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RESEARCH ARTICLE

Cytotoxic and nitric oxide inhibitory activities of methanol extracts of *Garcinia* species

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Abstract

The methanol extracts of 32 plant parts of 19 species of the genus *Garcinia* (Guttiferae) were collected from rainforests of the Malaysian Peninsula and the island of Sumatra, Indonesia, for evaluation of their *in vitro* cytotoxic and nitric oxide inhibitory activities. An end-point MTT cell viability assay was used to determine the 50% inhibitory concentration (IC_{50}) of the extracts in three human tumor cell lines representing tumors of the breast (MCF-7), lung (NCI-H460) and prostate (DU-145). Griess assay was performed to assess the nitric oxide (NO) inhibitory activity. Of the 32 extracts, 27 showed cytotoxic activity in at least one of the three tumor cell lines used in this study. Four extracts, *Garcinia opaca* King (fruit), *Garcinia maingayi* Hook.f. (stem), *Garcinia penangiana* Pierre (leaf) and *Garcinia urophylla* Scortech.ex King (leaf) extracts showed the most potent and selective cytotoxic activity against MCF-7 cells (IC_{50} 3-8 μg/mL). The extracts from *Garcinia cowa* Roxb. (stem), *Garcinia bancana* Miq. (stem) and *Garcinia malaccensis* Hook.f. (leaf) showed moderate activity and selectivity towards non-small lung tumor cells. The extracts from *Garcinia bancana* (stem), *Garcinia malaccensis* (stem), *Garcinia prainiana* King (leaf), *Garcinia rostrata* Hassk.ex Hook.f. (stem and leaf), *Garcinia cowa* (stem) and *Garcinia nervosa* Miq. (leaf) exhibited inhibition against NO production without affecting the viability of LPS and IFN-γ-induced RAW 264.7 macrophage cells. Among these, the most promising extracts were *G. bancana* (stem) and *G. malaccensis* (stem), as they showed the highest selectivity indices (> 50) for NO inhibition. In conclusion, these data provide evidence that some of the *Garcinia* species could potentially contain potent and selective cytotoxic and anti-inflammatory agents.

Keywords: *Breast cancer;* Garcinia; *Griess assay;* in vitro *cytotoxic; lung cancer; MTT assay; nitric oxide inhibition; prostate cancer; xanthones*

Introduction

Garcinia (Guttiferae) species are commonly found in the lowland areas of the rainforests. They consist of about 400 species within the paleotropical regions concentrated mainly in south-east Asia, and secondarily in India and west Africa (Corner, 1988). *Garcinia* species are typically small to medium dioecious evergreen fruit trees, although some occur as shrubs, and usually produce hard timber. Some traditional uses and medicinal properties of this species were documented by Burkill (1966). A list of the traditional uses of these plants as well as their local names is shown in Table 1.

In relation to the phytochemical studies, this genus is commonly reported to contain xanthones, benzophenones, triterpenes, biflavonoids and benzoquinone (Waterman & Hussain, 1983; Peres et al., 2000; Sadaquat et al., 2000; Kenji et al., 2003; Rukachaisirikul et al., 2008).

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The biological activities of the isolated compounds include anti-inflammatory (Nakatani et al., 2002), anti-HIV (Lin et al., 1997) and antibacterial (Permana et al., 2001; Rukachaisirikul et al., 2003, 2005). Xanthones are especially noted as potential anticancer agents (Thoison et al., 2000; Matsumoto et al., 2003a; Chiang et al., 2003; Ito et al., 2003a).

The phytochemistry of the popular *Garcinia* species, such as *Garcinia mangostana* Linn. and *Garcinia kola* Heckel, has been extensively investigated. However, only a few studies involving other less popular species such as those listed in Table 1 have been conducted. These include *Garcinia nigrolineata* Planch.ex T. Andres. (Rukachaisirikul et al., 2003), *Garcinia bancana* Miq. (Rukachaisirikul et al., 2005), *Garcinia cowa* Roxb. (Likhitwitayawuid et al., 1998) and *Garcinia parvifolia* Miq. (Xu et al., 1998; Rukachaisirikul et al., 2008). We have recently reported the cytotoxic constituents from *Garcinia penangiana* Pierre (Jabit et al., 2007), *Garcinia urophylla* Scortech.ex King (Khalid et al., 2007) and *Garcinia cantleyana* T.C. Whitmore (Shadid et al., 2007). A number of new prenylated and caged prenylated xanthones have been found to exhibit strong cytotoxic activities and thus present good potential as new cytotoxic agents.

