

Chamuangone-Enriched *Garcinia cowa* Leaf Extract with Rice Bran Oil: Extraction and Cytotoxic Activity against Cancer Cells

Pirunrat Sae-Lim¹, Supreeya Yuenyongsawad¹, Pharkphoom Panichayupakaranant^{1,2}

¹Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, ²Phytomedicine and Pharmaceutical Biotechnology Excellence Center, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songkhla, Thailand

Submitted: 17-09-2018

Revised: 29-10-2018

Published: 06-03-2019

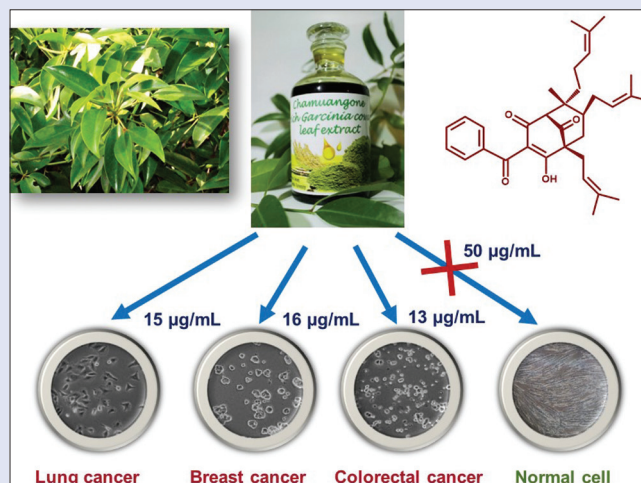
ABSTRACT

Background: Chamuangone has been isolated from *Garcinia cowa* leaves and exhibited various biological activities, i.e., antibacterial, anti-*Leishmania major*, and cytotoxic activity against cancer cells. *n*-Hexane has been reported to be the most suitable solvent for extraction of chamuangone. **Objectives:** Some vegetable oils were determined as an alternative green solvent for extraction of an anticancer compound, chamuangone from *G. cowa* leaf. The chamuangone-enriched extract was standardized and evaluated for cytotoxic activity against human cancer cell lines. **Materials and Methods:** Microwave-assisted extraction and high-performance liquid chromatography were used for extraction and standardization. The cytotoxic activity was determined using a sulforhodamine B assay. **Results:** The chamuangone-enriched extract was obtained using rice bran oil as the alternative green solvent and standardized to contain 1.97 mg/mL chamuangone. The extract exhibited cytotoxic activity against human lung adenocarcinoma, human breast adenocarcinoma, and human colorectal adenocarcinoma cell lines, with IC₅₀ values of 15.3, 15.9, and 12.8 µg/mL, respectively, but was nontoxic to human gingival fibroblasts, a normal cell line, at a concentration of 50 µg/mL. Moreover, the extract contained several natural antioxidants, including α-tocopherol (76.7 mg/100 g), γ-oryzanol (cycloartenol ferulate: 67.1 µg/mL and 24-methylenecycloartenol ferulate: 85.6 µg/mL), and antioxidant capacity determined as ascorbic acid (258.7-mM ascorbic acid equivalent per gram). **Conclusion:** Based on these findings, the chamuangone-enriched extract may be considered as a novel functional food in cancer chemopreventive action.

Key words: Anticancer, antioxidant, chamuangone, *Garcinia cowa*, green extraction

SUMMARY

- Chamuangone-enriched *Garcinia cowa* leaf extract was prepared using rice bran oil as a green solvent
- The chamuangone-enriched extract exhibited good cytotoxic activity against cancer cells
- The chamuangone-enriched extract contained high levels of natural antioxidants
- The chamuangone-enriched extract is a novel functional food for cancer prevention.



Abbreviations used: AAE/g: Ascorbic acid equivalent per gram; CEO: Chamuangone-enriched *Garcinia cowa* leaf extract with rice bran oil; DMEM: Dulbecco's Modified Eagle Medium; FBS: Fetal bovine serum; HGF: Human gingival fibroblasts; HPLC: High-performance liquid chromatography; IC₅₀: 50% Inhibitory concentration; MAE: Microwave-assisted extraction; MHz: Megahertz; MUFAs: Monounsaturated fatty acids; NMR: Nuclear magnetic resonance; ODS: Octadecylsilane; PUFAs: Polyunsaturated fatty acids; SD: Standard deviation; SFAs: Saturated fatty acids; SRB: Sulforhodamine B; TCA: Trichloroacetic acid; UFAs: Unsaturated fatty acids; UV: Ultraviolet; W: Watt.

