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Antioxidant Properties and Phytochemical Contents of *Garcinia schomburgkiana* Pierre.

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ABSTRACT

This study was conducted to determine the phytochemical contents and radical scavenging activities of twelve *Garcinia schomburgkiana* extracts from the leaves, roots, twigs and branches. Among all extracts, the root (RA) and branch (BA) acetone extract revealed high phenolic (427.83±4.84 and 390.15±7.89 mg GAE/g extract), flavonoid (626.32±59.97 and 414.49±15.99 mg QE/g extract) and xanthone (625.80±3.78 and 615.07±9.97 mg AME/g extract) contents and possessed greater antioxidant capacities and radical scavenging activities than the standard vitamin C and Trolox. Our results reveal that *G. schomburgkiana* were potential natural sources of antioxidant with high amount of phenolics, flavonoids xanthones and beneficial fatty acid derivatives.

INTRODUCTION

Theoretically, the reactive oxygen species (ROS) and reactive nitrogen species (RNS) such as, superoxide, hydroxyl radical, peroxyl radical and nitric oxide, are pro-oxidant and oxidant species from the nutrient metabolic processes (Limón-Pacheco and Gonsebatt, 2009; Limón-Pacheco *et al.*, 2006). They play an important role for biochemical processes at the low concentrations (Ahmed, 2005; Khansari *et al.*, 2009; Ratnam *et al.*, 2006). In one sense, the overproduction of the ROS and RNS species, from internal and external excitation such as, irradiation, chemical, pollution and stress (Poljšak and Fink, 2014; Sen *et al.*, 2010), is believed to be a cause of most human diseases, like cancer, chronic inflammation, aging, Parkinson's disease, Alzheimer's disease and hypertension (Ratnam *et al.*, 2006; Limón-Pacheco and Gonsebatt, 2009; Khansari *et al.*, 2009). Nowadays, antioxidants are scientifically attractive since they can prevent cell destructions caused by the action of free radicals (Poljšak and Fink, 2014; Sen *et al.*, 2010). Numerous scientists, therefore, attempt to search for the promising compounds from both natural resources and synthetics. Compounds are designed, synthesized and evaluated for their antioxidation properties. However, synthetic antioxidants are claimed to be industrially high-priced and toxic to the body (Tavasalkar *et al.*, 2012). Natural sources such as vegetables, fruits and medicinal plants which are relatively cheaper and render fewer side effects (Sen *et al.*, 2010), are of interest for the investigation of new antioxidants, like flavonoids, stilbenes, xanthones and phenolic acid (Leopoldini *et al.*, 2011; Pedraza-Chaverri *et al.*, 2008). Reportedly, plants in the Guttiferae family (Clusiaceae) such as, *Garcinia hombroniana*, *G. mangostana*, *G. brasiliensis*, *G. lateriflora* var. *javanica*, *G. combogia* and *G. virgate*, are known sources of a variety of biological active natural compounds, e.g., xanthone, terpenoid, benzophenone and flavonoid (Elya *et al.*, 2012; Gontijo *et al.*, 2012; Jung *et al.*, 2006; Kosema *et al.*, 2004; Merza *et al.*, 2004; Nargis *et al.*, 2013; Pedraza-Chaverri *et al.*, 2008; Subhashini *et al.*, 2011;Yu *et al.*, 2007).

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For instance, *G. mangostana* or magosteen, also recognized by the epithet "the queen of fruit", not only is a popular fruit, but also can be used as traditional medicines for diarrhea, infected wound, inflammation, chronic ulcer and as antioxidant, antitumor, anti-allergic, anti-inflammatory, antibacterial, antifungal and antiviral herbs (Pedraza-Chaverri *et al.,* 2008).

Currently, the consumption of mangosteen juice and mangosteen capsules as the dietary supplements dramatically increase in the market for health care people (Gutierrez-Orozco and Failla, 2013; Tang *et al.,* 2009).

Garcinia schomburgkiana, locally known as Ma-dan, is another interesting natural source of antioxidative compounds. This plant is widely distributed in the central and southern areas of evergreen forests in Thailand. Its sour-tasting fruit, besides being consumed fresh and fermented by local people, is also traditionally used for the treatment of laxation, cough, and diabetes (Lim, 2012).