Inflammation is a pathophysiological process mediated by a variety of signaling molecules produced mainly by leukocytes, macrophages and plasma cells. Macrophages play a crucial role in the generation of the pro-inflammatory molecule nitric oxide (NO). NO synthesized by the enzyme inducible nitric oxide synthase (iNOS) has been reported as a mediator of acute and chronic inflammation (Heras et al., 2001). Studies have shown that macrophages, upon stimulation with bacterial lipopolysaccharide (LPS), express iNOS to produce large amount of NO. iNOS is one of the essential components of the inflammatory response and is involved in the pathogenesis of several inflammatory diseases such as asthma and rheumatoid arthritis. In this study, using Griess assay to measure the level of NO produced by activated murine RAW 264.7 macrophage cells, plant extracts were tested for their anti-inflammatory activity. We also report the cytotoxic potential of plant extracts against three human tumor cell lines of the breast, lung and prostate, with the aim of identifying potent and tumor selective plant extracts. The main intention of this paper is to promote more research to be undertaken to isolate and identify bioactive compounds from the less popular *Garcinia* species that might be responsible for anticancer and anti-inflammatory activities.

Materials and methods

Plant materials

All *Garcinia* species were collected from the Peninsula Malaysia except *Garcinia cowa* and *Garcinia*

Table 1. List of *Garcinia* species and their traditional uses in Malaysia and West Sumatra, Indonesia.

Plants	Local name	Traditional uses
G. urophylla	Kandis hutan	The fruits are used to treat stomachache and the leaves are used to treat fever*
G. maingayi	Kandis gajah	Decoction of leaves is used as antifever*
G. penangiana	Kandis burung, manggis burung	The local people use the leaves to treat skin diseases and fever*
G. forbesii	Kandis	The fruits are eaten by local people*
G. eugenifolia	Tetulang merah	The fruits and young leaves are eaten by local people*
G. bancana	Tengkawang, beruas	Decoction of the leaves is used to treat fever*
G. opaca	Kandis	The fruits are eaten by local people and decoction of leaves is used to improve blood circulation*
G. rostrata	Lulai, loli	The fruits are eaten by local people*
G. parvifolia	Kandis, berunai cherry	The fresh fruits are eaten and dried fruits are used in Malay and Indonesian cuisines (Burkill, 1966)
G. nigrolineata	Kandis, kandis hutan	The fruits are eaten by the local people and decoction of leaves is used to treat eye diseases (Burkill, 1966)
G. cantleyana	Kandis	The fruits and leaves are eaten by local people*
G. prainiana	Chepu, button mangosteen	The fruits are eaten by local people (Burkill, 1966)
G. malaccensis	Manggis hutan	The fruits are used in Malay and Indonesian cuisines*
G. griffithii	Kandis, kandis gajah	The fruits are used in Malay and Indonesian cuisines (Burkill, 1966)
G. nervosa	Kandis gajah	The leaves are used to treat skin infections and wounds healing*
G. cowa	Asam kandis, chamuang	The fruits and leaves are eaten by local people. The bark has been used as antipyretic and antimicrobial agent. The latex has been used as antifever agent (Na Pattalung et al., 1994)
G. merguensis	Luli, lulai	The fruits are eaten by local people and the bark is used as a yellow dye while the leaves are used in folk medicine for the treatment of edema*

* Through personal communication.

merguensis Wight which were collected from West Sumatra, Indonesia in July toNovember 2001-2003. They were identified by Shamsul Khamis of the Institute of Bioscience, Universiti Putra Malaysia, and Rusdi Tamin of Andalas University, West Sumatra, Indonesia. The samples were deposited in the Herbarium of the Institute of Bioscience, Universiti Putra Malaysia and ANDA Herbarium, Andalas University (Table 2).

Preparation of crude extracts

About 100 g each of the sample (leaf, stem, bark and fruit) was ground to powder and macerated with methanol for three days and filtered. The filtrate was evaporated under reduced pressure at 40°C to obtain a crude extract. The procedure was repeated three times for each sample.