Correspondence:

Dr. Pharkphoom Panichayupakaranant, Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand.
E-mail: pharkphoom.p@psu.ac.th
DOI: 10.4103/pm.pm_472_18

Access this article online

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INTRODUCTION

Cancer is a major public health problem that causes billions of deaths in most countries throughout the world.^[1,2] Dietary chemoprevention using fruits and vegetables as a practical, economical, and effective approach for reducing the risk of cancers has previously been described.^[3,4] In 2016, cancer was the leading cause of death, accounting for 8.9 million deaths globally. The most common causes of cancer death for both sexes were tracheal, bronchus, and lung cancer (1.71 million people); stomach cancer (834,171 people); colorectal cancer (829,557 people); liver cancer (828,945 people); and breast cancer (545,590 people), respectively.^[5]

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Cite this article as: Sae-Lim P, Yuenyongsawad S, Panichayupakaranant P. Chamuangone-enriched *Garcinia cowa* leaf extract with rice bran oil: Extraction and cytotoxic activity against cancer cells. Phcog Mag 2019;15:183-8.

Although many anticancer drugs have been discovered, they have been unable to reduce the cancer mortality rate. As a result, many countries in the world are eager to find the means for decreasing the risk of cancers using dietary chemoprevention. Natural compounds from fruits and vegetables have provided many effective anticancer agents. Currently, over 50% of drugs used in clinical trials for anticancer activity have been isolated from natural products.^[3]

The cancer chemopreventive activity of vegetables, such as cabbage, broccoli, green tea, and tomatoes, along with some medicinal plants has been presented as an alternative route for cancer treatment and prevention.^[6,7] Vegetables are rich in vitamins, minerals, antioxidants, and phytochemicals, all of which help to protect against cancer.^[8] Presumably, the combination of these compounds in whole foods can reduce the risk of certain cancers. However, dietary antioxidant supplements for cancer prevention must be imported from abroad which can be costly. There is an enormous biodiversity in Thai vegetables that are easy to find and harvest, and it is possible that some could be great resources for detecting new anticancer agents.

Garcinia cowa Roxb. ex Choisy, called “Chamuang” in Thai, is an edible plant in the Guttiferae or Clusiaceae family. Its leaf is used in many Thai recipes, such as pork stew with chamuang leaves (in Thai “Mu-Chamuang”). Some anticancer compounds may be extracted from this recipe by lard oil obtained from streaky pork. Researchers have recently identified a new polyprenylated benzophenone named “chamuangone” that showed various biological activities, i.e., antibacterial, anti-*Leishmania major*, and strong cytotoxic activity against some cancer cell lines, including A549, SCB3, K562, and K562/ADM.^[9-11] In addition, a rapid method for quantitative analysis and the best extraction method of chamuangone have been described, and *n*-hexane has been found to be the most suitable solvent for extraction of chamuangone from *G. cowa* leaves using a microwave-assisted extraction (MAE).^[9] However, the use of *n*-hexane as a solvent for herbal industrial application was limited due to its toxicities to the respiratory and nervous systems as well as carcinogenicity.^[12]

Currently, the major concerns are reducing the risks during extraction and increasing the safety of the ingredients used. These concerns have drawn attention toward the need to use an alternative green solvent. Vegetable oils are usually used as a vehicle for nutraceutical products^[13] that are used with nonpolar active compounds.^[14] These oils have similar polarities to *n*-hexane but are considered to be safer and cheaper. Therefore, the present study focused on investigating the use of vegetable oils that contain different combinations of saturated, monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs), including palm oil, rice bran oil, soybean oil, and sunflower oil, as alternative green solvents for extraction of chamuangone from *G. cowa* leaves using MAE. In addition, the extraction processes were optimized to obtain the chamuangone-enriched *G. cowa* leaf extract with rice bran oil (CEO). Cytotoxicity of the standardized CEO against human cancer cell lines was also determined to obtain the preliminary anticancer activity information useful for nutraceutical applications.

MATERIALS AND METHODS

Plant materials

G. cowa leaves were collected from Trang Province, Thailand, in November 2013. The leaves were identified by comparison with the herbarium specimen (Voucher No. SKP 083 07 03 01) which was deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand. The leaves were washed and then dried in a hot air oven at 60°C for 24 h. The dried leaves were reduced to powder using a grinder, and the powder was passed through a no. 45 sieve.

Chemicals and reagents

All analytical grade chemicals and high-performance liquid chromatography (HPLC) grade solvents were obtained from LABScan Asia (Bangkok, Thailand). Phosphate-buffered saline, penicillin-streptomycin, and Dulbecco's Modified Eagle Medium (DMEM) were purchased from Gibthai (Bangkok, Thailand). Fetal bovine serum (FBS) was acquired from Seromed (Berlin, Germany). Tris was purchased from Gibco (New York, USA), sulforhodamine B (SRB) from Sigma-Aldrich Chemie GmbH (Steinheim, Germany), and trichloroacetic acid (TCA) from CARLO ERBA Reagent S. r. l. (Cornaredo, Italy).

Cell lines

The human lung adenocarcinoma cell line (A549, CLS No. 300114), the human breast adenocarcinoma cell line (MCF-7, CLS No. 300273), and the human colorectal adenocarcinoma cell line (HT-29, CLS No. 300215) were obtained from Gibthai (Bangkok, Thailand), and the human gingival fibroblasts (HGF) were acquired from Faculty of Dentistry, Prince of Songkla University, Thailand.

