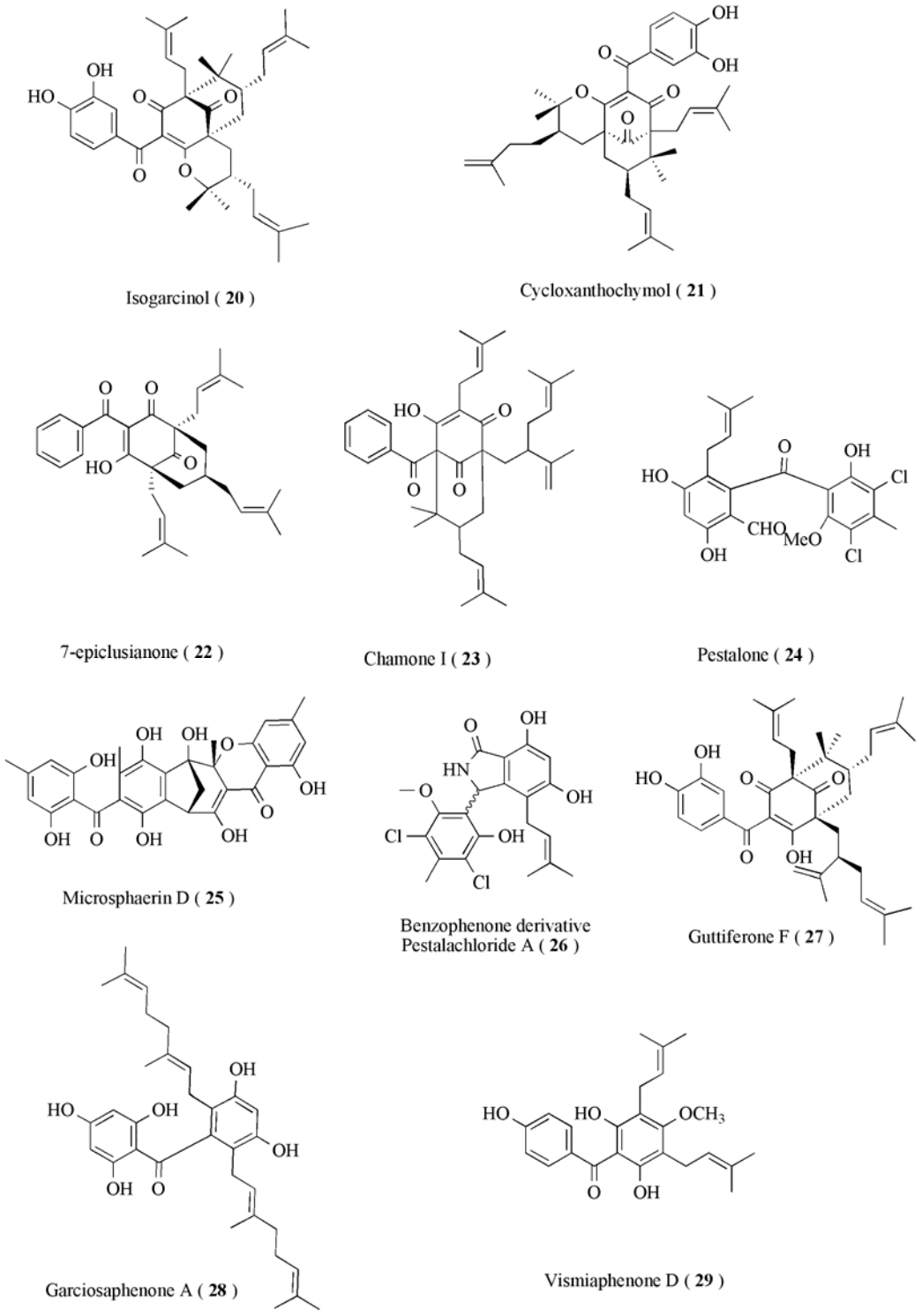
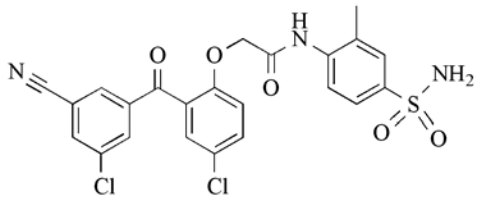
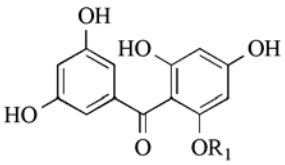


Figure 2.2. (Continued) Polyisoprenylated benzophenones from natural sources and synthetic analogues (compounds 1-34)

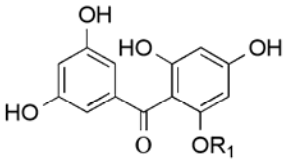


GW678248 (**30**)



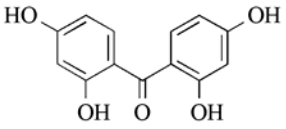
R_1 = β-Xyl

3',4.5',6-Tetrahydroxy-*2-O-β-D*-xylosylbenzophenone (**31**)

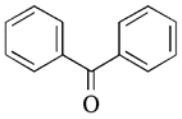


R_1 = 4-OAc-β-Xyl

3',4,5',6-Tetrahydroxy-2-O-(4-O-acetyl-*β-D*-xylosyl) benzophenone (**32**)



Benzophenone-2 (**33**)



Benzophenone (**34**)

Figure 2.2. (Continued) Polyisoprenylated benzophenones from natural sources and synthetic analogues (compounds 1-34)

Chapter 3
Benzophenones and Biflavonoids Antioxidants from *Garcinia intermedia* fruits (Clusiaceae)

3.1. Introduction

As part of our ongoing studies of antioxidants from tropical edible fruits, we have studied *Garcinia intermedia* (Pittier) Hammel.[88] *Garcinia intermedia* is a 30 m high tree with yellow latex, native to the Central American lowland tropical rain-forest. The leaves are opposite, leathery, dark-green on the adaxial surface, brownish on the abaxial surface, 8-15 cm long and 2-5 cm wide, although the leaf-shape can be elliptic, lanceolate or oblong. The flowers are white, joined in clusters and have four petals. The male flowers have 25-30 stamens. The fruits are yellow and are oval or oblong, 2-3 cm long, with a smooth thin skin. The seeds are 1-2. The fruit is sweet and matures from late January to March. It is the only Central American member of this genus with edible exocarp. The wood has numerous gum ducts and it is immune to insects and it is sometimes grown as an ornamental tree.[89] *Garcinia intermedia* has many different common names. It is called "waiki-plum" in Belize, "arrayán" and "palo de frutilla" in Guatemala, "chaparrón" in El Salvador, "caimito" in Honduras, "jorco" in Costa Rica and "sastra" in Panama. It is cultivated locally in Brazil and in the Philippines where it is called "limão do matto" or "berba", respectively.[89]

Guttiferone A, 8-desoxygartanin, podoscarpusflavone A, amentoflavone, and friedelin have previously been identified in the leaves of this species.[74] To our knowledge no previous study has been done on the fruits of this species. Previous studies have found that prenylated benzophenones have antiviral[23] and insecticidal activities.[90] We have found that prenylated benzophenone compounds from *Garcinia xanthochymus* fruits have antioxidant and weak cytotoxic effect against colon cancer cells, SW480.[25] Gene expression studies of cancer cells suggested that prenylated benzophenone compounds interfere with the mitochondrial membrane potential and subsequently

activates the endoplasmatic stress and cellular energy stress response pathways. Guttiferone compounds induce cell-cycle arrest in G_1-phase and induce apoptosis,[38] suggesting that these compounds exert chemopreventive effects against cancer by arresting their cell cycle of cancer cells.

It has been suggested that benzophenone compounds inhibit the disassembly of microtubules, thus inhibiting cell replication through yet another mechanism.[34] Nevertheless, the specific cellular target of benzophenones exerting cytotoxicity has not been described until now. Thus we tested the compounds isolated from *G. intermedia* for inhibitory activity of the following cell cycle regulators; DYRK1A, CK1, CDK5, and GSK3.

3.2. Results and discussion

In our study of antioxidants from the fruits of *G. intermedia* we have identified six benzophenones; guttiferone A **(1)**, guttiferone E **(2)**, isoxanthochymol **(3)**, xanthochymol **(4)**, aristophenone **(5)** maclurin **(6),** and two biflavonoids, volkensiflavone **(7)** and fukugetin **(8)**. Identification was made through comparison to previously isolated standards and based on literature data (Fig. **3.1**). Compound **9** was analyzed by HRESIMS and by 1D and 2D NMR experiments, which led to the identification and characterization of its structure (Fig. **3.2**). The compound showed the UV absorption at λ_{max} 233 and 279 nm, which is distinctive of benzophenone compounds. The HRESIMS spectrum showed a protonated molecule $[M+H]^+$ of *m/z* 619.3568, which corresponded to a molecular formula $C_{38}H_{50}O_7$ calculated for 618.3557 and containing 14 degrees of unsaturations.

The ^{1}H-spectrum of **9** (Fig **3.3**) showed an aromatic AMX splitting system with resonances at δ 7.19 ppm d (J = 1.8 Hz), 7.06 ppm dd (J = 8.2, 1.8 Hz), and 6.72

ppm d (J = 8.2 Hz). Three olefinic protons were detected at δ 5.03 ppm, 4.95 ppm and 4.90 ppm, indicating the presence of three isoprenylated substituting groups. The broad proton signals at δ 4.90 and 4.76 ppm suggested the presence of a terminal methylene. The ^{13}C-NMR spectrum (Fig. **3.4**) of compound **9** showed three signals at δ 192.0, 193.0 and 213.0 ppm, indicating the presence of three carbonyl groups**.** The resonance signals of substituted aromatic carbons were detected at δ 133.5, 145.7 and 150.0 ppm. The carbon signal at δ 75.6 ppm was assigned as C-34 and it corresponded to a methine signal in the DEPT experiment (Fig. **3.5**). The HSQC experiment (Fig. **3.6**) confirmed that the carbon at δ 75.6 ppm (C-34) was coupled to a proton at δ 3.94 ppm (d, J= 5.31). Moreover, the HMBC experiment (Fig. **3.7**) showed that the proton signal at δ 4.90 brs ppm (H-32) correlates with carbon at δ 18.0 ppm (C-33), and to carbon at δ 75.6 ppm (C-34). Furthermore, the proton at δ 1.67 ppm (H-33) showed strong correlations to carbons at δ 17.6 ppm (C-33), 75.6 ppm (C-34), 110.2 ppm (C-32) and 148.5 ppm (C-31). In the HMBC experiment, the proton at δ 4.76 brs ppm (H-32a), which appears as broad singlet in the proton spectrum, correlated to carbon at δ 17.6 ppm (C-33) as well as to carbon at δ 75.6 ppm (C-34). The COSY spectrum showed a correlation between proton at δ 3.94 (H-34) ppm and proton at δ 4.90 brs (H-32b) ppm. There are five methylene signals in the ^{13}C DEPT 135 spectrum.

The aromatic proton at δ 7.19 ppm (H-12) showed HMBC correlations to carbons at δ 123.9 ppm (C-16), 145.7 ppm (C-13), 150.0 ppm (C-14), and 199.0 ppm (C-10), this last correlation links the aromatic 3,4-dihydroxybenzoyl moiety to one of the carbonyl carbons (C-10). Similarly, the aromatic proton at 7.06 ppm (H-16) showed HMBC long-range correlations to carbons at δ 116.9 ppm (C-12), 150.0 ppm (C-14), and

199.0 ppm (C-10). Proton at δ 6.72 ppm (H-15) correlates to carbons at δ 133.5 ppm (C-11), 145.7 ppm (C-13), and 150 ppm (C-14). Moreover, proton at δ 2.80 ppm (H-30) correlates to carbons at δ 39.4 ppm (C-29). Proton at δ 2.60 ppm (H-17) correlates to carbon at δ 68.7 ppm (C-4), 132.7 ppm (C-19), 193.0 ppm (C-3), and 213.0 ppm (C-9). In the bicyclo[3.3.1]nonane structure proposed, the proton at δ 2.00 (H- 7b) ppm correlates to carbons at δ 39.4 ppm (C-29), 61.7 ppm (C-8), 192.0 ppm (C-1), and 213.0 ppm (C-9).

In addition, strong HMBC cross-peaks were identified from the methyl group at δ 1.22 ppm s (H-22) correlating to carbon at δ 40.6 ppm (C-6) and 50.2 ppm (C-5). Proton at δ 1.61 ppm was assigned as H-27 showed correlations coupling to carbon at δ 17.8 ppm (C-28), 125.9 ppm (C-25), and 132.6 ppm (C-26).

During isolation of benzophenones and biflavonoids, antioxidant activity was measured using the DPPH assay, previous to isolation of phytochemical constituents. The methanol extract displayed low antioxidant activity (IC_{50}= 118.1 ± 0.94 μg/ml) in the DPPH assay. The chloroform and ethyl acetate partitions showed high activity, IC_{50}= 23.4 ± 1.28 μg/ml and IC_{50}= 41.4 ± 3.66 μg/ml respectively, while the *n*-butanol partition only had a moderate activity (68.0 ± 1.41 μg/ml). The antioxidant activity of guttiferone A (IC_{50}= 46 μM) is comparable to that of gallic acid (IC_{50}= 35 μM). The antiproliferative effect of guttiferone A against HT-29 colon cancer cells was IC_{50}= 15.8 μM (tested by Dr. Linda Saxe-Einbond).

Fukugetin, volkensiflavone, and guttiferone A were analyzed for cyclin dependent kinase activity and cell cycle regulation by DYRK1A, CK1, CDK5, and GSK3; however, the tested compound did not show any activity on these cell cycle mediators.

Experimental Section

3.3. General experimental procedures

^{1}H, ^{13}C, DEPT, HSQC, and HMBC NMR spectra were measured using a Bruker Avance 300 MHz spectrometer. Sephadex LH-20 (Pharmacia Fine Chemicals, Piscataway, NJ) and reversed-phase C_{18} silica gel (J.T. Baker, Phillipsburg, NJ) was used for gel-filtration and column chromatography. MeOH (J.T Baker, Phillipsburg, NJ) was used for extraction and isolation. TLC plates RP_{18} F_{254} (Merck, Darmstadt, Germany) were used to monitor separation and isolation. Preparative HPLC was carried out to separate a mixture of guttiferone A and isoxanthochymol using a Waters 600 controller with Waters 486 tunable absorbance detector and a Phenomenex Luna® C_{18} column (250 x 21.2 mm, 10 μm) and Phenomenex Synergi® Hydro-RP C_{18} column (250 x 4.6 mm, 5 μm). A Waters 2695 separation module and a 996 photodiode array detector with a Phenomenex Luna® C_{18} column (250 x 4.6 mm, 5 μm) (Torrance, CA) were used to analyze the main constituents. Molecular weights were obtained using a ThermoFinnigan electrospray LCQ mass spectrometer in the negative mode. Absorbance was measured using a microplate reader (Molecular Devices Versa$_{max}$).