Typically, the fruit methanol extract has been reported to have high total phenolic content and possess antioxidant activity and ferric reducing ability (Wetwitayaklung *et al.,* 2012; Nanasombat *et al.,* 2012). In addition, several chemical constituents from Ma-dan extracts revealed the potent cytotoxic activities against various cancer cell lines (Häfner&Frahm, 1993; Wetwitayaklung *et al.,* 2012; Nanasombat *et al.,* 2012; Fun *et al.,* 2006; Vo *et al.,* 2012; Mungmee *et al.,* 2013).

To the best of our knowledge, there have been a few reports about Ma-dan on its total phytochemical content and antioxidant activity (Wetwitayaklung *et al.,* 2012; Nanasombat *et al.,* 2012). To supplement the current medical data with a new potential source of antioxidants, we report herein the extraction procedures, the antioxidant activities of the Ma-dan extracts against all of these radicals (DPPH, ABTS, nitric oxide and hydroxyl radicals), their phytochemical contents (total phenolic contents, total flavonoid contents, total xanthone contents) and the GC-MS analysis.

MATERIALS AND METHODS

Chemicals and reagents

Aluminium chloride hexahydrate, ferrous sulphateheptahydrate, Folin-Ciocalteu's phenol reagent, sodium carbonate, sodium nitroprussidedihydrate and *N*-1-naphthyl ethylene diaminedihydrochloride were purchased from LobaChemie Pvt. Ltd. (India), ascorbic acid (Vitamin C) and sulphanilamide from Carlo Erba Reagents S.r.l. (Italy), potassium persulphate and salicylic acid from Ajax Finechem, Thermo Fisher Scientific Pty. Ltd. (Australia), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), quercetin, α-mangostin and dimethylsulfoxide (DMSO) from Sigma-Aldrich Pte. Ltd. (products from Germany, Denmark, India, China and France, respectively), gallic acid from Merck KGaA (Germany) and AR grade methanol from Fisher Chemicals, Fisher Scientific UK Ltd. (United Kingdom). The commercial grade solvents were used for extraction.

Plant Materials and Extraction Procedure

The leaves, roots, twigs and branches of *Garcinia schomburgkiana* were collected from Trang province, Southern Thailand in August 2012. The samples were dried at room temperature for 5-7 days and cut into small size. Each part of the collected sample was macerated in triplicate with dichloromethane at room temperature for 3 days. The residue was subsequently extracted with acetone and finally with methanol. The extracts were then filtered and evaporated under reduced pressure to obtain 12 crude extracts (Table 1).

Table 1: Abbreviation of Ma-dan extracts.

Parts	Solvent extractions	Abbreviations
Leaves	Dichloromethane	LD
	Acetone	LA
	Methanol	LM
Roots	Dichloromethane	RD
	Acetone	RA
	Methanol	RM
Twigs	Dichloromethane	TwD
	Acetone	TwA
	Methanol	TwM
Branches	Dichloromethane	BD
	Acetone	BA
	Methanol	BM

Determination of Total Phenolic Content (TPC)

Folin–Ciocalteu's method with slight modification was applied to determine the total phenolic content (Panyathepa *et al.,* 2013). In a 96-well plate, 12.5 μL of extract solutions (250 μg/mL in DMSO) or standard gallic acid solutions were added, followed by 50 μL of DI water and 12.5 μL of Folin-Ciocalteu (50 % v/vin DI water). After 10 min, 125 μL of 7 % Na_2CO_3 and 100 μL of DI water were added. The mixture was allowed to stand for 15 min at 45 °C and the absorbance was determined at 765 nm. Total phenolic content was calculated from gallic acid standard curve with linear relation of r^2=0.997. Data were expressed as mg of gallic equivalent (GAE) per 1 g of extract.