Cell culture

Three types of human tumor cell lines, DU-145 (prostate), MCF-7 (breast) and NCI-H460 (non-small cell lung) were used in the cytotoxic studies. RAW 264.7 (murine monocytic macrophage) cells were used in the Griess assay. The cells were purchased from the American Type Culture Collection (Manassas, VA, USA). The cancer cells were cultured in RPMI-1640 medium (Life Technologies, Paisley, Scotland) with 10% v/v fetal calf serum (PAA Laboratories, Linz, Austria), 100 IU/mL penicillin and 100 µg/mL streptomycin (Life Technologies). The RAW 264.7 cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Life Technologies) with phenol red containing HEPES, L-glutamine supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic/antimycotic solution (Gibco/BRL). All cells were grown in a humidified environment

Table 2. Different plant parts of *Garcinia* species used for bioassays, their weights and percentage yields of the methanol extracts.

Garcinia species	Location	Plant parts	Herbarium code	% yield (w/w)
G. urophylla	FH	Stem	SK97/01	8.3
G. urophylla	FH	Leaf	SK97/01	9.9
G. maingayi	HS	Stem	SK96/01	20.4
G. maingayi	HS	Leaf	SK96/01	9.2
G. rostrata	HS	Stem	SK104/01	8.7
G. rostrata	HS	Leaf	SK104/01	8
G. penangiana	FH	Stem	SK95/01	8.1
G. penangiana	FH	Leaf	SK95/01	5.5
G. forbesii	PN	Stem	PK4/01	4.5
G. forbesii	PN	Leaf	PK4/01	21.6
G. eugenifolia	PN	Stem	SK3/01	5.6
G. eugenifolia	PN	Leaf	SK3/01	10
G. bancana	PN	Stem	SK2/01	3.6
G. bancana	PN	Leaf	SK2/01	8.9
G. opaca	PN	Stem	SK8/01	9.6
G. opaca	PN	Leaf	SK8/01	11.2
G. opaca	PN	Fruit	SK8/01	5.5
G. parvifolia	FH	Leaf	SK94/01	10.5
G. nervosa	HS	Stem	SK183/02	6
G. nervosa	HS	Leaf	SK183/02	23
G. nigrolineata	HS	Stem	NS28	4.9
G. nigrolineata	HS	Leaf	NS27	8.9
G. cantleyana	CH	Stem	SK58/01	7.1
G. cantleyana	CH	Leaf	SK58/01	16.9
G. malaccensis	HS	Stem	SK175/02	8.1
G. malaccensis	HS	Leaf	SK175/02	12.9
G. prainiana	IBS	Stem	SK06/01	3.9
G. prainiana	IBS	Leaf	SK06/01	11.9
G. griffithii	HS	Stem	SK182/02	23
G. griffithii	HS	Leaf	SK182/02	18
G. cowa	HV	Stem	DR-180	3
G. merguensis	AU	Stem	DR-181	4.1

Note: CH, Cameron Highland Forest Reserve, Malaysia; HV, Harau valley, Sumatra, Indonesia; FH, Fraser's Hill Forest Reserve, Malaysia; AU, Biological Research and Development Forest, Andalas University, Sumatra, Indonesia; HS, Hulu Selangor Forest Reserve, Malaysia; PN, Panang Forest Reserve (Pekan), Malaysia; IBS, Institute of Bioscience, Universiti Putra Malaysia.

containing 5% CO_2 at 37°C. The cells were maintained in 25 cm^2 flask (TPP, Trasadingen, Switzerland) using 10 mL of medium.

In vitro test for cytotoxic activity - 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) cell viability assay

Subconfluent DU-145, MCF-7 and NCI-H460 cells were trypsinized, detached and made into suspensions of single cells before seeding them into 96-well microculture plates. The cell concentrations were set at 3,000, 4,000, and 2,500 cells/well, respectively. Crude extracts were tested at 0.1, 1, 10, and 100 μg/mL concentrations and each concentration was tested in four replicates. The culture plates were incubated at 37°C in a 5% CO_2 humidified environment for 96 h.

The fraction of viable cells after treatment with the extracts was determined by the ability of the cells to metabolize MTT. Fifty μL of MTT (Sigma, St. Louis, MO) solution (2 mg/mL) was added to yield a final concentration of 0.4 mg/mL and the plates were further incubated at 37°C (95% air, 5% CO_2) for 4 h to allow viable cells to convert soluble MTT into insoluble formazan. The medium containing MTT was aspirated and the formazan was dissolved by adding DMSO (100 μL). The absorbance of the formazan solutions was determined at 550 nm using a microplate reader (VersaMax, Molecular Devices, Inc., Sunnyvale, CA, USA). The IC_{50} values (concentration of drugs that will produce a 50% reduction in the absorbance compared with untreated controls) were determined from the dose-response curves (Stanslas et al., 2000).