3.3.1. Plant material

Fruits of *Garcinia intermedia* were obtained from the Broward County Rare Fruit and Vegetable Council, Florida. The collected fruits were shipped overnight and stored at -20 °C at the Phytochemistry Laboratory, Lehman College, City University of New York, Bronx, NY. A voucher specimen is kept at The William and Lynda Steere Herbarium at the New York Botanical Garden, Bronx, NY.

3.3.2. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay

The DPPH antioxidant assay was performed according to previously described procedures.[91] Samples were dissolved in DMSO and further diluted before being transferred to a 96-well microtiter plate. Each well contained 150 µL ethanolic solution of DPPH (400 mM) and 50 µL of sample solution. The microtiter plates were incubated at 37°C for 30 min and their absorbance were measured at UV_{max} 515 nm using a microplate reader (Molecular Devices $Versa_{max}$). The obtained data were used to determine the concentration of the sample required to scavenge 50% of the DPPH free radicals (IC_{50}). The percent inhibition was plotted against concentration and the IC_{50} was obtained from the fitted linear curve. In the experiments, the negative controls, ethanol and DMSO, showed no antioxidant activity. Ascorbic acid was used as a positive control (IC_{50} = 15 µg/mL). The $CHCl_3$ partition displayed highest antioxidant activity in the DPPH assay (IC_{50} = 23.4 ± 1.28 µg/mL) and was further analyzed. The antioxidant activity of guttiferone A (IC_{50} = 46 µM) is comparable to that of gallic acid (IC_{50} = 35 µM).

3.3.3. Extraction and isolation

To obtain minor benzophenone constituents, the seeds of *G. intermedia* (358 g) were extracted three times in 2000 mL MeOH at room temperature. The MeOH extract was evaporated *in vacuo,* and it yielded a residue, which was suspended in water and then sequentially partitioned with 300 x 3mL $CHCl_3$, EtOAc, and BuOH. The EtOAc partition (6.95 g) was chromatographed over an open column with silica gel reversed-phase C_{18}, using a gradient solvent system composed of ammonium acetate (10 mM) and MeOH, starting at (50:50)→(0:100). Thirteen fractions were obtained and analyzed. Fractions D through F were combined (8.6 mg). This sample contained a binary mixture

of benzophenone compounds and was analyzed by 1D and 2D NMR. Structure elucidation and identification of compound **9** was achieved from these experiments.

Analytical HPLC was used for further purification and to obtain compound **9**, using a Synergi® Hydro-RP column with a solvent system composed of ammonium acetate buffer (10 mM) and MeOH. A linear gradient system was used; starting from (50:50)→(35:65), over 5 minutes, then running from (35:65)→(25:75), over 18 minutes. Then, finally, the isocratic condition (5:95) was run for 5 minutes, before restoring initial conditions (50:50) for 10 min. The flow rate was 1 mL/min. Separation was monitored at λ_{max} 280 nm. A sample of 1.5 mg containing compound **9** was obtained for further NMR analysis to identify compound **9**.

The separations and fractions were monitored and combined using RP_{18}-TLC, developed in a mixture composed of ammonium acetate buffer (10 mM): MeCN (5:95). The constituents were detected by spraying the TLC plate with a reagent composed of 10% H_2SO_4 and 1% vanillin in EtOH and followed by heating. Analysis during separations was performed using reversed-phase HPLC, with a solvent system composed of ammonium acetate buffer (10 mM) and MeCN; a linear gradient of the mixture (95:5→0:1) was run over 45 minutes at a flow rate of 1 mL/minute, holding 100% MeCN for 10 minutes. In addition, fractions were monitored by LC-MS. ^{1}H, ^{13}C, DEPT, HSQC and HMBC experiments were recorded in acetone-*d*6.

Guttiferone A **(1)**: The UV (MeOH) absorption was λ_{max} 233 and 279 nm; ^{1}H-NMR (300 MHz, acetone-d_6) The molecular weight obtained by negative electrospray ionization mass spectrometry was *m/z* 601. The structure was identified by NMR and in compared to data in previously published literature.[68]

Guttiferone E **(2)**: The UV (MeOH) absorption was λ_{max} 241 and 279 nm. It was identified by HPLC spiking experiment using available standards.

Isoxanthochymol **(3)**: The UV (MeOH) absorption was λ_{max} 233 and 279 nm. The molecular weight obtained by negative electrospray ionization mass spectrometry was *m/z* 601. The structure was identified by NMR and by comparison to previously published literature data.[68]

Xanthochymol **(4)**: The UV (MeOH) absorption was λ_{max} 241 and 279 nm. It was identified by HPLC spiking experiment using available standards.

Aristophenone **(5)**: The UV (MeOH) absorption was λ_{max} 243 and 310 nm. The molecular weight obtained, by negative electrospray ionization spectroscopy, was *m/z* 534.

Maclurin **(6)**: The UV (MeOH) absorption was λ_{max} 228, 279 and 360 nm. The molecular weight obtained, by negative electrospray ionization spectroscopy, was *m/z* 262.

Volkensiflavone **(7)**: The UV (MeOH) absorption was λ_{max} 215, 288 and 323 nm. The molecular weight obtained, by negative electrospray ionization spectroscopy, was *m/z* 540.

Fukugetin **(8)**: The UV (MeOH) absorption was λ_{max} 214, 289 and 329 nm. The molecular weight obtained, by negative electrospray ionization spectroscopy, was *m/z* 556.

Compound **(9):** yellow oil; UV (MeOH) absorption was λ_{max} 213, 230 and 280 nm; ^{1}H-NMR (300 MHz, acetone-d_6) and ^{13}C-NMR (300 MHz, acetone-d_6). See Table 3.1; HRESIMS was *m/z* 619.3568 $[M+H]^+$ (calcd for $C_{38}H_{50}O_7$, 618.3557).

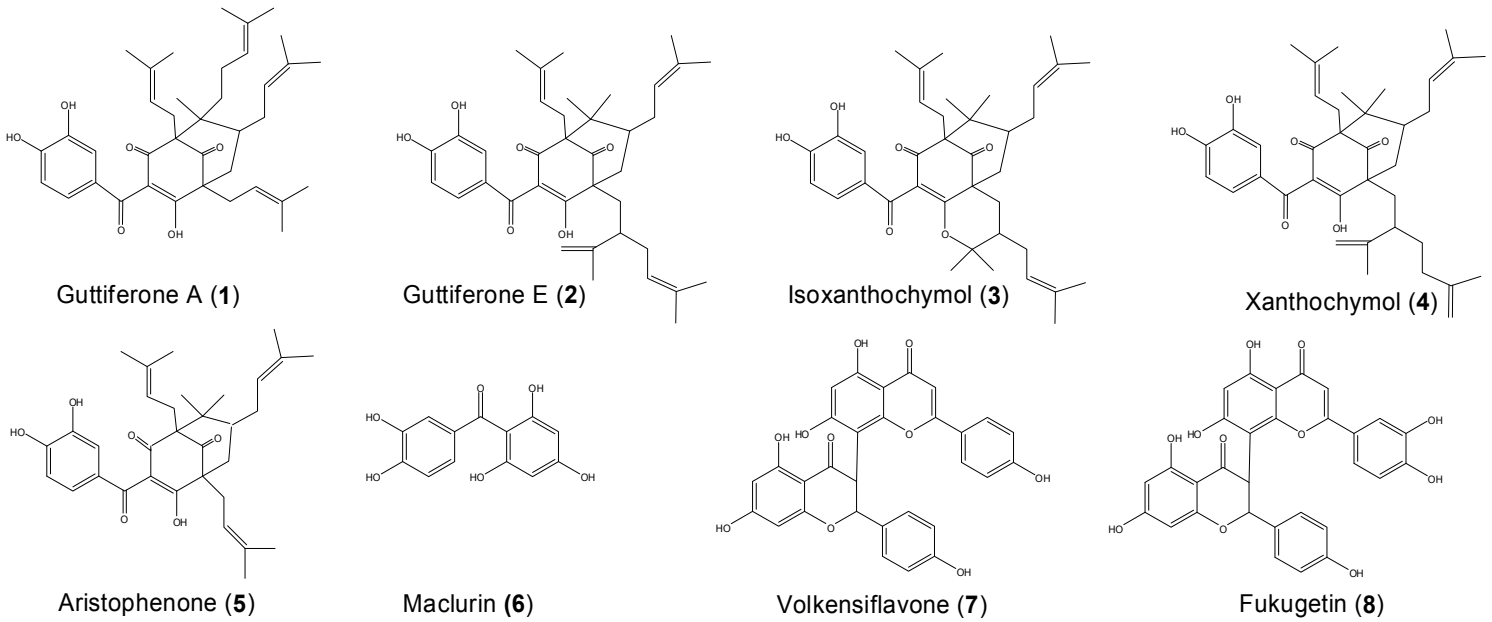


Figure 3.1. Previously known compounds identified in fruits of *G. intermedia*

Table 3.1. NMR Data for compound **9** (in acetone-d_6)

Position	^{13}C	^{1}H	^{1}H HMBC connectivities[b]
1	192		7A, 29B
2	119.3		
3	193		17AB
4	68.7		17AB
5	50.2		7B, 22, 23
6	40.6	1.76 m	7A, 7B, 22, 23
7	38	2, 1.97 m	6, 24 AB, 29A
8	61.7		7A, 7B
9	213		7B, 17AB
10	199		12, 16
11	133.5		15
12	116.9	7.19 d (1.8)	16
13	145.7		12, 15
14	150		12, 15, 16
15	114.6	6.72 d (8.2)	
16	123.9	7.06 dd (8.2, 1.8)	12
17	26.1	2.6	
18	122	5.03	20, 21
19	132.7		17AB, 20, 21
20	25.9	1.69 s	18, 21
21	17.9	1.64 s	18, 20
22	19.3	1.221 s	23
23	30.1	1.216 s	22
24	30.1	2.07	6, 7AB
25	125.9	4.95	27, 28
26	132.6		27, 28
27	25.8	1.61 s	28
28	17.8	1.48 s	27
29	39.4	2.15, 1.90	7AB, 30
30	42.6	2.80	29A, 29B, 34
31	148.5		33
32	110.2	4.90 brs, 4.76 brs	33
33	17.6	1.67	32B
34	75.6	3.94	29A, 32A, 32B, 33
35	125.8	4.89	37, 38
36	132.5		37, 38
37	25.8	1.61 s	38
38	17.7	1.68 s	37

[a]Proton coupling constants are given in parentheses; carbon types were confirmed with DEPT and edited-HSQC experiments. [b]Protons correlating with carbon resonance.

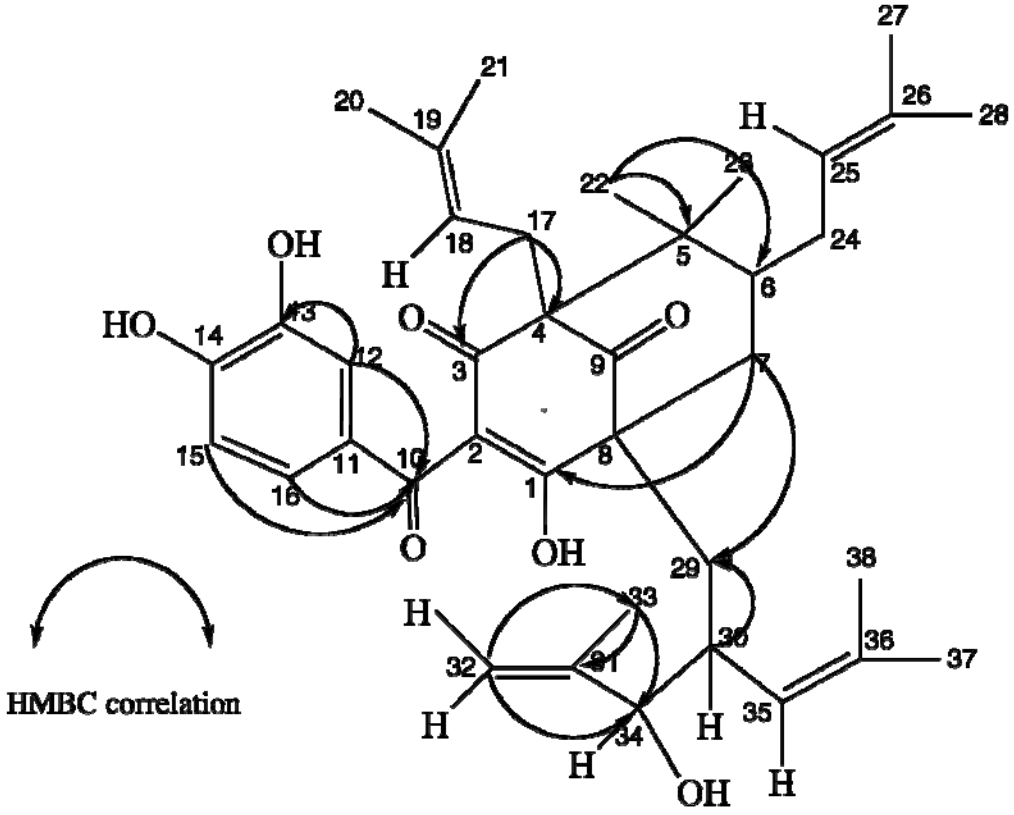


Figure 3.2. Proposed structure of new polyisoprenylated benzophenone **9**

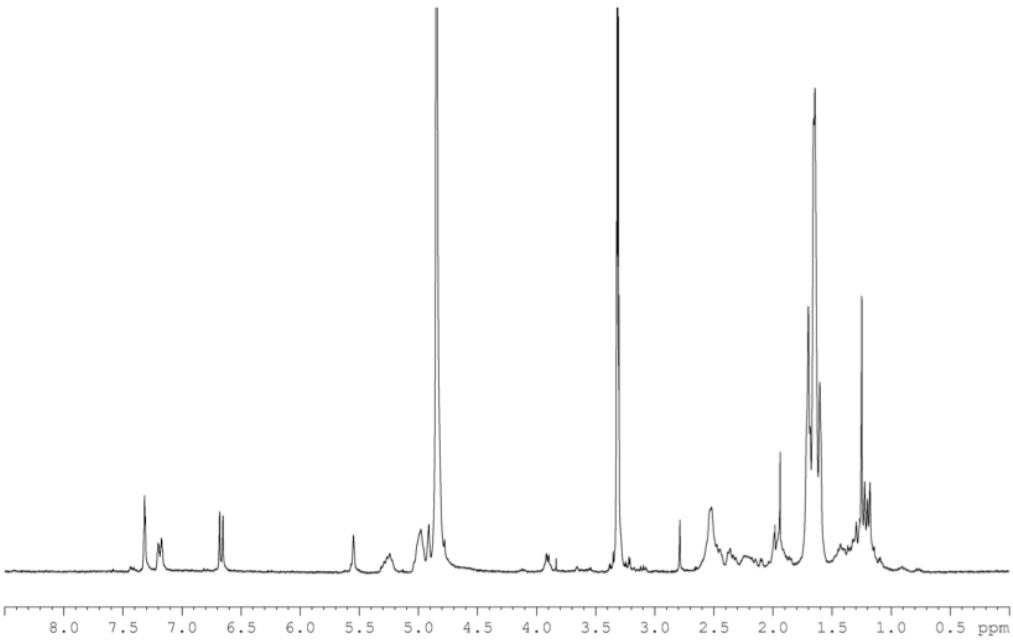


Figure 3.3. ^{1}H spectrum of compound **9** in CD_3OD (300 MHz)