Determination of Total Flavonoid Content (TFC)

In order to investigate the total flavonoid content, a colorimetric method was applied (Zongo *et al.,* 2010). In a 96-well plate, 100 μL of the extracts (100 μg/mL in DMSO) or standard quercetin solutions and 100 μL of 2 % $AlCl_3$ in methanol were added and mixed thoroughly. The reaction mixture was kept at room temperature for 15 min and the absorbance was recorded at 435 nm. The total flavonoid content was calculated using quercetin standard curve with linear relation of r^2=0.999. Data were expressed as mg quercetin equivalent (QE) per 1 g of extract.

Determination of Total Xanthone Content (TXC)

The total xanthone content was evaluated with the use of UV-visible spectrophotometric method (Aisha *et al.,* 2013; Pothitirat and Gritsanapan., 2008). With a microplate reader, the

absorbance was determined at 320 nm. The total xanthone content was expressed as mg of the α-mangostin equivalent (AME) per 1 g of extract using α-mangostin standard curve with linear relation of r^2=0.999.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

In order to perform the GC-MS analysis, a Trace GC Ultra gas chromatography coupled with ISQ mass spectrometer (Thermo Scientific Inc., USA) was applied. A TR-5MS capillary column (30 m x 0.25 mm I.D., 0.25 mm film thickness) was employed to separate the volatile and semi-volatile compounds. In the gas chromatography, 1 μL of the extracts (dissolved in acetone) was injected in splitless mode with the injector temperature at 250 °C. The GC oven operating conditions were 50 (6 min) to 280 ºC at 5 ºC/min and 280 ºC (5 min). Helium was used as carrier gas with constant flow mode at 1.0 mL/min. The transfer line was set at 250 °C and ion source temperature was set to 230 ºC. GC-MS was operated in the 70 eV electron ionization (EI) mode with a collected mass range of 35 to 500 amu. The components were identified by comparison with wiley9 database.

Antioxidant Capacity Assay

The diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay was performed with a microplate reader (Zongo *et al.*, 2010). In the reaction well, 100 μL of the extracts (250 μg/mL in DMSO) or standard solution was mixed with 100 μL of the DPPH-radical (100 μg/mL in methanol) and left to stand at room temperature for 15 min in the dark. The absorbance was measured at 517 nm. Vitamin C was used as the standard. The percentage of DPPH-radical scavenging was calculated from the equation 1 and the antioxidant capacity of the extracts was expressed as vitamin C equivalent antioxidant capacity (VCEAC).

The 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) cation radical scavenging assay was performed with a slight modification (Re *et al.*, 1999). The working ABTS cation radical reagent was prepared by mixing ABTS and potassium persulfate ($K_2S_2O_8$) in DI water to obtain the final concentration of 7 mM and 2.45 mM, respectively. The mixture was stored in the dark at 4 °C for 12–16 h. Before being used, this prepared reagent was diluted with DI water until its absorbance value reached 0.7 ± 0.02 at 734 nm. To assess its free radical scavenging activity, 20 μL of the extracts (250 μg/mL in DMSO) or standard solution was mixed with 180 μL of the working ABTS cation radical reagent. The absorbance was measured at 734 nm after 3 min incubation at room temperature. To calculate the percentage of ABTS cation radical scavenging, the equation 1 was applied. With the application of the Trolox standard curve, the antioxidant capacity of the extracts was expressed as Trolox Equivalent Antioxidant Capacity (TEAC).

% Radical scavenging activity = $[1-(A_{sample}/A_{control})]$ x100(1)

Where, A_{sample}: The absorbance of the extracts or standards mixed with DPPH or $ABTS^{+\cdot}$.

$A_{control}$: The absorbance of the DMSO mixed with DPPH or $ABTS^{+\cdot}$.

Determination of Nitric Oxide Scavenging Activity

The nitric oxide scavenging activity in which vitamin C and quercetin were used as the standards was spectrophotometrically performed at 577 nm (Harput *et al.*, 2011; Ho *et al.*, 2010). To a 96-well plate, 50 μL of the extracts of varying concentrations ranging from 0 to 1,000μg/mL and 50 μL of 10 mM sodium nitroprusside in phosphate buffer saline (PBS), pH 7.4, were added. The reaction mixture was incubated at room temperature for 150 min under light condition, and 100 μL of Griess reagent (1 % sulfanilamide and 0.1 % *N*-(1-Naphthyl)ethylenediaminedihydrochloride in 2.5 % H_3PO_4) was then added. The absorbance was recorded after being left at room temperature for 10 min. Equation 2 was used to calculate the percentage of NO-scavenging activity. The result was reported as a sufficient concentration to obtain 50 % of a maximum nitric oxide scavenging capacity (SC_{50}).