In vitro test for NO inhibitory activity - Griess assay

To evaluate the inhibition of NO production by the extracts, Griess assay was employed according to the modified method of Dirsch et al. (1998). Briefly, RAW 264.7 cells were stimulated to produce inflammation using recombinant mouse interferon (IFN)-γ (BD Pharmingen, San Diego, CA, USA) and lipopolysaccharide (LPS) from *Escherichia coli* (Sigma). To evaluate the NO inhibitory activity, Griess reagent (1% sulfanilamide/0.1% *N*-(1-naphtyl)ethylenediamine dihydrochloride in 2.5% H_3PO_4) was mixed with an equal part of the cell culture medium of control or extract treated RAW 264.7 cells. The concentrations of the extract used to treat RAW 264.7 cells were 100, 10 and 1 μg/mL. The color development corresponding to NO level was assessed at 550 nm with a microplate reader (SpectraMax, Plus 384, Molecular Devices, Inc., Sunnyvale, CA, USA) and the percentage NO inhibition was determined according to the formula below. This was followed by cell viability determination using the MTT assay as described above.

$$\text{Percentage NO inhibition} = \frac{\text{NO level of control cells} - \text{NO level of extract treated cells}}{\text{NO level of control cells}} \times 100$$

Results

Yields of methanolic extract of different plant parts

The yields of the extracts obtained from different parts of *Garcinia* species used are shown in Table 2. The *Garcinia maingayi* (stem), *G. forbesii* King (leaf), *G. nervosa* (leaf) and *G. griffithii* (stem) produced among the highest yields with 20% and more recovery. *G. urophylla* (leaf), *G. eugenifolia* Wall (leaf), *G. opaca* (stem), *G. opaca* (leaf), *G. parvifolia* (leaf), *G. cantleyana* (leaf), *G. malaccensis* (leaf), *G. prainiana* (leaf) and *G. griffithii* (leaf) demonstrated percentage yields of between 10 and 20%. The rest of the extracts had yields less than 10%.

Cytotoxic activity of extracts against tumor cell lines

The 32 crude methanol extracts obtained from different parts of *Garcinia* species were tested for cytotoxic effect on three human tumor cell lines, representing tumors of the breast, lung and prostate. An extract was considered active if it had a mean $IC_{50} < 100$ μg/mL in any of the three cell lines. Among the active extracts, $IC_{50} < 10$ μg/mL in at least one tumor cell line was classified as strong activity, whereas extracts with IC_{50} in the range of 10-100 μg/mL were considered to have moderate activity. In addition, an extract was considered to have selective cytotoxic effect if it showed a pronounced difference in the activity among the three cell lines.

Based on the activity criteria set as above, the extracts were divided into 4 categories designated as A (strong and selective activity), B (strong activity without selectivity), C (moderate activity) and D (not active) (Table 3). From the results of our study, group A consisted of eight extracts and among these, *G. opaca* (fruit), *G. urophylla* (leaf), *G. maingayi* (stem) and *G. maingayi* (leaf) were selective towards MCF-7 cells with IC_{50} values of 8 ± 4, 3 ± 1, 6 ± 3 and 10 ± 9 μg/mL, respectively. The extract *G. urophylla* (leaf) showed approximately 12- and 11-fold selectivity towards MCF-7 cells as compared with DU-145 and NCI-H460 cells, respectively. Meanwhile the stem extract of *G. maingayi* displayed approximately 6-fold selectivity towards MCF-7 cells as compared with DU-145 and NCI-H460 cells. *G. opaca* fruit extract was approximately 5-fold and 4-fold selectivity towards MCF-7 cells as compared with DU-145 and NCI-H460 cells, respectively.

Table 3. IC_{50} values of *Garcinia* extracts in three human tumor cell lines.