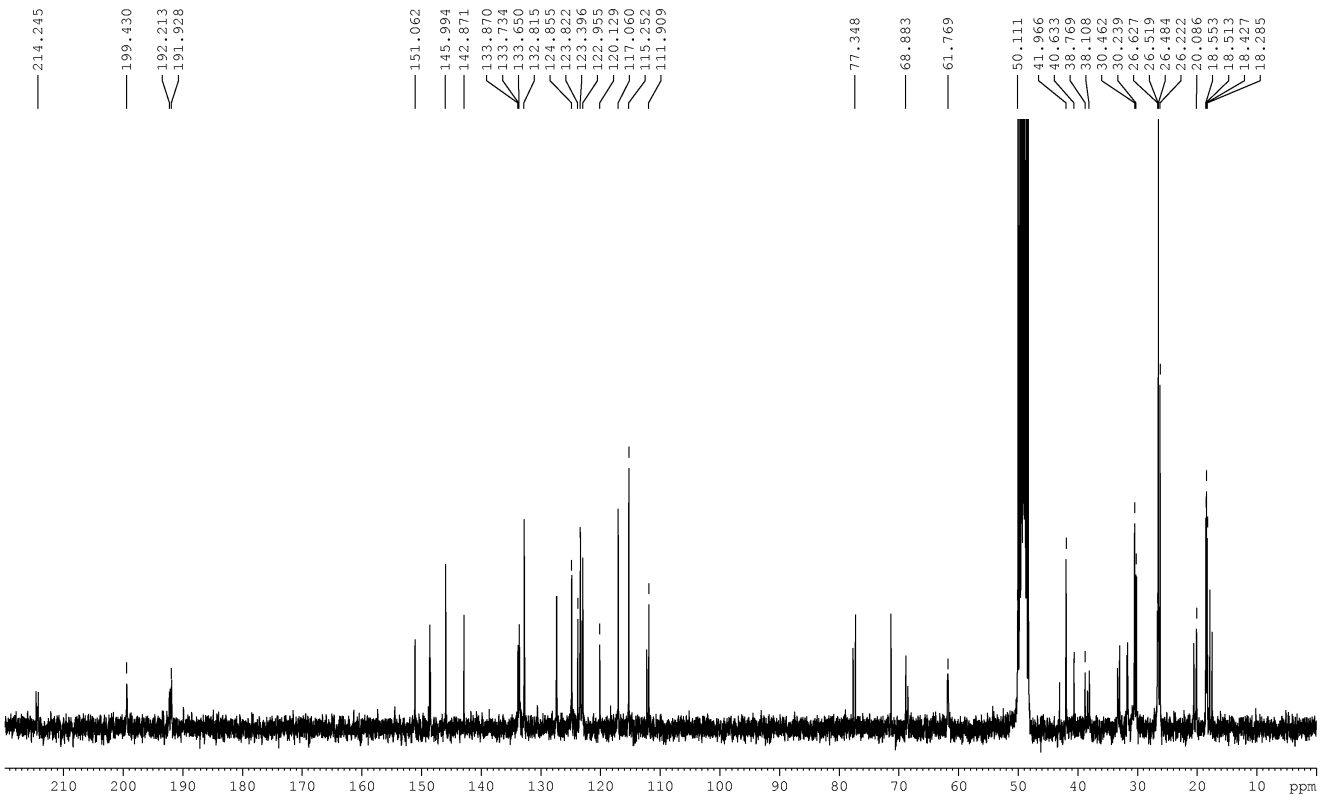


Figure 3.4. ^{13}C spectrum of compound **9** in CD_3OD (300 MHz)

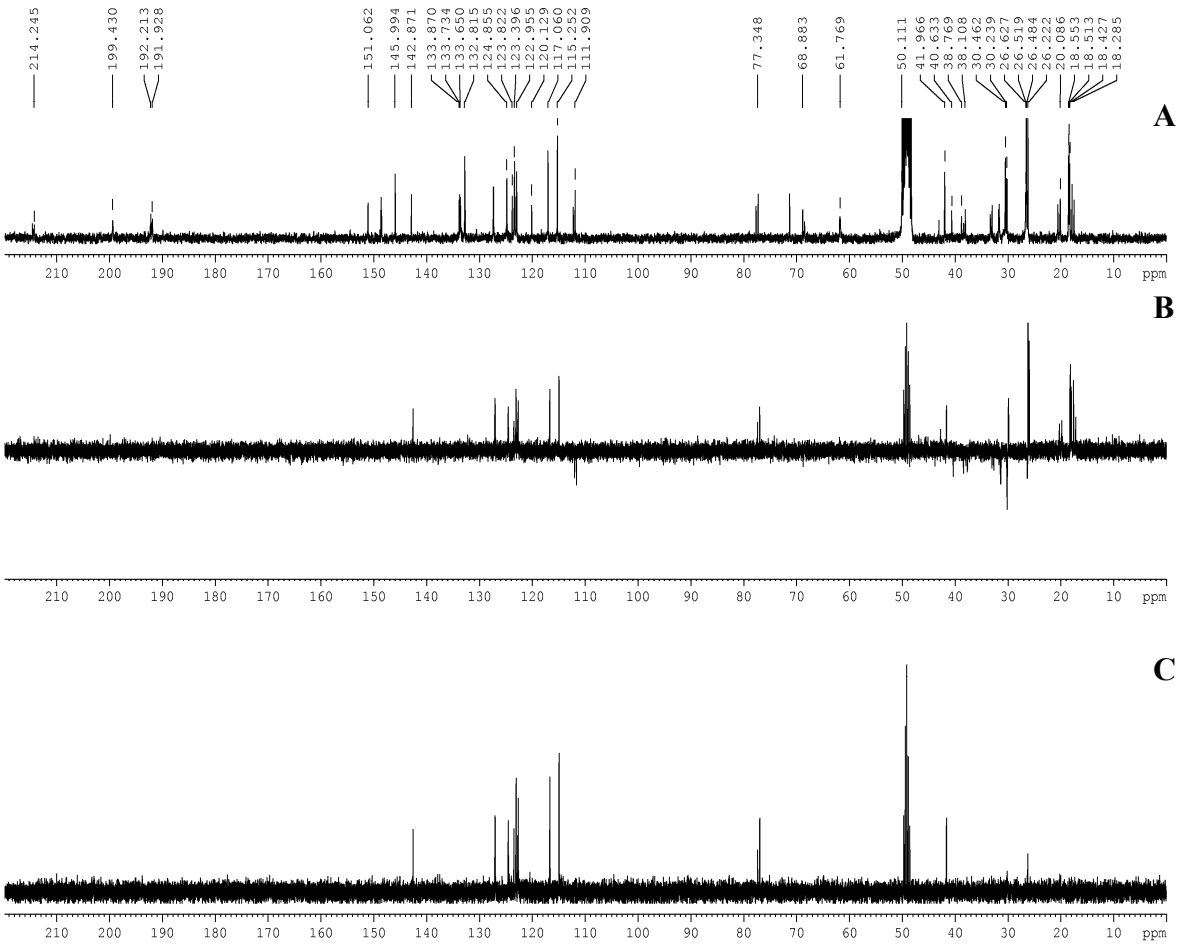


Figure 3.5. Spectra of compound **9** in CD_3OD (300 MHz): **A** ^{13}C spectrum **B** DEPT 135 **C** DEPT 90

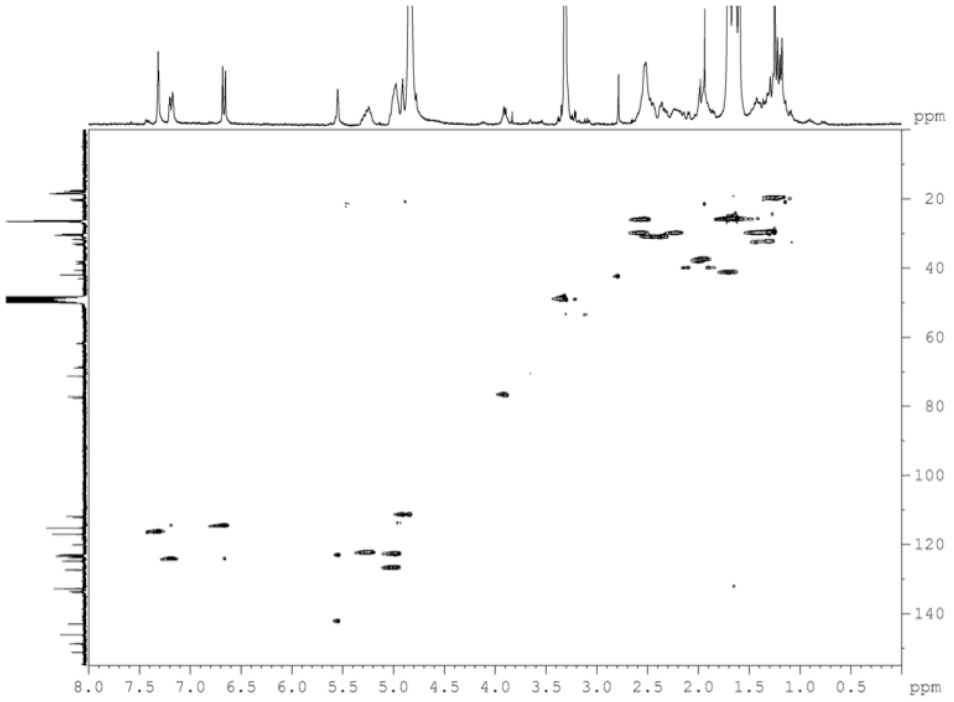


Figure 3.6. HSQC spectrum of compound **9** in CD_3OD (300 MHz)

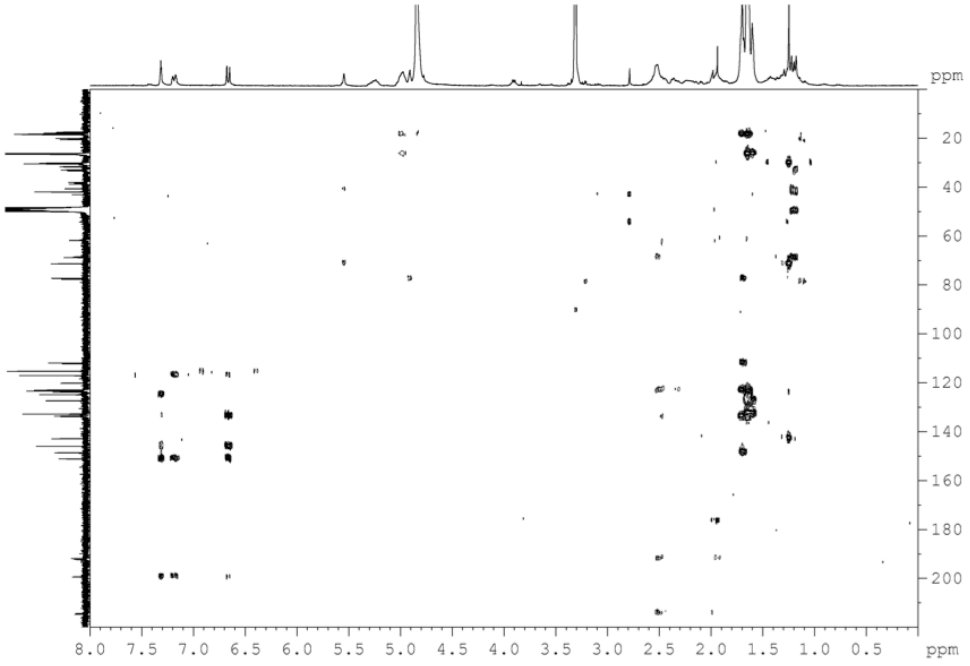


Figure 3.7. HMBC spectrum of compound **9** in CD_3OD (300 MHz)

Chapter 4
Quantitative HPLC-PDA Analysis of Benzophenones and Biflavonoids in Eight *Garcinia* Species

4.1. Introduction

Fruits and vegetables are rich in essential antioxidant nutrients, including the pro-vitamin β-carotene and vitamins C and E. Additional antioxidants, such as polyphenols ingested through the daily diet increase the antioxidant capacity of plasma[1] and are an important source of compounds protective against the damaging effects of free-radicals[2]. The benefits of certain non-nutritional antioxidants such as flavonoids have been evaluated in several epidemiological studies.[3, 4, 5] In particular, it has been shown that a diet rich in fruits and vegetables significantly reduces the incidence and mortality rate of cardiovascular diseases,[6] and a high intake of fruit and vegetables has protective effects against cancer.[7, 8]