% NO- scavenging activity = $[1-(A_{sample} - A_{sample\ blank}/A_{control} - A_{control\ blank})]$ x100(2)

Where, $(A_{sample} - A_{sample\ blank})$: The difference in the absorbance of the extracts or standards, with or without sodium nitroprusside.

$(A_{control} - A_{control\ blank})$: The difference in the absorbance of DMSO, with or without sodiumnitroprusside.

Determination of Hydroxyl Radical Scavenging Assay

A slightly modified method was used to determine the hydroxyl radical scavenging activity inwhich Trolox and gallic acid were used as the standards (Omwamba *et al.*, 2013; Sudha *et al.*, 2011). Into a 96-well plate, 90 μL of the extracts (the concentrations ranging from 0 to 1,000 μg/mL), 45 μL of 8 mM $FeSO_4.7H_2O$, 63 μL of 5.7 mM salicylic acid and 72 μL of 6 mM H_2O_2 were mixed and the absorbance was measured at 562 nm after a 30 min incubation at 37 ºC. Equation 3 was applied to calculate the percentage of the hydroxyl scavenging activity. The data was reported as a sufficient concentration to obtain 50 % of the maximum hydroxyl radical scavenging capacity (SC_{50}).

% OH-scavenging activity = $[1-(A_{sample} - A_{sample\ blank}/A_{control} - A_{control\ blank})]$ x100(3)

Where, $(A_{sample} - A_{sample\ blank})$: The difference in the absorbance of the extracts or standards, with or without salicylic acid.

$(A_{control} - A_{control\ blank})$: The difference in the absorbance of DMSO, with or without salicylic acid.

Statistical Analysis

All analyses were tested in triplicate and represented as the means ± standard deviation **(**SD**)**. The *t*-test method was used to differentiate the samples with a significance level of 0.05 **(***p*< 0.05**)**.

RESULTS AND DISCUSSIONS

According to the phytochemical determination, *G. schomburgkiana* was considered to be a vital source of phenolic, flavonoid and xanthone constituents. Table 2, displaying the values of antioxidant substances TPC, TFC and TXC of twelve *G. schomburgkiana* extracts, showed that the RA extract provided the

highest amount of TPC, TFC and TXC with 427.83±4.84 mg GAE/g extract, 626.32±59.97 mg QE/g extract and 625.80±3.78 mg AME/g extract, respectively. In addition, the BA extract exhibited another minor source of TPC (390.15±7.89 mg GAE/g extract) and TFC (414.49±15.99 mg QE/g extract). TXC in BA extract was comparable to that in RA extract with the value of 615.07±9.97 mg AME/g extract. For the other extracts, ascending order of TPC was TwM>TwA> BM> RM, RD,TwD> BD> LM and LD, the order of TFC was RD,TwA, RM >TwM, BM TwD> LD > LA > BD, and the order of TXC was LD >TwA>TwD> RD > BM, BD, RM > LM >TwM. The LA extract had the lowest amount of TPC and TXC, and the least value of TFC was LM.

The levels of antioxidant capacity, investigated by the DPPH, $ABTS^+$, nitric oxide and hydroxyl radical scavenging assay were also reported in Table 2. In this study, The RA and BA extracts provided the values of VCEAC and TEAC better than the other extracts. The BA extract which had lesser phytochemical contents than RA extract displayed the highest DPPH and $ABTS^+$ radical scavenging activities with the values of 197.56±4.43 VCEAC mg /g extract and 283.37 ± 2.09 TEAC mg /g extract, respectively. DPPH and $ABTS^+$ radical scavenging activities of RA extract were 190.89±2.22 VCEC mg /g extract and 259.11 ± 4.85 TEAC mg/g extract, respectively. The other extracts, the VCEAC value was followed by RD, TwA, RM, TwD, TwM, BD, BM, LM, LD, and LA, the TEAC value was as follows: RD, TwA, TwD, LD, BD, TwM, BM, LM, RM and LA.