Group	Extracts	IC_{50} (μg/mL)		
		MCF-7	DU-145	H460
A	*G. opaca*, fruits	8 ± 4	37 ± 3	32 ± 13
	G. urophylla, leaf	3 ± 1	37 ± 8	32 ± 10
	G. maingayi, stem	6 ± 3	35 ± 23	35 ± 5
	G. maingayi, leaf	10 ± 9	37 ± 8	35 ± 5
	G. nigrolineata, stem	43 ± 18	5 ± 1	3 ± 1
	G. cantleyana, stem	31 ± 20	10 ± 7	4 ± 1
	G. cantleyana, leaf	28 ± 11	4 ± 1	2 ± 1
	G. penangiana, leaf	5 ± 1	18 ± 8	8 ± 2
B	*G. nigrolineata*, leaf	5 ± 4	3 ± 1	4 ± 1
C	*G. cowa*, stem	>100	>100	11 ± 4
	G. bancana, stem	>100	>100	45 ± 5
	G. *malaccensis*, leaf	>100	>100	58 ± 10
	G. bancana, leaf	42 ± 4	>100	>100
	G. rostrata, leaf	65 ± 40	>100	>100
	G. forbesii, leaf	27 ± 5	38 ± 8	48 ± 18
	G. opaca, stem	26 ± 7	60 ± 20	57 ± 25
	G. parvifolia, leaf	22 ± 6	47 ± 3	47 ± 15
	G. nervosa, stem	>100	33 ± 13	50 ± 1
	G. nervosa, leaf	>100	48 ± 13	34 ± 3
	G. prainaina, leaf	>100	32 ± 5	31 ± 12
	G. urophylla, stem	70 ± 28	52 ± 18	47 ± 6
	G. forbesii, stem	92 ± 8	>100	57 ± 25
	G. penangiana, stem	49 ± 27	>100	50 ± 5
	G. griffithii, leaf	35 ± 21	48 ± 3	33 ± 4
	G. griffithii, stem	32 ± 19	47 ± 7	21 ± 5
	G. opaca, leaf	15 ± 3	48 ± 8	37 ± 15
	G. prainaina, stem	37 ± 2	32 ± 6	36 ± 13
D	*G. rostrata*, stem	>100	>100	>100
	G. eugenifolia, leaf	>100	>100	>100
	G. eugenifolia, stem	>100	>100	>100
	G. malaccensis, stem	>100	>100	>100
	G. merguensis, stem	>100	>100	>100
E	Doxorubicin	0.01 ± 0.001	0.07	0.12 ± 0.05
	Cytosine arabinoside	0.04 ± 0.02	4.8	2.8
	Vincristine	<0.01	<0.01	<0.01

A: strong and selective activity; B: strong activity; C: moderate activity; D: not active; E: positive controls (IC_{50} values in μM).
Note: Extracts were considered active if they show IC_{50}<100 μg/mL and in addition, extracts with IC_{50}<10 μg/mL were categorized to have strong activity; IC_{50} 10-100 μg/mL were considered to be moderate in activity; IC_{50} > 100 μg/mL were considered inactive.

G. maingayi leaf extract was roughly 4-fold selective in inhibiting the growth of MCF-7 cells as compared with DU-145 and NCI-H460 cells. The extracts of *G. nigrolineata* (stem) and *G. cantleyana* (leaf and stem) showed pronounced preferences in their cytotoxicity towards NCI-H460 and DU-145 in comparison to MCF-7 cells, with the activity being 3- to14-fold selective. *G. penangiana* (leaf) extract was selective towards MCF-7 cells and NCI-H460 cells with IC_{50} values of 5 ± 1 and 8 ± 2 μg/mL, respectively.

The extract of *G. nigrolineata* (leaf) was placed in group B, which was equipotent in all cell lines. There were 18 extracts with IC_{50} values in the range of 10-100 μg/mL and therefore were placed into group C. Among these, the extracts of *G. cowa* (stem), *G. bancana* (stem) and *G. malaccensis* (leaf) were selective toward NCI-H460 as compared with MCF-7 and DU-145 cells. However, the extracts of *G. bancana* (leaf), *G. rostrata* (leaf), *G. forbesii* (leaf), *G. opaca* (stem) and *G. parvifolia* (leaf) were selective towards MCF-7 compared with DU-145 and NCI-H460 cells. Nine extracts were selective toward two types of tumor cell lines, of which the extracts of *G. nervosa* (stem and leaf), *G. prainaina* (leaf) and *G. urophylla* (stem) were selective towards DU-145 and NCI-H460 cells, while the extracts of *G. forbesii* (stem), *G. penangiana* (stem), *G. griffithii* (leaf and stem) and *G. opaca* (leaf) showed selectivity towards MCF-7 and NCI-H460 tumor cells. Only *G. prainaina* was equiactive in all the three tumor cell lines.