The *Garcinia* fruits are used frequently as food and sold in local markets in the tropics. *Garcinia mangostana* L., native to Southeast Asia and commonly called "mangosteen" and the "queen of fruits",[92] is highly regarded among connoisseurs who consider it as one of the best tasting fruits.[89] Mangosteen has been marketed to consumers as a "superfruit" with health-beneficial effects in functional food and in dietary supplements.[93] The nonedible pericarp of *G. mangostana* has been used in Thai traditional medicine to treat wounds, ulcers and dysentery.[94] The fruit of *G. xanthochymus* Hook. f. or "gamboge" are native to India and is used in traditional medicine for treating diarrhea and dysentery.[25] The fruit is traditionally used as jellies or preserve, as a tamarind substitute in cooking and as a yellow dye for fabric and watercolors. Similarly, the fruits of *G. spicata* Hook.f. are native to South India and are traditionally used to dye silk.[95] The fruit of *G. livingstonei* T. Anderson is also called "imbe" and is native to East Africa. It is widely distributed from Durban in South Africa to Somalia and near the valleys of the Zambezi and Limpopo rivers.[96,97] *Garcinia*

intermedia (Pittier) Hammel is the only species of the genus native to Central America. The fruits are used for jellies and preserves.[89] The fruits are acidic and have two to three seeds. The fruits of *G. hombroniana* Pierre are native to Malaysia, commonly called "seashore mangosteen" and are the closest relative of "mangosteen".[12] *Garcinia kola* Heckel is traditionally used by Western African healers, and is commonly used in West Africa as a chewing stick to maintain dental health.[98,99] The fruits of *Garcinia aristata* Borhidi, native to Cuba, are also called the "Cuban mangosteen", and have been found growing in endangered habitats.[64] *Garcinia* species display a high diversity of floral morphology which led to the recognition of several segregated genera; however, a recent taxonomic revision and a phylogenetic studies support a broad circumscription of *Garcinia,* including the earlier segregated genera *Ochrocarpus* Thouars, *Pentaphalagium* Warb., *Rheedia* L., and *Tripetalum* Schumann.[12]

In contrast to polyphenols, which are common in edible fruits from a large number of plant families, the production of polyisoprenylated benzophenones and xanthones is more restricted to families such as Clusiaceae and the Moraceae. In this work the distribution of these constituents is analyzed in fruits of eight species of *Garcinia* by high-performance liquid chromatography with a photo-diode array detector (HPLC-PDA). Antioxidant properties and DPPH activity were tested, and the total phenolic content (TPC) was determined by the Folin-Ciocalteau assay. This is the largest comparative study of benzophenones and biflavonoids in eight *Garcinia* species.

4. 2. Materials and methods

4.2.1. Chemicals

The purity of the standards (Fig. **4.1**) was determined by HPLC and the following values were obtained, xanthochymol = 78.8%, guttiferone E = 79.7%, guttiferone A = 96.0%, fukugetin = 100%, volkensiflavone = 100%, amentoflavone = 99.3%, and fukugeside = 98.1%. The standards, guttiferone E, xanthochymol, guttiferone A, volkensiflavone, fukugetin, and fukugeside (Fig. **4.1**) have been previously isolated and identified.[25] The standard amentoflavone was purchased from ChromaDex (Santa Ana, CA). Ammonium acetate was obtained from Sigma Aldrich. HPLC grade methanol and acetonitrile solvents were obtained from J.T. Baker (Philipsburg, NJ). Buffer was prepared using, deionized water.

4.2.2. Plants

Fruits of *G. xanthochymus*, *G. spicata*, *G. hombroniana*, *G. aristata*, and *G. intermedia* were collected by Margaret J. Basile at the Rare Fruit and Vegetable Council of Broward County (FL) and the Fruit and Spice Park (Homestead, FL). The fruits were shipped overnight on dry ice and frozen, and stored at - 20 ºC in the laboratory until extraction. Voucher specimens were prepared and were deposited at the Lynda Steere Herbarium at the New York Botanical Garden (Bronx, NY). The fruits of *G. mangostana* were purchased in New York City. The chewing sticks of *G. kola* were a donation from the Lewis' collection and had been purchased from local markets in Cameroon.

4.2.3. Methods

Separation and identification were achieved using a Waters 2695 separation module (Milford, MA) and a 2996 photodiode array detector operated with Empower software. Separation was carried out on a Phenomenex Synergi® Hydro RP-18 column

(250 x 2.00 mm id, 4 μm) (Torrance, CA) using a mobile phase and solvent system composed of 10 mM ammonium acetate buffer (A) and acetonitrile (B) at a flow rate of 0.2 ml / min, and a temperature of 30 º C. The gradient profile consisted of three periods (0 - 15 min) 25 - 30 % B; (15 - 30 min) 30 - 51 % B; (30 - 100 min) 51 B %, maintaining isocratic conditions for the last period. Prior to the next sample injection, the column was washed with 100 % B for 10 min before being equilibrated at the initial conditions for 15 minutes. The UVvis spectra were measured from 200 nm to 500 nm. Chromatograms were extracted at λ_{max} = 280 nm. A sample of 2 μl was injected into the column in triplicate (Fig. **4.2**). The retention times are presented in Table **4.1**. Limits of detection (LOD) were determined based on a signal-to-noise ratio of 3:1. The limits of detection was 260 ng/ml for amentoflavone and 330 ng/ml for guttiferone A (Table **4.1**).

4.2.4. Standard dilutions

Stock solutions were prepared by dissolving individual standards in HPLC grade MeOH. The concentrations were xanthochymol, 3.4 mg/ml; guttiferone E, 2.2 mg/ml; 7.2 mg/ml for guttiferone A; 2.7 mg/ml for volkensiflavone; 10.1 mg/ml for fukugetin; 6.5 mg/ml for amentoflavone and 12.7 mg/ml for fukugeside. Stock solutions were then diluted with MeOH to provide ten concentration levels to determine the standard calibration curves. The samples were filtered using Phenex RC 0.45 μm membrane filter (Millipore, Germany) and injected in triplicate into the HPLC.

4.2.5. Sample preparation

The fruits (10.00 g) were lyophilized and dried before being extracted three times in 200 ml MeOH for 1 hour at room temperature. The extracts were then dried *in vacuo* at 40 ºC and then dissolved to 100 ml in a volumetric flask. Aliquots of 2 ml were stored for

each extract in the freezer at – 20º C before HPLC analysis. Samples were filtered through 0.45 μm membrane filters, before injection into the HPLC.

4.2.6. Validation

Recovery tests were performed according to American Association of Analytical Chemists guidelines for single laboratory validation (Section 3.3.2). Accuracy of the method was achieved by determination of the recovery through standard addition. Three different concentrations of amentoflavone (3.1, 1.5, and 0.8 mg/ml) were used to spike 0.50 g of lyophilized *G. spicata* fruit. Three different concentrations of guttiferone A (6.8, 3.4, and 1.7 mg/ml) were used to spike 0.50 g of lyophilized *G. spicata* fruit. The recoveries were determined by subtracting the values obtained from the control (unspiked matrix) from the sample containing added spiked standard, divided by the amounts added. Recovery was 96.2%, 98.7%, and 113.0% for amentoflavone, and for guttiferone A recovery was 96.1%, 98.3%, and 98.7%. Guttiferone A was added at three levels, 150%, 70%, and 30%. The obtained values indicated that MeOH is an acceptable extraction solvent.

4.2.7. Precision

The spiked samples were injected three times and the relative standard deviation values (RSD %) were calculated for the peak area and were considered as a measure of precision. The relative standard deviations (RSD %) from the recovery experiments ranged from 0.07 % - 0.59 %.

4.2.8. DPPH assay

The DPPH antioxidant assay was performed according to procedures described by Smith *et al*.[91] Samples for the experiments (10 mg) were dissolved in 6 ml

dimethylsulfoxide (DMSO) and further diluted to appropriate concentrations (15.6, 31.3, 62.5, 125, and 250 μg/ml) before being transferred to a 96-well microtiter plate. The experiments were performed in duplicate. Each well contained 150 μl ethanolic solution of DPPH (400 μM) and 50 μl of sample solution. Controls were prepared with 150 μl ethanol and DMSO. The microtiter plates were incubated at 37 °C for 30 min and the absorbance was measured at 515 nm using a microplate reader (Molecular Devices Versa$_{max}$). In the experiments, the negative controls, ethanol and DMSO, showed no antioxidant activity. Ascorbic acid was used as a positive control. Percent inhibition by sample exposure was determined by comparison with a DMSO-treated control group.

4.2.9. Total phenolic content

The aliquots of the extracted fruits were used for testing the total phenolic content present in samples. A sample of 100 μl of was added to 1 ml of 10% (v/v) Folin-Ciocalteau reagent incubated for 5 minutes and then 1 ml of 10% $NaCO_3$ was added. The mixture was incubated for 90 minutes before reading absorbance at 765 nm using a microplate reader (Molecular Devices Versa$_{max}$). All aliquots of analyzed samples and gallic acid standards were analyzed in triplicate. Gallic acid equivalents (GAE) were established for 1 gram of dry fruit.

4.2.10. Statistical analysis

Values are averages of three determinations and the standard deviation. The results were analyzed for variation (ANOVA) and statistical significance by Tukey-Kramers test. Software JMP version 8 was used for data analysis. Pearson´s correlation coefficient was calculated for DPPH activity.

4.3. Results and discussion

In our quest to identify novel sources of antioxidant from uncommonly used edible plants, extracts of *Garcinia* species were investigated for their composition and antioxidant properties for the first time, and compared to the well-known related species *G. mangostana*.

The antioxidant activity of seven *Garcinia* fruits and the wood of *G. kola* were tested using the DPPH scavenging assay (Table **4.2**). The antioxidant activity of *Garcinia* extracts was in the following order *G. intermedia* (IC_{50}= 60.1 ± 27.3 μg/ml), and *G. mangostana* (IC_{50}= 64.3 ± 11.3 μg/ml) > *G. livingstonei* (IC_{50}= 108.4 ± 12.9 μg/ml), *Garcinia hombroniana* (IC_{50}= 116.8 ± 66.8 μg/ml), *G. aristata* (IC_{50}= 128.9 ± 13.6 μg/ml), and *G. xanthochymus* (IC_{50}= 152.7 ± 12.1 μg/ml) > *G. kola* (IC_{50}= 205.1 ± 38.8 μg/ml) > *G. spicata* (IC_{50}= 185.3 ± 20.9 μg/ml) (Table **4.2**). The scavenging activities displayed by *G. intermedia* and *G. mangostana* were significantly higher than the other tested *Garcinia* extracts.

The total phenolic content (TPC) *Garcinia* extracts was assessed using the Folin-Ciocalteau method, and the results are shown in Table **4.2**. The TPC decreased in the following order *G. intermedia* (476.9 ± 40.8 GAE per g dry fruit) > *G. hombroniana* (326.9 ± 8.1 GAE per g of dry fruit), *G. xanthochymus* (283.6 ± 65.3 GAE per g of dry fruit), *G. mangostana* (263.3 ± 6.4 GAE per g of dry fruit), *G. kola* (248.3 ± 48.7 GAE per g dry fruit), *G. spicata* (237.6 ± 15.6 GAE per g of dry fruit), and *G. aristata* (237.1 ± 6.3 GAE per g of dry fruit) > *G. livingstonei* (115.5 ± 34.1 GAE per g of dry fruit). *Garcinia intermedia* has the highest amount of TPC compared to other *Garcinia* extracts. Multivariate analysis did not show any correlation between TPC and antioxidant activity

(r = -0.39), indicating that compounds other than the seven polyphenols quantified, contribute significantly to antioxidant activity.[100] However, there was significant correlation between the contents of guttiferone A and TPC (r = 0.78).

This is the first study in which both benzophenones and biflavonoids are quantified simultaneously in eight *Garcinia* species. The amounts of benzophenones and biflavonoids were quantified by HPLC-PDA and a good linearity was achieved over the concentration range presented, the seven calibration curves exhibited linear regressions of at least R^2>0.966. The limits of detection (LOD) and of quantitation (LOQ) were determined under the operational conditions of the method (Table **4.1**).

Polyisoprenylated benzophenones were present in the fruits of *G. spicata*, *G. xanthochymus, G. intermedia, G. livingstonei,* and *G. aristata*. The amounts in mg/g of dry fruit of polyisoprenylated benzophenones and biflavonoid in seven *Garcinia* species are presented in Table **4.3**. The content of xanthochymol decreased in the following order *G. spicata* (70.50 ± 1.10 mg/ g dry weight) > *G. xanthochymus* (65.10 ± 0.03 mg/g dry weight) > *G. intermedia* (2.90 ± 0.20 mg/g dry weight). Previous studies quantifying xanthochymol, found 35.60 ± 3.60 mg/g of present in fruits of *G. indica.*[101] The amount of guttiferone E decreased in the following order, *G. spicata* (50.00 ± 2.10 mg/g dry weight) and *G. xantochymus* (48.10 ± 2.60 mg/g dry weight) > *G. intermedia* (3.50 ± 0.20 mg/g dry weight). The content of guttiferone A decreased in the following order in the fruits; *G. intermedia* (43.00 ± 0.30 mg/g dry weight) > *G. aristata* (31.30± 0.30 mg/g dry weight) > *G. livingstonei* (11.20 ±0.50 mg/g dry weight) > *G. spicata* (4.80 ± 0.07 mg/g dry weight). High amounts of guttiferone A in *Garcinia* fruits correlate with high levels of DPPH activity (r= -0.79).

Among the biflavonoids analyzed, fukugetin decreased in the following order:

G. xanthochymus (12.90 ± 0.70 mg/g dry weight) > *G. spicata* (4.90 ± 0.20 mg/g dry weight) > *G. intermedia* (4.60 ± 0.04 mg/g dry weight) > *G. livingstonei* (2.90 ± 0.10 mg/g dry weight). The content of volkensiflavone decreased in the following order *G. hombroniana* (12.40 ± 0.20 mg/g dry weight) > *G. kola* (5.60 ± 0.10 mg/g dry weight) > *G. xanthochymus* (1.50 ± 0.01 mg/g dry weight) > *G. intermedia* (0.90 ± 0.08 mg/g dry weight) and *G. livingstonei* (0.80 ± 0.10 mg/g dry weight). The content of fukugeside decreased in the following order *G. xanthochymus* (9.90 ± 0.60 mg/g dry weight) > *G. spicata* (5.40 ± 0.10 mg/g dry weight) > *G. intermedia* (3.20 ± 0.20 mg/g dry weight > *G. livingstonei* (1.10 ± 0.06 mg/g dry weight). The content of amentoflavone decreased in the following order *G. spicata* (0.30 ± 0.10 mg/g dry weight) > *G. livingstonei* (0.20 ± 0.03 mg/g dry weight) > *G. xanthochymus* (0.10 ± 0.01 mg/g dry weight).