The SC_{50} values of nitric oxide and hydroxyl radical scavenging activities from BA extract were 113.18±14.63 and 933.93±9.15 μg/mL, respectively and RA extract were 125.39±8.15 and 978.44±9.02 μg/mL, respectively. BA and RA extracts had more potent nitric oxide and hydroxyl radical scavenging activities than vitamin C but less than quercetin. For hydroxyl radical scavenging activity, BA and RA extracts were more potent than Trolox but less than gallic acid. For the other extracts, the rank of nitric oxide scavenging activity was TwA> LD > RM >TwM, BM and TwD, whereas, LA, LM, RD and BD have SC_{50} with more than 1000 μg/mL. The BA and RA extracts with high phytochemical contents and antioxidant activities values were subjected for the GC-MS analysis. A comparison of the mass spectra with Wiley9 library and the relative percentages as shown in Table 3 revealed that there were fatty acid derivatives which have been reported to be potential antioxidant (Ismail *et al.*, 2010; Meechaona *et al.*, 2007), anti-cardiovascular and anti-inflammatory agents (Rustan and Drevon, 2005) in the BA and RA extracts. For the RA extracts, 7 fatty acid derivatives consisting of methyl palmitate, palmitic acid, methyl oleate, methyl stearate, linoleic acid, 9-octadecenoic acid and stearic acid, were found in the peak area range of 1.69-17.52%. In the BA extracts, 2 phytosterols, stigmast-5-en-3-ol (3.99%) andstigmastane-3,6-dione (7.15%), 2 phenolic compounds, isovanillic acid (0.39%) and 2,6-dihydroxy-4-methoxybenzophenone (0.71%), 5 major fatty acid derivatives(1.68-12.46%), palmitic acid, linoleic acid, ethyl linoleate, 9-octadecenoic acid and ethyl (*Z*)-9-octadecenoate were detected. Consequently, the observed antioxidant properties of *G. schomburgkiana* should be derived from the detected phytochemical contents and beneficial fatty acid of the extracts.

Table 2 Total phytochemical contents and antioxidant activities of 12 Ma-dan extracts.

Extracts	Phenolic content	Flavonoid content	Xanthone content	DPPH scavenging	ABTS+· scavenging	Nitric oxide scavenging	Hydroxyl radical scavenging
	GAE (mg/g extract)1	QE (mg/g extract)2	AME (mg/g extract)3	VCEAC (mg/g extract)4	TEAC (mg/g extract)5	SC50 (μg/mL)	SC50 (μg/mL)
LD	58.84±3.51j	111.06±2.21f	448.02±8.05b	93.93±2.43h	151.51±5.86e	315.60±18.76d	>1,000
LA	37.68±1.81k	92.68±3.51g	89.66±0.77i	87.21±5.75h	31.21±6.47j	>1,000	>1,000
LM	97.97±2.66i	44.55±1.18i	131.16±1.17g	96.75±6.85g,h	93.19±3.56h	>1,000	>1,000
RD	140.29±6.53f,g	219.47±26.97c	324.39±8.40e	168.17±2.65b	219.51±7.45c	>1,000	>1,000
RA	427.83±4.84a	626.32±59.97a	625.80±3.78a	190.89±2.22a	259.11±4.85b	125.39±8.15b	978.44±9.02b
RM	145.80±3.05f	195.64±8.98c	137.04±1.17f	141.13±6.85d	85.64±2.65i	392.93±11.95e	>1,000
TwD	133.33±3.62g	145.79±0.81e	338.44±6.38d	131.28±0.88d	174.34±8.21d	941.50±45.80h	>1,000
TwA	235.65±5.43d	205.30±7.27c	357.85±0.77c	150.98±3.31c	187.44±12.91d	245.61±8.65c	>1,000
TwM	359.13±13.67c	159.97±0.54d	115.71±2.76h	123.99±3.62e	121.47±6.73f,g	688.07±28.12f	>1,000
BD	103.77±5.59h	59.81±0.47h	144.96±5.10f	114.72±2.21f	131.95±8.35f	>1,000	>1,000
BA	390.15±7.89b	414.49±15.99b	615.07±9.97a	197.56±4.43a	283.37±2.09a	113.18±14.63b	933.93±9.15c
BM	160.58±2.51e	156.23±4.54d	147.13±7.46f	104.56±3.76g	113.69±1.88g	625.50±77.59f	>1,000
Vitamin C*	ND	ND	ND	ND	ND	711.84±153.40g	ND
Trolox*	ND	ND	ND	ND	ND	ND	>1,000
Gallic acid*	ND	ND	ND	ND	ND	ND	428.53±6.38a
Quercetin*	ND	ND	ND	ND	ND	202.41±5.38a	ND