The extracts of those which did not show cytotoxicity were placed in group D (IC_{50} > 100 μg/mL). These include *G. rostrata* (stem), *G. eugenifolia* (leaf and stem), *G. malaccensis* (stem) and *G. merguensis* (stem).

Inhibition of LPS and IFN-γ-activated NO production by RAW 264.7 cells

The 32 extracts were also tested for nitric oxide inhibitory activity in RAW 264.7 macrophage cell line. The results were divided into 4 categories: A (strong NO inhibitory activity with IC_{50}<30 μg/mL and not cytotoxic); B (moderate NO inhibitory activity with IC_{50} 30-100 μg/mL and not cytotoxic; C (false inhibition of NO with IC_{50}<100 μg/mL but cytotoxic; D (not active) (Table 4). Group A consisted of seven extracts and among these, *G. bancana* (stem) and *G. malaccensis* (stem) extracts showed a strong NO inhibitory activity with IC_{50} 2 μg/mL. In addition these extracts had the highest selectivity indices of >50 (lowest cytotoxicity). Group B consisted of six extracts, which include the extracts from *G. maingayi* (leaf), *G. penangiana* (stem), *G. bancana* (leaf), *G. opaca* (leaf), *G. eugeniifolia* (leaf) and *G. forbesii* (stem). The extracts of *G. maingayi* (stem), *G. urophylla* (leaf), *G. penangiana* (leaf), *G. opaca* (fruits), *G. parvifolia* (leaf), *G. nigrolienata* (stem and leaf), *G. cantleyana* (stem and leaf) *G. merguensis* (stem), *G. griffithii* (stem and leaf), *G. prainiana* (stem) and *G. malaccensis* (leaf) belonged to group C. The extracts represented in group D consisted of

Table 4. *Nitric oxide inhibitory activity of plant extracts.

Group	Extract	Mean IC_{50} (μg/mL) values		Selectivity index
		NO inhibition	RAW 264.7 cell viability	$IC_{50\ cell\ viability}/IC_{50\ NO\ inhibition}$
A	*G. bancana,* stem	2	> 100	> 50
	G. malaccensis, stem	2	> 100	> 50
	G. prainiana, leaf	11	> 100	> 9.1
	G. rostrata, stem	24	> 100	> 4.2
	G. rostrata, leaf	24	> 100	> 4.2
	G. cowa, stem	25	> 100	> 4
	G. nervosa, leaf	29	> 100	> 3.4
B	*G. maingayi,* leaf	33	> 100	> 3
	G. penangiana, stem	33	> 100	> 3
	G. bancana, leaf	34	> 100	> 2.9
	G. opaca, leaf	43	> 100	> 2.3
	G. eugeniifolia, leaf	48	> 100	> 2.1
	G. forbesii, stem	80	> 100	> 1.2
C	*G. maingayi,* stem	23	39	1.7
	G. urophylla, leaf	15	22	1.5
	G. penangiana, leaf	37	41	1.1
	G. opaca, fruits	17	6.2	0.4
	G. parvifolia, leaf	14	29	2.1
	G. nigrolienata, stem	< 1	< 1	1
	G. nigrolienata, leaf	21	35	1.7
	G. cantleyana, stem	< 1	6.5	6.5
	G. cantleyana, leaf	< 1	< 1	1
	G. merguensis, stem	20	45	2.2
	G. griffithii, stem	25	6	0.2
	G. griffithii, leaf	23	35	1.5
	G. prainiana, stem	1	25	25
	G. malaccensis, leaf	9	50	5.6
D	*G. opaca,* stem	> 100	> 100	1
	G. urophylla, stem	> 100	> 100	1
	G. eugeniifolia, stem	> 100	> 100	1
	G. nervosa, stem	> 100	> 100	1
	G. forbesii, leaf	> 100	27	0.3

*Positive control used in this study was N(G)-nitro-L-arginine methyl ester (L-NAME), whereby at 250 μM it inhibited 73% NO production.
A: strong NO inhibitory activity with IC50 < 30 μg/mL and not cytotoxic
B: moderate NO inhibitory activity with IC50 30-100 μg/mL and not cytotoxic
C: false inhibition of NO with IC50<100 μg/mL but cytotoxic
D: not active
Note: All extracts were tested three times.