Garcinia spicata contained all the seven phytochemical constituents. Four biflavonoids were detected in each of the following species: *Garcinia livingstonei*, *G. xanthochymus,* and *G. spicata.* Biflavonoids are known for anti-inflammatory and analgesic effects.[102] *Garcinia intermedia* showed the presence of xanthochymol and gutiferone E, although guttiferone A is the major constituent present in the fruit.

Garcinia mangostana and *G. hombroniana* show significant differences from the other tested *Garcinia* species in their phytochemistry (lacking polyisoprenylated benzophenones in their fruits), morphology (having hard rinds), and biogeography (both native to Southeast Asia). These differences are in agreement with Sweeney, who divides the genus *Garcinia* into two phylogenetic lineages, namely A (containing *G. spicata*, *G. kola*, *G. intermedia*, and *G. livingstonei*) and B (containing *G. mangostana* and *G. hombroniana*) based on morphology and genetic variation.[12] Based on our limited sample set, lineage A produces polyprenylated benzophenones, whereas lineage B does not,

instead making the related compounds xanthones. However, the phytochemistry of additional species in lineages A and B needs to be tested for this hypothesis to be confirmed. In addition, the role of environmental factors on *Garcinia* fruits has not been considered in this study.

In previous studies we have found that guttiferone E displays a higher activity than its double-bond isomer xanthochymol against colon cancer.[25] In this study the separation between the double bond isomers was only achieved on a reversed-phase C_{18} column using a long run-time with a mobile phase composed of ammonium acetate buffer and acetonitrile. This led to a total run time of 100 min. The selectivity factor α for the adjacent bands of the pair of double bond isomers was $\alpha = 1.03$ for guttiferone E, and xanthochymol, but the resolution required isocratic conditions of 49 % A and 51 % B for 70 min. Separation of the structurally related double-bond isomers, guttiferone E and xanthochymol was achieved. The only difference is that guttiferone E has a double bond between carbon C-32 and C-33, while xanthochymol has a terminal double bond between carbon C-33 and C-34 (Fig. **4.1**). Thus the only structural difference is the *sp* - 2 hybridization on C-32 and C-34. Separation of closely related olefinic isomers is challenging and different stationary phases for separation and improvement of the resolution of olefinic isomers of estrogens have previously been evaluated.[103] Low retention time was achieved for early eluting fukugeside and the biflavonoids. The compounds were identified based on retention time and UV absorbance in comparison with individual standards. The seven peaks were identified based on the UV data and absorbance spectra characteristic of polyisoprenylated benzophenones and biflavonoids. The chromatographic method worked for the eight analyzed extracts.

Dietary supplements containing *G. mangostana* are increasingly marketed to consumers with claims of potential health beneficial effects; however, these claims need to be clinically evaluated.[104] The major constituent present in *G. mangostana* is α-mangostin.[93] A recent study has shown that the human plasma antioxidant capacity increases after one oral dose of mangosteen product. The bioavailablity of α-mangostin reaches its maximum concentration in plasma (C_{max}) 3.12 ±1.47 ng /ml at t_{max} of 1 h after consumption of mangosteen dietary product.[105]

Previous studies have shown that *G. mangostana* is a rich source of xanthones, anthocyanins, and oligomeric proanthocyanidins.[92] New xanthone compounds have been isolated from *G. mangostana.*[106] Polyisoprenylated benzophenones were detected in the stem bark and heartwood of *G. mangostana.* [107,108] Studies *in vitro* have shown that the isolated compounds from *G. mangostana* display antioxidant, anti-inflammatory, antimicrobial, and cytotoxic activity.[104] A crude methanolic extract showed antiproliferative effects [104] and recent studies suggest that α-mangostin is a potential cancer chemopreventive compound.[106] Toxicity studies in mice have shown that orally administered *G. mangostana* extract showed LD_{50} = 9.37 g/kg weight.[109]

Garcinia species may be important in the prevention and/or treatment of cancer.[15] For example, *G. xanthochymus* has shown activity against colon cancer cells.[25] There is evidence that suggests that polyisoprenylated benzophenones induce apoptosis and arrest the cell cycle early in G_1 phase, and prevent the transcription and translation of important cell-cycle regulators involved in cell progression in cancerous cells.[38] Thus they arrest the cell cycle and interrupt cell division and inhibit the growth of cancer cells, up-regulating mediators in the ER stress pathway.[38] The mechanism of action and cellular pathways

through which the benzophenones exert chemopreventive effects is still under investigation.

Conclusions

The fruit of *G. intermedia* has significantly higher antioxidant capacity and total phenolic content than the "superfruit" *G. mangostana*. Therefore, a new source of antioxidants has been identified, which might be of great potential to human health. However, further biological studies on the safety and efficacy of the fruit are warranted. The fruits of *G. spicata* and *G. xanthochymus* contain the highest amounts of guttiferone E and xanthochymol. These polyisoprenylated benzophenones are reported to have chemopreventive effects against the early stages of carcinogenesis and thus have health beneficial effects. The HPLC-PDA method used for identification and quantification of polyisoprenylated benzophenones and biflavonoids was sensitive, reproducible and fully validated.

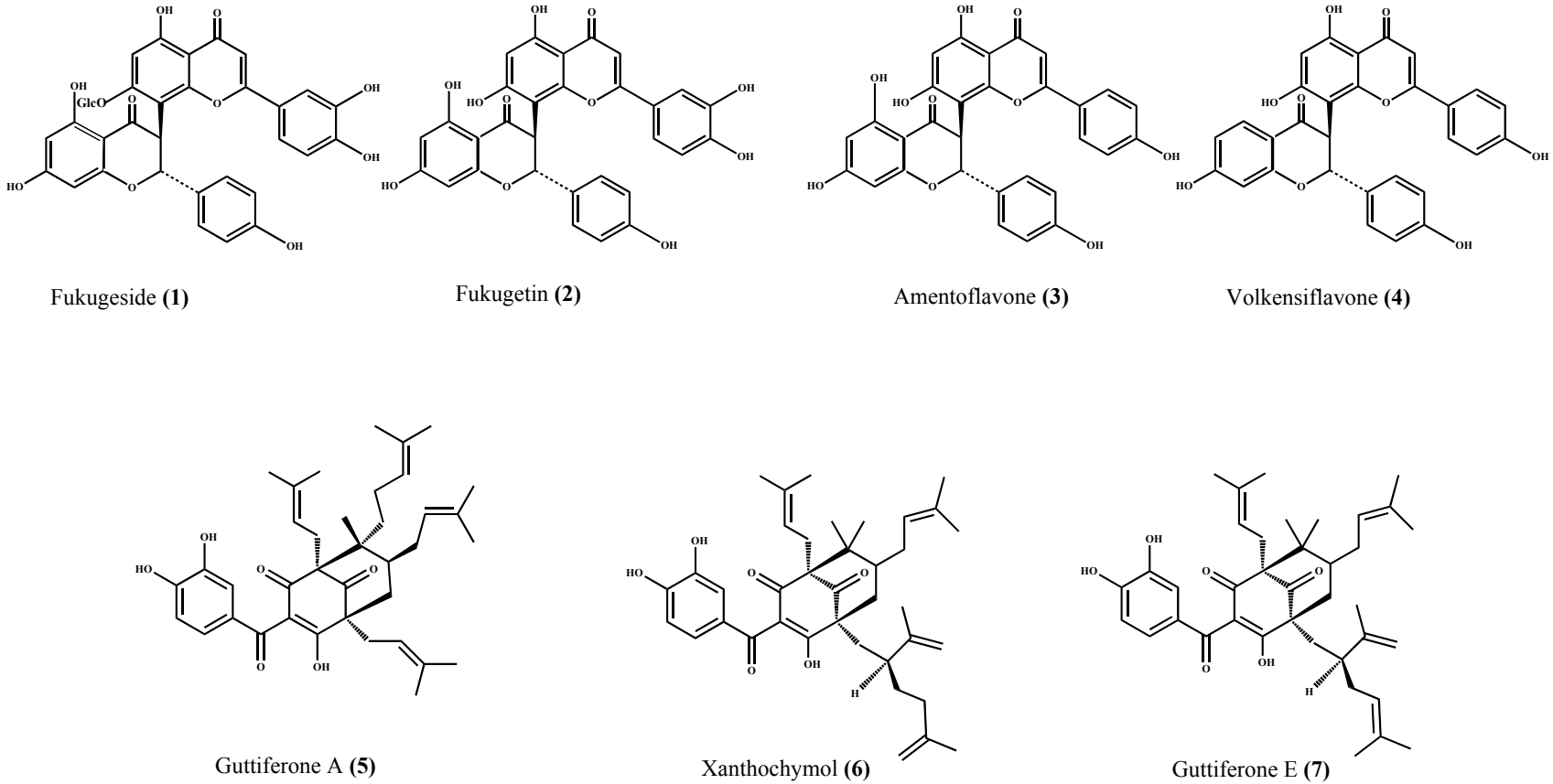


Figure 4.1. Polyisoprenylated benzophenones and biflavonoids quantified in the fruits of seven *Garcinia* species in this study.

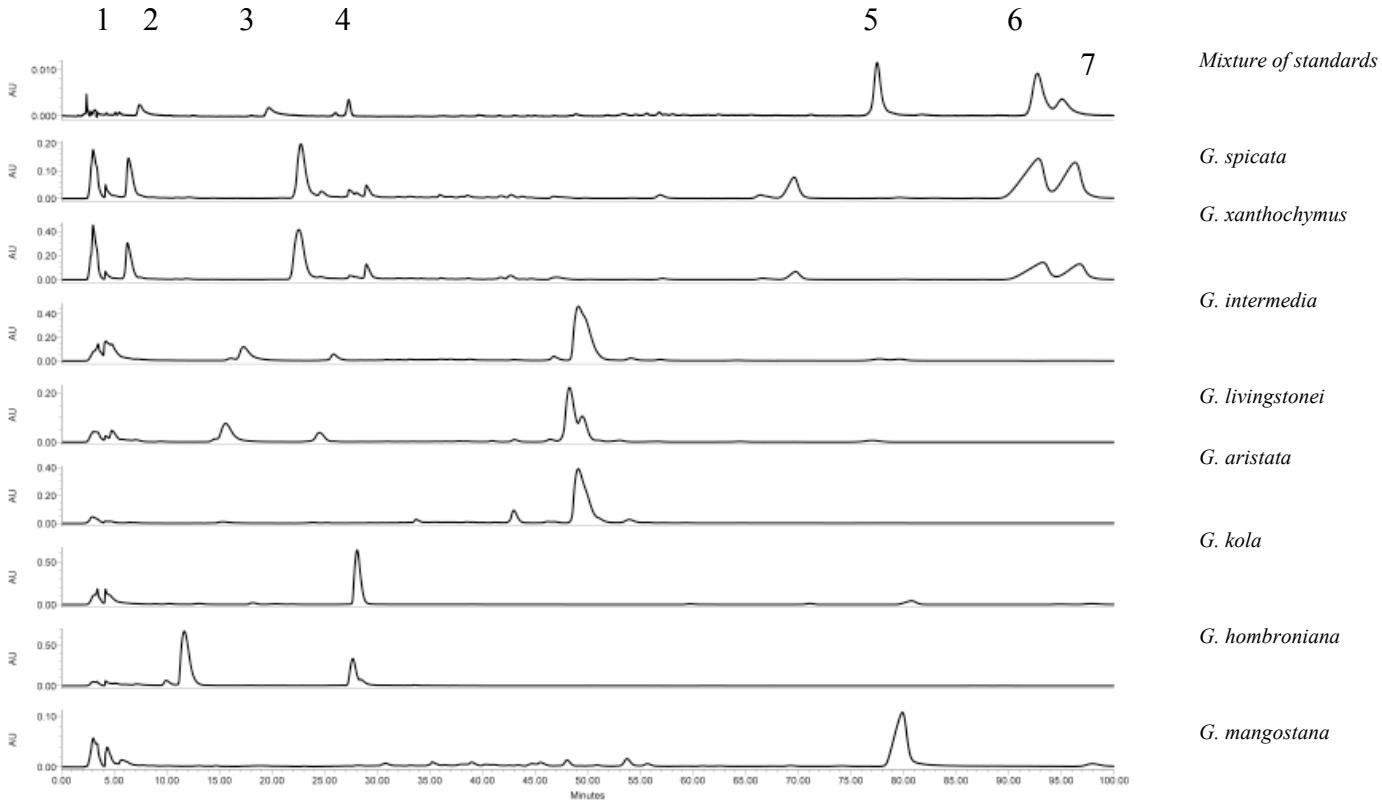


Figure 4.2. Chromatograms of eight *Garcinia* extracts analyzed at 280 nm. **(1)** Fukugeside **(2)** Fukugetin **(3)** Amentoflavone **(4)** Volkensiflavone **(5)** Guttiferone A **(6)** Xanthochymol **(7)** Guttiferone E.

Table 4.1. Concentration range of analyzed standards, equations of the calibration curves and linear regression coefficients.