Data are expressed as means ± S.D., n =3, ND; Not detected.
Different letters (a-k) with in the same column indicate significant differences at $p< 0.05$ by t-test. The values are descending order as a > k.
*; antioxidant standards.
1GAE (mg/g extract); Gallic acid equivalent (mg of gallic acid/g extract).
2QE (mg/g extract); Quercetin equivalent (mg of quercetin/g extract).
3AME (mg/g extract); α-Mangostin equivalent (mg of α-mangostin /g extract).
4VCEAC (mg/g extract); Vitamin C equivalent antioxidant capacity (mg of vitamin C/ g extract).
5TEAC (mg/g extract); Trolox equivalent antioxidant capacity (mg of Trolox/ g extract).

Table 3 The volatile compounds of BA and RA from GC-MS analysis.

No.	Compound	RT (min)	MW ($g.mol^{-1}$)	BA extract (%PA)	RA extract (%PA)
1	Mesity oxide	15.52	98	0.86	-
2	Furfural	6.80	96	0.77	0.32
3	Diacetone alcohol	6.98	116	0.45	0.25
4	Furfuryl alcohol	16.86	98	0.64	-
5	2-Carboxylmethyl-3-n-hexyl-maleic acid anhydride	26.85	240	0.31	-
6	Levoglucosan	28.45	162	14.63	5.77
7	Isovanillic acid	29.38	168	0.39	-
8	5-acetyl barbituric acid	31.47	170	2.36	-
9	Myristic acid	33.23	228	-	0.14
10	Methyl palmitate	36.50	270	-	15.53
11	Palmitic acid	37.36	256	12.01	11.73
12	Ethyl palmitate	37.80	284	1.83	0.17
13	Margaric acid	39.20	270	0.59	0.36
14	Methyl linoleate	39.71	294	-	0.21
15	Methyl oleate	39.82	296	-	12.65
16	Methyl octadec-8-enoate	39.92	296	-	0.48
17	Methyl stearate	40.28	298	-	2.74
18	Linoleic acid	40.57	280	6.03	5.11
19	Oleic acid	40.67	282	12.46	17.54
20	Ethyl linoleate	40.91	308	1.68	-
21	Ethyl (Z)-9-Octadecenoate	41.02	310	3.39	-
22	Stearic acid	41.05	284	-	1.69
23	Ethyl stearate	41.47	312	0.33	-
24	Methyl (E,E)-6,9-octadecadienoate	41.48	294	-	0.49
25	2,6-Dihydroxy-4-methoxybenzophenone	42.46	244	0.71	-
26	Methyl arachidonate	43.76	326	-	0.26
27	Stigmast-5-en-3-ol	50.96	414	3.99	-
28	Stigmastane-3,6-dione	52.82	428	7.15	-

%PA: Percent Peak area; - : Not appeared; MW: Molecular weight; RT: Retention time.

CONCLUSION

The RA and BA extracts of *Garcinia schomburgkiana* revealed high phenolic, flavonoid and xanthone contents, and exhibited beneficial fatty acids. They possessed more promising antioxidant capacities and radical scavenging activities than vitamin C and Trolox. Thus, such highly active antioxidant compounds would be interesting areas for further phytochemical investigation and nutraceutical promotion as a possible antioxidant source.

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