G. opaca (stem), *G. urophylla* (stem), *G. eugeniifolia* (stem), *G. nervosa* (stem) and *G. forbesii* (leaf).

Discussion

A substantial number of studies on the antitumor activities of *Garcinia* species have been reported and their activities were often attributed to the presence of xanthones, triterpenes, depsidones and benzophenones (Cao et al., 1998; Xu et al., 1998, 2000; Mackeen et al., 2000; Thoison et al., 2000; Matsumoto et al., 2003b). The chemopreventive effects of xanthones and benzophenones from *G. assigu* and *G. fusca* were also reported (Ito et al., 2003a, 2003b). The extracts as potential cytotoxic agents are those categorized in group A (Table 3), which include *G. urophylla* (leaf), *G. maingayi* (stem), *G. maingayi* (leaf), *G. opaca* (fruit), *G. nigrolineata* (stem), *G. cantleyana* (leaf), *G. penangiana* (leaf) and *G. cantleyana* (stem). *G. urophylla* (leaf), *G. maingayi* (stem) and *G. opaca* (fruit) extracts were especially interesting due to their strong and selective *in vitro* cytotoxic activity against the hormone-dependent breast tumor cells, MCF-7. The extract from *G. cowa* (stem) also showed promising results, with more than 10-fold selectivity towards the non-small lung cancer cells (NSCL) (NCI-H460) compared with MCF-7 and DU-145 cells.

This is intriguing considering the fact there are no drugs presently available to effectively treat NSCL tumors. Cytotoxic chemotherapeutic drugs currently employed in the management of NSCL cancer patients often fail because of the intrinsic resistance of the cancer cells. Based on this study, it can be concluded that these plants have cytotoxic properties, and although the plants are not usually used traditionally to treat cancer, this discovery is indeed invigorating for further studies to be undertaken on them. It is interesting to note we recently reported the isolation of potent cytotoxic xanthones from *G. urophylla* (Khalid et al., 2007), *G. penangiana* (Jabit et al., 2007) and *G. cantleyana* (Shadid et al., 2007), which goes on to prove that the active plant extracts do contain prospective anticancer agents.

A search of the literature revealed that there have not been many studies dealing with anti-inflammatory activities of *Garcinia* species. Some xanthones and their derivatives, which are commonly found in *Garcinia* have been shown to be effective as allergy inhibitors and bronchodilators in the treatment of asthma (Balasubramaniam & Rajagopalan, 1988). A study by Nakatani et al. (2002) showed that γ-mangostin isolated from *G. mangostana* inhibited cyclooxygenase and prostaglandin E_2 synthesis, properties relating to anti-inflammatory effect. In the present study, the group A extracts (Table 4), especially those exhibiting high selectivity indices for inhibition of NO, such as *G. bancana* (stem) and *G. malaccensis* (stem) are expected to contain potent lead anti-inflammatory agents which could pave the way in the discovery of novel clinical candidates. A recent study reported the isolation of garcinol, isogarcinol and [1,1'-biphenyl]-2-(3-methyl-2-butenyl)-3-methoxy-4,4',5,6-tetraol as antibacterial agents from *G. bancana* twigs (Rukachaisirikul et al., 2005) and since the plant showed NO inhibitory activity, we propose these compounds could also potentially be responsible for NO inhibition. Nevertheless, other extracts in this group should also be studied further for isolation of their phytochemicals responsible for the NO inhibitory activity. However, although some of the extracts from group C showed good inhibition of NO production, this effect is strongly believed to be due to the extracts' cytotoxic effect on RAW 264.7 cells, thus termed as "false" potent NO inhibitory effect.

In conclusion, several *Garcinia* species exhibited remarkable *in vitro* cytotoxic and nitric oxide inhibitory activities based on this preliminary study and warrant further detailed investigation to isolate and identify the phytochemicals responsible for the biological activities. The extracts with potential selective cytotoxic agents are *G. opaca* (fruits), *G. maingayi* (stem and leaf), *G. nigrolineata* (stem), *G. penangiana* (leaf) and *G. cowa* (stem). For identification of prospective lead compounds for development of clinically active novel anti-inflammatory agents, extracts of *G. bancana* (stem) and *G. malaccensis* (stem) ought to be considered as the most promising extracts, as these extracts selectively inhibited NO production without killing the normal RAW 264.7 cells. Currently, studies are in progress in our laboratory to isolate the bioactive compounds from some of the *Garcinia* species that showed superior biological activities found in this study and the outcome will be reported elsewhere.

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