Analytes			Linear regression data				
	t_R, (min)	UV data (λ, nm)	Regressive equation	Test range (μg ml^{-1})	R^2	LOD (ng ml^{-1})	LOQ (ng ml^{-1})
Fukugeside **(1)**	6	256, 329	$y = 10^7x+355580$	$24.9\text{-}1.3\times10^5$	1.000	720	2397
Fukugetin **(2)**	17	288, 327	$y = 2\times10^7x-10^6$	$19.6\text{-}1.0\times10^5$	0.999	109	363
Amentoflavone **(3)**	20	212, 292	$y = 2\times10^7x+458366$	$12.6\text{-}6.5\times10^3$	0.999	260	867
Volkensiflavone **(4)**	28	212, 292, 328	$y = 2\times10^7x+456891$	$2.6\text{-}2.7\times10^3$	0.998	337	1124
Guttiferone A **(5)**	53	229, 280	$y = 10^7x+870060$	$14.0\text{-}7.2\times10^3$	0.998	330	1100
Xanthochymol **(6)**	90	233, 279	$y = 3\times10^6x+340315$	$6.5\text{-}3.4\times10^3$	0.966	707	2357
Guttiferone E **(7)**	93	229, 279	$y=3\times10^7x+108058$	$6.6\text{-}2.2\times10^3$	0.998	482	1607

Table 4.2. DPPH activity[a] and total phenolic content[a] (TPC) in eight *Garcinia* species

Sample	DPPH (μg/ml)	TPC (GAE/g)
G. mangostana	64.3±11.3[a]	263.3±6.4[a]
G. hombroniana	116.8±66.8[ab]	326.9±8.1[a]
G. livingstonei	108.4±12.9[ab]	115.5±34.1[b]
G. xanthochymus	152.7±12.1[ab]	283.6±65.3[a]
G. intermedia	60.1±27.3[a]	476.87±40.8[c]
G. aristata	128.9±13.6[ab]	237.1±6.3[a]
G. spicata	185.3±20.9[c]	237.6±15.6[a]
G. kola	205.1±38.8[b]	248.3±48.7[a]

[a] Values are expressed as means ± SD of three replicate analysis.
Means in the same column with different letters are not significantly different ($p< 0.05$)

Table 4.3. Benzophenones and biflavonoids (mg/gram of dry weight) contents of eight G*arcinia* fruits and *G.kola* wood species.[a]

	Fukugeside **(1)**	Fukugetin **(2)**	Amentoflavone **(3)**	Volkensiflavone **(4)**	Guttiferone A **(5)**	Xanthochymol **(6)**	Guttiferone E **(7)**
G. spicata	5.40±0.10[a]	4.90±0.20[a]	0.30±0.10[a]	0.40±0.01[a]	4.80±0.07[a]	70.50±1.10[a]	50.00±2.10[a]
G. xanthochymus	9.90±0.60[b]	12.90±0.70[b]	0.10±0.01[b]	1.50±0.01[b]	nd	65.10±0.03[b]	48.10±2.60[a]
G. intermedia	3.20±0.20[c]	4.60±0.04[c]	nd	0.90±0.08[c]	43.00±0.30[b]	2.90±0.20[c]	3.50±0.20[b]
G. livingstonei	1.10±0.06[d]	2.90±0.10[d]	0.20±0.03[a]	0.80±0.10[c]	11.20±0.50[c]	nd	nd
G. aristata	nd	nd	nd	nd	31.30±0.30[d]	nd	nd
G. hombroniana	nd	nd	nd	12.40±0.20[d]	nd	nd	nd
G. kola	nd	nd	nd	5.60±0.10[e]	nd	nd	nd
G. mangostana	nd	nd	nd	nd	nd	nd	nd

[a] Values are expressed as means ± SD of three replicate analysis. Means in the same column with different letters are significantly different ($p< 0.05$)
nd= not detected

Chapter 5
Conclusions

By high performance liquid chromatography photo-diode array (HPLC-PDA) we have quantified the amounts of three major bioactive benzophenones, guttiferone A, guttiferone E, and xanthochymol, in seven species of *Garcinia* fruits, including the "superfruit" *Garcinia mangostana*. In addition, four biflavonoids were quantified: amentoflavone, fukugeside, fukugetin, and volkensiflavone. The quantitative HPLC method was validated with respect to linearity, accuracy, recovery, and precision. The amounts of benzophenones and biflavonoids were quantified by HPLC-PDA and a good linearity was achieved over the concentration range presented, the seven calibration curves exhibited linear regressions of at least $R^2 > 0.966$. The limit of detection LOD was determined. The antioxidant activity of the *Garcinia* fruits and the wood of *G. kola* were tested. The highest antioxidant activity was displayed by *G. intermedia* (IC_{50}= 60.1 ± 27.3 μg/ml) and *G. mangostana* (IC_{50}= 64.3 ± 27.3 μg/ml). In comparison, the wood of *G. kola* showed the lowest antioxidant activity (IC_{50}=205.1 ± 38.8 μg/ml) in this study.

The total phenolic content was 476.9 ± 40.8 GAE per gram dry fruit for *G. intermedia*. This value was followed by followed by *G. hombroniana* which showed a TPC of 326.9 ± 8.1 GAE per gram of dry fruit. "The superfruit" *G. mangostana* had a TPC of 263.3 ± 6.4 GAE per gram of dry fruit. *G. kola* had 248.3 ± 48.7 GAE per gram of dry fruit. We have found that the tropical fruits of *G. intermedia*, *G. mangostana,* and *G. hombroniana* display high antioxidant activity and contain a high level of total phenolic compounds. The highest amounts of guttiferone E and xanthochymol were found in both *G. spicata* and *G. xanthochymol*. Highest amount of guttiferone E was found in *G. spicata* (50.0 ± 2.1 mg/g dry weight). In this study xanthochymol was present

in three of the analyzed fruits and quantified. The highest amount was detected in *G. spicata* (70.5 ± 1.1 mg/g dry weight). Guttiferone A was found at the highest levels in *G. intermedia* (43.0 ± 0.3 mg/g dry weight), followed by *G. aristata* (31.3 ± 0.3 mg/g dry weight), while the lowest levels of guttiferone A were observed in *G. livingstonei* (11.2 ± 0.5 mg/g dry weight).

We have found that the fruits of *G. intermedia* (IC_{50}= 60.1 ± 27.3 μg/ml) have higher antioxidant capacity than *G. mangostana* (IC_{50}= 64.3 ± 11.3 μg/ml) and thus we have identified a new sustainable source of compounds with higher antioxidant activity than the "superfruit" mangosteen.

Overall the chemical profile and the content of polyisoprenylated benzophenones are different among species of *Garcinia* fruits. The fruits with a hard pericarp do not show the presence of polyisoprenylated benzophenones. Both the fruit extracts of *G. mangostana* and *G. intermedia*, displayed high content of total phenolic content and high antioxidant capacity. Noteworthy is the fact that the Central American species *G. intermedia* showed higher antioxidant activity than the "superfruit" *G. mangostana.*

In the fruits of *Garcinia intermedia* the following known compounds were identified: guttiferone A, guttiferone E, isoxanthochymol, xanthochymol, aristophenone, maclurin, fukugetin, and volkensiflavone. One new benzophenone isomer of *m/z* 618 was isolated and tentatively identified using 1D and 2D NMR experiments.

The antioxidant activity for guttiferone A (IC_{50}= 46 μM) is comparable to gallic acid (IC_{50}= 35 μM). Guttiferone A, a major constituent in *G. intermedia,* showed antiproliferative effects (9 μg/ml). Further it did not only enhance the effects of the chemopreventive agent sulindac sulfide against colon cancer HT-29 cells but also showed

strong synergistic activity in combination with the chemopreventive agent. Guttiferone A is a possible new lead compound for a potentially new chemopreventive agent of colon cancer.

Many new polyisoprenylated benzophenones with a bicyclo[3.3.1]-nonane-2,4,9-trione core structure have been isolated from plants in the Clusiaceae family, and their potent biological properties have been the subject of several studies. The cellular target through which guttiferone A exerts antiproliferative effects, and interrupts the cell cycle of cancer cells, needs to be investigated. The target of guttiferone A in colon caner cells needs to be identified, in order to be able to produce effective benzophenone analogues for chemoprevention. It is possible that guttiferone A affects the inflammatory mediation during the initiation of carcinogenesis, having an effect on the inducible enzymes iNOS and COX-2. This may be the mechanism of action through which polyisoprenylated benzophenones exert their antiproliferative effects *in vitro*. Evidence presented by independent investigators suggests that polyisoprenylated benzophenones affect the mediators in the Akt/mTOR stress pathway, although the specific target remains to be discovered. In addition, benzophenones isolated from plants display high antioxidant capacity and protect cells from oxidative stress, and the formation of ROS involved during the inflammatory process.

There is clinical evidence suggesting that breast cancer can be prevented in women with relative high risk of developing the disease, by means of pharmacological treatment and chemoprevention.[110] This shows that pharmacologic treatment, with the purpose of arresting and/or reversing the process of carcinogenesis, is a potential alternative for future efficient prevention of cancer. [111] During the early stages of colon

carcinogenesis there is over-expression of the cyclooxygenase-2 (COX-2). The mediators in the inflammatory response are up regulated in the initial stages of carcinogenesis in colon cancer cells. It is possible that guttiferone A also suppresses the inflammatory response during the initiation of carcinogenesis.

New approaches to control the disease are critically needed, and the prevention of the initiation of cancerogenesis is essential for the reduction in the incidence of the disease. New classes of compounds with chemopreventive effects, with the potential of being developed into lead compounds to prevent cancer are needed for future efficacious pharmacological treatment. Moreover, the discovery of a putative target might lead to more effective treatments against cancer, and possibly find an alternative approach through which cancer can be prevented.

Fruits of *Garcinia* plants are a rich dietary source of phenolic compounds. These findings suggest that fruits of *G. intermedia* which are rich in guttiferone A, might prevent colon cancer, and that the consumption of the fruit might have potential chemopreventive effects. *Garcinia* fruits are a rich dietary source of new phenolic compounds, particularly polyisoprenylated benzophenones high in antioxidant capacity. The polyisoprenylated benzophenones might prevent the damaging effects of free radicals involved in the etiology of atherosclerosis and other inflammatory processes. In addition, these compounds have shown antiproliferative effects. The phenolic compounds in *Garcinia* fruits have antioxidant activity and antiproliferative effects and may possibly prevent certain types of cancer.

BIBLIOGRAPHY

(1) Scalbert, A.; Williamson, G. Dietary Intake and Bioavailability of Polyphenols. *J. Nutr.* **2000**, *130*, 2073S-2085S.

(2) Cao, G.; Sofic, E.; Prior, R. Antioxidant Capacity of Tea and Common Vegetables. *J. Agric. Food Chem* **1996**, *44*, 3426-3431.

(3) Hertog, M. G.; Kromhout, D.; Aravanis, C. Flavonoid Intake and Long-Term Risk of Coronary Heart Disease and Cancer in the Seven Countries Study. *Arch. Intern. Med.* **1995**, *155*, 381-248.

(4) Hollman, P. C.; Katan, M. B. Bioavailability and Health Effects of Dietary Flavonols in Man. *Arch. Toxicol. Suppl.* **1998**, *20*, 237-248.

(5) Kelly, F. J. Use of Antioxidants in the Prevention and Treatment of Disease. *J. Int. Fed. Clin. Chem.* **1998**, *10*, 21-23.

(6) Hertog, M. G.; Feskens, E. J.; Hollman, P. C.; Katan, M. B.; Kromhout, D. Dietary Antioxidant Flavonoids and Risk of Coronary Heart Disease: The Zutphen Elderly Study. *Lancet* **1993**, *342*, 1007-1011.

(7) Mills, P. K.; Beeson, W. L.; Phillips, R. L.; Fraser, G. E. Dietary Habits and Breast Cancer Incidence among Seventh-Day Adventists. *Cancer* **1989**, *64*, 582-590.

(8) Key, T. J.; Thorogood, M.; Appleby, P. N.; Burr, M. L. Dietary Habits and Mortality in 11,000 Vegetarians and Health Conscious People: Results of a 17 Year Follow Up. *Br. Med. J.* **1996**, *313*, 775-779.

(9) Pietta, P. G. Flavonoids as Antioxidants. *J. Nat. Prod.* **2000**, *63*, 1035-1042.

(10) Smith, N.; Mori, S. A.; Henderson, A.; Stevenson, D. W.; Heald, S. V. Flowering Plants of the Neotropics, New Jersey: Princeton University Press; 2004.

(11) Gustafsson, M. H. G.; Bittrich, V.; Stevens, P. F. Phylogeny of Clusiaceae Based on Rbcl Sequences. *Int. J. Plant Sci.* **2002**, *163*, 1045-1054.

(12) Sweeney, P. W. Phylogeny and Floral Diversity in the Genus *Garcinia* (Clusiaceae) and Relatives. *Int. J. Plant Sci.* **2008**, *169*, 1288-1303.

(13) Hay, A. E.; Aumond, M. C.; Mallet, S.; Dumontet, V.; Litaudon, M.; Rondeau, D.; Richomme, P. Antioxidant Xanthones from *Garcinia vieillardii*. *J. Nat. Prod.* **2004**, *67*, 707-709.

(14) Wang, J. F.; Schramm, D. D.; Holt, R. R.; Ensunsa, J. L.; Fraga, C. G.; Schmitz, H. H.; Keen, C. L. A Dose-Response Effect from Chocolate Consumption on Plasma Epicatechin and Oxidative Damage. *J. Nutr.* **2000**, *130*, 2115S-2119S.

(15) Acuña, U. M.; Jancovski, N.; Kennelly, E. J. Polyisoprenylated Benzophenones from Clusiaceae: Potential Drugs and Lead Compounds. *Curr. Top. Med. Chem.* **2009**, *9*, 1560-1580.

(16) Newman, D. J.; Cragg, G. M. Natural Products as Sources of New Drugs over the Last 25 Years. *J. Nat. Prod.* **2007**, *70*, 461-477.

(17) Gulick, R. M.; McAuliffe, V.; Holden-Wiltse, J.; Crumpacker, C.; Liebes, L.; Stein, D. S.; Meehan, P.; Hussey, S.; Forcht, J.; Valentine, F. T. Phase I Studies of Hypericin, the Active Compound in St. John's Wort, as an Antiretroviral Agent in HIV-Infected Adults. Aids Clinical Trials Group Protocols 150 and 258. *Ann. Intern. Med.* **1999**, *130*, 510-514.

(18) Beerhues, L. Benzophenone Synthase from Cultured Cells of *Centaurium erythraea*. *FEBS Lett.* **1996**, *383*, 264-266.

(19) McCandlish, L. E.; Hanson, J. C.; Stout, G. H. The Structures of Two Derivatives of Bicyclo[3.3.1]Nonane-2,4,9-Trione. A Natural Product: Clusianone, $C_{33}H_{42}O_4$, and Trimethylated Catechinic Acid, $C_{18}H_{20}O_6$. *Acta Crystallogr.* **1976**, *B32*, 1793-1801.

(20) Kumar, S.; Chattopadhyay, S. K.; Darokar, M. P.; Garg, A.; Khanuja, S. P. Cytotoxic Activities of Xanthochymol and Isoxanthochymol Substantiated by LC-MS/MS. *Planta Med.* **2007**, *73*, 1452-1456.

(21) Kuete, V.; Komguem, J.; Beng, V. P.; Meli, A. L.; Tangmouo, J. G.; Etoa, F. X.; Lontsi, D. Antimicrobial Components of the Methanolic Extract from the Stem Bark of *Garcinia smeathmannii* Oliver (Clusiaceae). *S. Afr. J. Bot.* **2007**, *73*, 347-354.

(22) Xiao, Z. Y.; Mu, Q.; Shiu, W. K.; Zeng, Y. H.; Gibbons, S. Polyisoprenylated Benzoylphloroglucinol Derivatives from *Hypericum sampsonii*. *J. Nat. Prod.* **2007**, *70*, 1779-1782.

(23) Fuller, R. W.; Blunt, J. W.; Boswell, J. L.; Cardellina, J. H., 2nd; Boyd, M. R. Guttiferone F, the First Prenylated Benzophenone from *Allanblackia stuhlmannii*. *J. Nat. Prod.* **1999**, *62*, 130-132.

(24) Liao, C. H.; Ho, C. T.; Lin, J. K. Effects of Garcinol on Free Radical Generation and No Production in Embryonic Rat Cortical Neurons and Astrocytes. *Biochem. Biophys. Res. Commun.* **2005**, *329*, 1306-1314.

(25) Baggett, S.; Protiva, P.; Mazzola, E. P.; Yang, H.; Ressler, E. T.; Basile, M. J.; Weinstein, I. B.; Kennelly, E. J. Bioactive Benzophenones from *Garcinia xanthochymus* Fruits. *J. Nat. Prod.* **2005**, *68*, 354-360.

(26) Yamaguchi, F.; Saito, M.; Ariga, T.; Yoshimura, Y.; Nakazawa, H. Free Radical Scavenging Activity and Antiulcer Activity of Garcinol from *Garcinia indica* Fruit Rind. *J. Agric. Food Chem.* **2000**, *48*, 2320-2325.

(27) Hong, J.; Kwon, S. J.; Sang, S.; Ju, J.; Zhou, J. N.; Ho, C. T.; Huang, M. T.; Yang, C. S. Effects of Garcinol and Its Derivatives on Intestinal Cell Growth: Inhibitory Effects and Autoxidation-Dependent Growth-Stimulatory Effects. *Free Radic. Biol. Med.* **2007**, *42*, 1211-1221.

(28) Wu, C. C.; Weng, J. R.; Won, S. J.; Lin, C. N. Constituents of the Pericarp of *Garcinia subelliptica*. *J. Nat. Prod.* **2005**, *68*, 1125-1127.

(29) Pecchio, M.; Solís, P. N.; López-Pérez, J. L.; Vásquez, Y.; Rodríguez, N.; Olmedo, D.; Correa, M.; San Feliciano, A.; Gupta, M. P. Cytotoxic and Antimicrobial Benzophenones from the Leaves of *Tovomita longifolia*. *J. Nat. Prod.* **2006**, *69*, 410-413.

(30) Tanaka, N.; Takaishi, Y.; Shikishima, Y.; Nakanishi, Y.; Bastow, K.; Lee, K. H.; Honda, G.; Ito, M.; Takeda, Y.; Kodzhimatov, O. K.; Ashurmetov, O. Prenylated Benzophenones and Xanthones from *Hypericum scabrum*. *J. Nat. Prod.* **2004**, *67*, 1870-1875.

(31) Chaturvedula, V. S.; Schilling, J. K.; Kingston, D. G. New Cytotoxic Coumarins and Prenylated Benzophenone Derivatives from the Bark of *Ochrocarpos punctatus* from the Madagascar Rainforest. *J. Nat. Prod.* **2002**, *65*, 965-972.

(32) Cao, S.; Brodie, P. J.; Miller, J. S.; Ratovoson, F.; Birkinshaw, C.; Randrianasolo, S.; Rakotobe, E.; Rasamison, V. E.; Kingston, D. G. I. Guttiferones K and L, Antiproliferative Compounds of *Rheedia calcicola* from the Madagascar Rain Forest. *J. Nat. Prod.* **2007**, *70*, 686-688.

(33) Williams, R. B.; Hoch, J.; Glass, T. E.; Evans, R.; Miller, J. S.; Wisse, J. H.; Kingston, D. G. A Novel Cytotoxic Guttiferone Analogue from *Garcinia macrophylla* from the Suriname Rainforest. *Planta Med.* **2003**, *69*, 864-866.

(34) Roux, D.; Hadi, H. A.; Thoret, S.; Guenard, D.; Thoison, O.; Paies, M.; Sevenet, T. Structure-Activity Relationship of Polyisoprenyl Benzophenones from *Garcinia pyrifera* on the Tubulin/Microtubule System. *J. Nat. Prod.* **2000**, *63*, 1070-1076.

(35) Seo, E. K.; Wani, M. C.; Wall, M. E.; Navarro, H.; Mukherjee, R.; Farnsworth, N. R.; Kinghorn, A. D. New Bioactive Aromatic Compounds from *Vismia guianensis*. *Phytochemistry* **2000**, *55*, 35-42.

(36) Diaz-Carballo, D.; Malak, S.; Freistuhler, M.; Elmaagacli, A.; Bardenheuer, W.; Reusch, H. P. Nemorosone Blocks Proliferation and Induces Apoptosis in Leukemia Cells. *Int. J. Clin. Pharmacol. Ther.* **2008**, *46*, 428-439.

(37) Matsumoto, K.; Akao, Y.; Kobayashi, E.; Ito, T.; Ohguchi, K.; Tanaka, T.; Iinuma, M.; Nozawa, Y. Cytotoxic Benzophenone Derivatives from *Garcinia* Species Display a Strong Apoptosis-Inducing Effect against Human Leukemia Cell Lines. *Biol. Pharm. Bull.* **2003**, *26*, 569-571.

(38) Protiva, P.; Hopkins, M. E.; Baggett, S.; Yang, H.; Lipkin, M.; Holt, P. R.; Kennelly, E. J.; Bernard, W. I. Growth Inhibition of Colon Cancer Cells by Polyisoprenylated Benzophenones Is Associated with Induction of the Endoplasmic Reticulum Response. *Int. J. Cancer* **2008**, *123*, 687-694.

(39) Balasubramanyam, K.; Altaf, M.; Varier, R. A.; Swaminathan, V.; Ravindran, A.; Sadhale, P. P.; Kundu, T. K. Polyisoprenylated Benzophenone, Garcinol, a Natural

Histone Acetyltransferase Inhibitor, Represses Chromatin Transcription and Alters Global Gene Expression. *J. Biol. Chem.* **2004**, *279*, 33716-33726.

(40) Martins, F. T.; Assis, D. M.; Dos Santos, M. H.; Camps, I.; Veloso, M. P.; Juliano, M. A.; Alves, L. C.; Doriguetto, A. C. Natural Polyprenylated Benzophenones Inhibiting Cysteine and Serine Proteases. *Eur. J. Med. Chem.* **2008**, *44*, 1-10.

(41) Wu, C. C.; Lu, Y. H.; Wei, B. L.; Yang, S. C.; Won, S. J.; Lin, C. N. Phloroglucinols with Prooxidant Activity from *Garcinia subelliptica*. *J. Nat. Prod.* **2008**, *71*, 246-250.

(42) Hong, J.; Kwon Seok, J.; Sang, S.; Ju, J.; Zhou, J.-N.; Ho, C.-T.; Huang, M.-T.; Yang Chung, S. Effects of Garcinol and Its Derivatives on Intestinal Cell Growth: Inhibitory Effects and Autoxidation-Dependent Growth-Stimulatory Effects. *Free Radic. Biol. Med.* **2007**, *42*, 1211-1221.

(43) Pan, M. H.; Chang, W. L.; Lin-Shiau, S. Y.; Ho, C. T.; Lin, J. K. Induction of Apoptosis by Garcinol and Curcumin through Cytochrome C Release and Activation of Caspases in Human Leukemia Hl-60 Cells. *J. Agric. Food Chem.* **2001**, *49*, 1464-1474.

(44) Márquez Hernández, I.; Campo Fernandez, M.; Cuesta-Rubio, O.; Piccinelli, A. L.; Rastrelli, L. Polyprenylated Benzophenone Derivatives from Cuban Propolis. *J. Nat. Prod.* **2005**, *68*, 931-934.

(45) Hou, A.; Fukai, T.; Shimazaki, M.; Sakagami, H.; Sun, H.; Nomura, T. Benzophenones and Xanthones with Isoprenoid Groups from *Cudrania cochinchinensis*. *J. Nat. Prod.* **2001**, *64*, 65-70.

(46) Li, E.; Jiang, L.; Guo, L.; Zhang, H.; Che, Y. Pestalachlorides A-C, Antifungal Metabolites from the Plant Endophytic Fungus *Pestalotiopsis adusta*. *Bioorg. Med. Chem.* **2008**, *16*, 7894-7899.

(47) Merza, J.; Aumond, M. C.; Rondeau, D.; Dumontet, V.; Le Ray, A. M.; Seraphin, D.; Richomme, P. Prenylated Xanthones and Tocotrienols from *Garcinia virgata*. *Phytochemistry* **2004**, *65*, 2915-2920.

(48) Kumar, S.; Chattopadhyay, S. K. High-Performance Liquid Chromatography and LC-ESI-MS Method for the Identification and Quantification of Two Biologically Active Polyisoprenylated Benzophenones Xanthochymol and Isoxanthochymol in Different Parts of *Garcinia indica*. *Biomed. Chromatogr.* **2007**, *21*, 139-163.

(49) Xu, G.; Feng, C.; Zhou, Y.; Han, Q. B.; Qiao, C. F.; Huang, S. X.; Chang, D. C.; Zhao, Q. S.; Luo, K. Q.; Xu, H. X. Bioassay and Ultraperformance Liquid Chromatography/Mass Spectrometry Guided Isolation of Apoptosis-Inducing Benzophenones and Xanthone from the Pericarp of *Garcinia yunnanensis* Hu. *J. Agric. Food Chem.* **2008**, *56*, 11144-11150.

(50) Seo, E. K.; Wall, M. E.; Wani, M. C.; Navarro, H.; Mukherjee, R.; Farnsworth, N. R.; Kinghorn, A. D. Cytotoxic Constituents from the Roots of *Tovomita brevistaminea*. *Phytochemistry* **1999**, *52*, 669-674.

(51) Krishnamurthy, N.; Lewis, Y. S.; Ravindranath, B. On the Structure of Garcinol, Isogarcinol, and Camboginol. *Tetrahedron Lett.* **1981**, *22*, 793-796.

(52) 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. *J. Nat. Prod.* **2003**, *66*, 206-209.

(53) Sang, S.; Pan, M.-H.; Cheng, X.; Bai, N.; Stark, R. E.; Rosen, R. T.; Lin-Shiau, S.-Y.; Lin, J.-K.; Ho, C.-T. Chemical Studies on Antioxidant Mechanism of Garcinol: Analysis of Radical Reaction Products of Garcinol and Their Antitumor Activities. *Tetrahedron* **2001**, *57*, 9931-9938.

(54) Yu, S.; Shen, G.; Khor, T. O.; Kim, J. H.; Kong, A. N. Curcumin Inhibits Akt/Mammalian Target of Rapamycin Signaling through Protein Phosphatase-Dependent Mechanism. *Mol. Cancer Ther.* **2008**, *7*, 2609-2620.

(55) Lin, H. Y.; Chen, C. S.; Lin, S. P.; Weng, J. R. Targeting Histone Deacetylase in Cancer Therapy. *Med. Res. Rev.* **2006**, *26*, 397-413.

(56) Martins, F. T.; Cruz, J. W., Jr.; Derogis, P. B. M. C.; dos Santos, M. H.; Veloso, M. P.; Ellena, J.; Doriguetto, A. C. Natural Polyprenylated Benzophenones: Keto-Enol Tautomerism and Stereochemistry. *J. Braz. Chem. Soc.* **2007**, *18*, 1515-1523.

(57) Kumazawa, E.; Hirotani, K.; Burford, S. C.; Kawagoe, K.; Miwa, T.; Mitsui, I.; Ejima, A. Synthesis and Antitumor Activity of Novel Benzophenone Derivatives. *Chem. Pharm. Bull* **1997**, *45*, 1470-1474.

(58) Tanaka, T.; Kohno, H.; Shimada, R.; Kagami, S.; Yamaguchi, F.; Kataoka, S.; Ariga, T.; Murakami, A.; Koshimizu, K.; Ohigashi, H. Prevention of Colonic Aberrant Crypt Foci by Dietary Feeding of Garcinol in Male F344 Rats. *Carcinogenesis* **2000**, *21*, 1183-1189.

(59) Iinuma, M.; Tosa, H.; Tanaka, T.; Asai, F.; Kobayashi, Y.; Shimano, R.; Miyauchi, K. Antibacterial Activity of Xanthones from Guttiferaeous Plants against Methicillin-Resistant *Staphylococcus aureus*. *J. Pharm. Pharmacol.* **1996**, *48*, 861-865.

(60) Rukachaisirikul, V.; Naklue, W.; Sukpondma, Y.; Phongpaichit, S. An Antibacterial Biphenyl Derivative from *Garcinia bancana* Miq. *Chem. Pharm. Bull.* **2005**, *53*, 342-343.

(61) Almeida, L. S.; Murata, R. M.; Yatsuda, R.; Dos Santos, M. H.; Nagem, T. J.; Alencar, S. M.; Koo, H.; Rosalen, P. L. Antimicrobial Activity of *Rheedia brasiliensis* and 7-Epiclusianone against *Streptococcus mutans*. *Phytomedicine* **2008**, *15*, 886-891.

(62) Negi, P. S.; Jayaprakasha, G. K.; Jena, B. S. Antibacterial Activity of the Extracts from the Fruit Rinds of *Garcinia cowa* and *Garcinia pedunculata* against Food Borne Pathogens and Spoilage Bacteria. *Food Sci. Tech.* **2008**, *41*, 1857-1861.

(63) Lokvam, J.; Braddock, J. F.; Reichardt, P. B.; Clausen, T. P. Two Polyisoprenylated Benzophenones from the Trunk Latex of *Clusia grandiflora* (Clusiaceae). *Phytochemistry* **2000**, *55*, 29-34.

(64) Cuesta-Rubio, O.; Padron, A.; Castro, H. V.; Pizza, C.; Rastrelli, L. Aristophenones a and B. A New Tautomeric Pair of Polyisoprenylated Benzophenones from *Garcinia aristata*. *J. Nat. Prod.* **2001**, *64*, 973-975.

(65) Cuesta-Rubio, O.; Frontana-Uribe, B. A.; Ramirez-Apan, T.; Cardenas, J. Polyisoprenylated Benzophenones in Cuban Propolis; Biological Activity of Nemorosone. *Z. Naturforsch.* **2002**, *57*, 372-378.

(66) Cueto, M.; Jensen, P. R.; Kauffman, C.; Fenical, W.; Lobkovsky, E.; Clardy, J. Pestalone, a New Antibiotic Produced by a Marine Fungus in Response to Bacterial Challenge. *J. Nat. Prod.* **2001**, *64*, 1444-1446.

(67) Yoganathan, K.; Cao, S.; Crasta, S. C.; Aitipamula, S.; Whitton, S. R.; Ng, S.; Buss, A. D.; Butler, M. S. Microsphaerins A-D, Four Novel Benzophenone Dimers with Activity against MRSA from the Fungus *Microsphaeropsis* sp. *Tetrahedron* **2008**, *64*, 10181-10187.

(68) Gustafson, K. R.; Blunt, J. W.; Munro, M. H. G.; Fuller, R. W.; McKee, T. C.; Cardellina, J. H., 2nd; McMahon, J. B.; Cragg, G. M.; Boyd, M. R. HIV Inhibitory Natural Products. 8. The Guttiferones, HIV-Inhibitory Benzophenones from *Symphonia globulifera*, *Garcinia livingstonei*, *Garcinia ovalifolia* and *Clusia rosea*. *Tetrahedron* **1992**, *48*, 10093-10102.

(69) Rukachaisirikul, V.; Pailee, P.; Hiranrat, A.; Tuchinda, P.; Yoosook, C.; Kasisit, J.; Taylor, W. C.; Reutrakul, V. Anti-HIV-1 Protostane Triterpenes and Digeranylbenzophenone from Trunk Bark and Stems of *Garcinia speciosa*. *Planta Med.* **2003**, *69*, 1141-1146.

(70) Fuller, R. W.; Westergaard, C. K.; Collins, J. W.; Cardellina, J. H., 2nd; Boyd, M. R. Vismiaphenones D-G, New Prenylated Benzophenones from *Vismia cayennensis*. *J. Nat. Prod.* **1999**, *62*, 67-69.

(71) Ferris, R. G.; Hazen, R. J.; Roberts, G. B.; St Clair, M. H.; Chan, J. H.; Romines, K. R.; Freeman, G. A.; Tidwell, J. H.; Schaller, L. T.; Cowan, J. R.; Short, S. A.; Weaver, K. L.; Selleseth, D. W.; Moniri, K. R.; Boone, L. R. Antiviral Activity of GW678248, a

Novel Benzophenone Nonnucleoside Reverse Transcriptase Inhibitor. *Antimicrob. Agents Chemother.* **2005**, *49*, 4046-4051.

(72) Romines, K. R.; Freeman, G. A.; Schaller, L. T.; Cowan, J. R.; Gonzales, S. S.; Tidwell, J. H.; Andrews, C. W., 3rd; Stammers, D. K.; Hazen, R. J.; Ferris, R. G.; Short, S. A.; Chan, J. H.; Boone, L. R. Structure-Activity Relationship Studies of Novel Benzophenones Leading to the Discovery of a Potent, Next Generation HIV Non-Nucleoside Reverse Transcriptase Inhibitor. *J. Med. Chem.* **2006**, *49*, 727-739.

(73) Chan, J. H.; Freeman, G. A.; Tidwell, J. H.; Romines, K. R.; Schaller, L. T.; Cowan, J. R.; Gonzales, S. S.; Lowell, G. S.; Andrews, C. W., 3rd; Reynolds, D. J.; St Clair, M.; Hazen, R. J.; Ferris, R. G.; Creech, K. L.; Roberts, G. B.; Short, S. A.; Weaver, K.; Koszalka, G. W.; Boone, L. R. Novel Benzophenones as Non-Nucleoside Reverse Transcriptase Inhibitors of HIV-1. *J. Med. Chem.* **2004**, *47*, 1175-1182.

(74) Abe, F.; Nagafuji, S.; Okabe, H.; Akahane, H.; Estrada-Muñiz, E.; Huerta-Reyes, M.; Reyes-Chilpa, R. Trypanocidal Constituents in Plants 3. Leaves of *Garcinia intermedia* and Heartwood of *Calophyllum brasiliense*. *Biol. Pharm. Bull.* **2004**, *27*, 141-143.

(75) Lenta, B. N.; Vonthron-Senecheau, C.; Weniger, B.; Devkota, K. P.; Ngoupayo, J.; Kaiser, M.; Naz, Q.; Choudhary, M. I.; Tsamo, E.; Sewald, N. Leishmanicidal and Cholinesterase Inhibiting Activities of Phenolic Compounds from *Allanblackia monticola* and *Symphonia globulifera*. *Molecules* **2007**, *12*, 1548-1557.

(76) Alves, T. M.; Alves, R.; Romanha, A. J.; Zani, C. L.; dos Santos, M. H.; Nagem, T. J. Biological Activities of 7-Epiclusianone. *J. Nat. Prod.* **1999**, *62*, 369-371.

(77) Marti, G.; Eparvier, V.; Moretti, C.; Susplugas, S.; Prado, S.; Gréllier, P.; Retailleau, P.; Gueritte, F.; Litaudon, M. Antiplasmodial Benzophenones from the Trunk Latex of *Moronobea coccinea* (Clusiaceae). *Phytochemistry* **2008**.

(78) Ngouela, S.; Lenta, B. N.; Noungoue, D. T.; Ngoupayo, J.; Boyom, F. F.; Tsamo, E.; Gut, J.; Rosenthal, P. J.; Connolly, J. D. Anti-Plasmodial and Antioxidant Activities of Constituents of the Seed Shells of *Symphonia globulifera* Linn F. *Phytochemistry* **2006**, *67*, 302-306.

(79) Yamaguchi, F.; Ariga, T.; Yoshimura, Y.; Nakazawa, H. Antioxidative and Anti-Glycation Activity of Garcinol from *Garcinia indica* Fruit Rind. *J. Agric. Food Chem.* **2000**, *48*, 180-185.

(80) Neves, J. S.; Coelho, L. P.; Cordeiro, R. S.; Veloso, M. P.; Rodrigues e Silva, P. M.; dos Santos, M. H.; Martins, M. A. Antianaphylactic Properties of 7-Epiclusianone, a Tetraprenylated Benzophenone Isolated from *Garcinia brasiliensis*. *Planta Med.* **2007**, *73*, 644-649.

(81) Hong, J.; Sang, S.; Park, H.-J.; Kwon Seok, J.; Suh, N.; Huang, M.-T.; Ho, C.-T.; Yang Chung, S. Modulation of Arachidonic Acid Metabolism and Nitric Oxide Synthesis by Garcinol and Its Derivatives. *Carcinogenesis* **2006**, *27*, 278-286.

(82) Koeberle, A.; Northoff, H.; Werz, O. Identification of 5-Lipoxygenase and Microsomal Prostaglandin E2 Synthase-1 as Functional Targets of the Anti-Inflammatory and Anti-Carcinogenic Garcinol. *Biochem. Pharmacol.* **2009**, *77*, 1513-1521.

(83) Demirkiran, O.; Ahmed Mesaik, M.; Beynek, H.; Abbaskhan, A.; Iqbal Choudhary, M. Cellular Reactive Oxygen Species Inhibitory Constituents of *Hypericum thasium* Griseb. *Phytochemistry* **2009**, *70*, 244-249.

(84) Seidlova-Wuttke, D.; Jarry, H.; Wuttke, W. Pure Estrogenic Effect of Benzophenone-2 (BP2) but Not of Bisphenol a (BPA) and Dibutylphthalate (DBP) in Uterus, Vagina and Bone. *Toxicology* **2004**, *205*, 103-112.

(85) Schlecht, C.; Klammer, H.; Frauendorf, H.; Wuttke, W.; Jarry, H. Pharmacokinetics and Metabolism of Benzophenone 2 in the Rat. *Toxicology* **2008**, *245*, 11-17.

(86) Jeon, H. K.; Sarma, S. N.; Kim, Y. J.; Ryu, J. C. Toxicokinetics and Metabolisms of Benzophenone-Type Uv Filters in Rats. *Toxicology* **2008**, *248*, 89-95.

(87) Mitcheva, M.; Kondeva, M.; Vitcheva, V.; Nedialkov, P.; Kitanov, G. Effect of Benzophenones from *Hypericum annulatum* on Carbon Tetrachloride-Induced Toxicity in Freshly Isolated Rat Hepatocytes. *Redox. Rep.* **2006**, *11*, 3-8.

(88) Balick, M., Nee, M., Atha, D. Checklist of the Vascular Plants of Belize. Bronx, NY: The New York Botanical Garden Press; 2000.

(89) Morton, J. F. Fruits of Warm Climates. Greensboro, N.C.: Julia F. Morton; 1987.

(90) De Oliveira, C. M. A.; Porto, A. L. M.; Bittrich, V.; Marsaioli, A. J. Two Polyisoprenylated Benzophenones from the Floral Resins of Three *Clusia* Species. *Phytochemistry* **1999**, *50*, 1073-1079.

(91) Smith, R. C.; Reeves, J. C.; Dage, R. C.; Schnettler, R. A. Antioxidant Properties of 2-Imidazolones and 2-Imidazolthiones. *Biochem. Pharmacol.* **1987**, *36*, 1457-1460.

(92) Fu, C.; Loo, A. E.; Chia, F. P.; Huang, D. Oligomeric Proanthocyanidins from Mangosteen Pericarps. *J. Agric. Food Chem.* **2007**, *55*, 7689-7694.

(93) Chin, Y.-W.; Jung, H.-A.; Chai, H.; Keller, W. J.; Kinghorn, A. D. Xanthones with Quinone Reductase-Inducing Activity from the Fruits of *Garcinia mangostana* (Mangosteen). *Phytochemistry* **2008**, *69*, 754-758.

(94) Farnsworth, N. R., Bunyapraphatsara N. Thai Medicinal Plants Recommended for Primary Health Care System. Prachachon Co. LB, editor: Prachachon Co.,Ltd: Bangkok; 1992.

(95) Konoshima, M.; Ikeshiro, Y. Fukugiside, the First Biflavonoid Glycoside from *Garcinia spicata* Hook. F. *Tetrahedron Lett.* **1970**, *20*, 1717-1720.

(96) Sordat-Diserens, I.; Rogers, C.; Sordat, B.; Hostettmann, K. Prenylated Xanthones from G*arcinia livingstonei*. *Phytochemistry* **1991**, *31*, 313-316.

(97) Samuelsson, G.; Farah, M. H.; Claeson, P.; Hagos, M.; Thulin, M.; Hedberg, O.; Warfa, A. M.; Hassan, A. O.; Elmi, A. H.; Abdurahman, A. D.; et al. Inventory of Plants Used in Traditional Medicine in Somalia. Ii. Plants of the Families Combretaceae to Labiatae. *J. Ethnopharmacol.* **1992**, *37*, 47-70.

(98) Taiwo, O.; Xu, H. X.; Lee, S. F. Antibacterial Activities of Extracts from Nigerian Chewing Sticks. *Phytother. Res.* **1999**, *13*, 675-679.

(99) Han, Q. B.; Lee, S. F.; Qiao, C. F.; He, Z. D.; Song, J. Z.; Sun, H. D.; Xu, H. X. Complete NMR Assignments of the Antibacterial Biflavonoid Gb1 from *Garcinia kola*. *Chem. Pharm. Bull.* **2005**, *53*, 1034-1036.

(100) Reynertson, K. A.; Yang, H.; Jiang, B.; Basile, M. J.; Kennelly, E., J. Quantitative Analysis of Antiradical Phenolic Constituents from Fourteen Edible Myrtaceae Fruits. *Food Chem.* **2008**, *109*, 883-890.

(101) Chattopadhyay, S. K.; Kumar, S. Identification and Quantification of Two Biologically Active Polyisoprenylated Benzophenones Xanthochymol and Isoxanthochymol in *Garcinia* Species Using Liquid Chromatography-Tandem Mass Spectrometry. *J. Chromatogr. B.* **2006**, *844*, 67-83.

(102) Luzzi, R.; Guimaraes, C. L.; Verdi, L. G.; Simionatto, E. L.; Delle Monache, F.; Yunes, R. A.; Floriani, A. E. O.; Cechinel-Filho, V. Isolation of Biflavonoids with Analgesic Activity from *Rheedia gardneriana* Leaves. *Phytomedicine* **1997**, *4*, 141-144.

(103) Reepmeyer, J. C.; Brower, J. F.; Ye, H. Separation and Detection of the Isomeric Equine Conjugated Estrogens, Equilin Sulfate and $\Delta^{8,9}$-Dehydroestrone Sulfate, by Liquid Chromatography-Electrospray-Mass Spectrometry Using Carbon-Coated Zirconia and Porous Graphitic Carbon Stationary Phases. *J. Chromatogr. A* **2005**, *1083*, 42-51.

(104) Obolskiy, D.; Pischel, I.; Siriwatanametanon, N.; Heinrich, M. *Garcinia mangostana* L.: A Phytochemical and Pharmacological Review. *Phytother. Res.* **2009**, *23*, 1047-1065.

(105) Kondo, M.; Zhang, L.; Ji, H.; Kou, Y.; Ou, B. Bioavailability and Antioxidant Effects of a Xanthone-Rich Mangosteen (*Garcinia mangostana*) Product in Humans. *J. Agric. Food Chem.* **2009**, *57*, 8788-8792.

(106) Jung, H. A.; Su, B. N.; Keller, W. J.; Mehta, R. G.; Kinghorn, A. D. Antioxidant Xanthones from the Pericarp of *Garcinia mangostana* (Mangosteen). *J. Agric. Food Chem.* **2006**, *54*, 2077-2082.

(107) Nilar; Harrison, L. J. Xanthones from the Heartwood of *Garcinia mangostana*. *Phytochemistry* **2002**, *60*, 541-548.

(108) Nilar; Nguyen, L.-H. D.; Venkatraman, G.; Sim, K.-Y.; Harrison, L. J. Xanthones and Benzophenones from *Garcinia griffithii* and *Garcinia mangostana*. *Phytochemistry* **2005**, *66*, 1718-1723.

(109) Pongphasuk N, K. W., Chitcharoenthum M. Antiinflammatory and Analgesic Activities of the Extract from *Garcinia mangostana* Linn. *Acta Hort* **2005**, *680*, 125-130.

(110) Sporn, M. B.; Suh, N. Chemoprevention of Cancer. *Carcinogenesis* **2000**, *21*, 525-530.

(111) Corpet, D. E.; Tache, S. Ranking Chemopreventive Agents on Rat Colon Carcinogenesis. *IARC Sci. Publ.* **2002**, *156*, 381-384.