Apparatus

The Agilent 1100 liquid chromatographic system equipped with a photodiode array detector and autosampler (Agilent, USA) was used for the quantitative HPLC analysis. ¹H- and ¹³C-nuclear magnetic resonance spectra were determined using a JEOL JNM α -400 spectrometer (JEOL Ltd., Japan). A microwave (LG Electronics Inc., Thailand) was used for extraction. A microplate reader (Bio TEK Instruments Inc., Germany) and a CO₂ incubator (Sheldon Manufacturing Inc., USA) were used for anticancer assay.

Quantitative high-performance liquid chromatography determination of chamuangone

HPLC analysis of chamuangone was performed using the method previously described.^[9] Separation was achieved on a Tosoh Bioscience[®] TSK-gel octadecylsilane-80Tm column (5- μ m particle size, 4.6 mm \times 150 mm). The mobile phase consisted acetonitrile and 2% v/v phosphoric acid in Milli-Q grade water (97:3, v/v), and was pumped at a flow rate of 1 mL/min. The injection volumes were 20 μ L, and quantitative detection was by ultraviolet at a wavelength of 245 nm.

Purification of chamuangone

The dried powder of *G. cowa* leaf (1 kg) was extracted three times with *n*-hexane (10 L \times 3) using an MAE. MAE was carried out in a laboratory scale microwave extraction apparatus, with a microwave frequency of 2450 MHz, power of 600 W, and three irradiation cycles (one cycle was 60-s power-on and 30-s power-off). The filtered solution was evaporated to dryness under reduced pressure at 40°C. The *n*-hexane extract was subjected to isolation and identification of chamuangone using the method previously described,^[9] with some modifications, to produce a light yellowish oil of chamuangone (81 mg). The structure of chamuangone was determined by ¹³C- and ¹H-NMR at 100 MHz and 400 MHz, respectively, and compared with the previous report.^[11] The NMR data are as follows: ¹³C NMR (CDCl₃, 100 MHz) 17.7 (CH₃, C-17), 17.8 (CH₃, C-22), 17.9 (CH₃, C-32), 18.1 (CH₃, C-27), 21.9 (CH₂, C-19), 25.7 (CH₃, C-23), 25.7 (CH₃, C-28), 26.0 (CH₃, C-33), 28.1 (CH₂, C-24), 30.4 (CH₂, C-29), 38.5 (CH₂, C-18), 40.9 (CH, C-7), 42.6 (CH₂, C-6), 48.5 (C, C-8), 64.6 (CH, C-5), 64.8 (C, C-1), 115.6 (C, C-3), 119.6 (CH, C-30), 121.9 (CH, C-25), 123.8 (CH, C-20), 127.8 (CH, C-13), 127.8 (CH, C-15), 128.5 (CH, C-12), 128.5 (CH, C-16), 132.1 (C, C-21), 132.4 (CH, C-14), 133.4 (C, C-26), 134.8 (C, C-31), 137.1 (C, C-11), 191.4 (C, C-2), 194.6 (C, C-4), 198.0 (C, C-10), 206.5 (C, C-9). ¹H NMR

(CDCl₃, 400 MHz) 0.95 (1H, s, H-17), 1.36 (1H, dt, *J* = 3.7, 12.7 Hz, H-18a), 1.44 (1H, t, *J* = 13 Hz, H-6ax), 1.50 (3H, overlapped, H-28), 1.55 (1H, m, H-7), 1.60 (1H, overlapped, H-24a), 1.60 (3H, overlapped, H-27), 1.63 (3H, overlapped, H-32), 1.64 (1H, overlapped, H-18b), 1.64 (3H, overlapped, H-23), 1.69 (3H, overlapped, H-22), 1.73 (3H, overlapped, H-33), 1.95 (1H, m, H-19), 2.05 (1H, dd, *J* = 3.2, 13 Hz, H-6eq), 2.10 (1H, overlapped, H-24b), 2.34 (1H, overlapped, H-19b), 2.35 (1H, overlapped, H-29a), 2.52 (1H, dd, *J* = 9.5, 13.9 Hz, H-29b), 3.4 (1H, s, H-1), 4.87 (1H, m, H-25), 5.07 (1H, m, H-20), 5.17 (1H, m, H-30), 7.35 (1H, t, *J* = 7.8 Hz, H-13), 7.35 (1H, t, *J* = 7.8 Hz, H-15), 7.45 (1H, d, *J* = 7.3 Hz, H-12), 7.45 (1H, d, *J* = 7.3 Hz, H-16), and 7.50 (1H, t, *J* = 7.3 Hz, H-14).

Determination of suitable vegetable oil for extraction

The powders of *G. cowa* leaves (2 g) were extracted with various vegetable oils (20 mL), including palm oil, rice bran oil, soybean oil, and sunflower oil, using the MAE with a microwave frequency of 2450 MHz, power of 600 W, and an irradiation period of 60 s. The leaf extracts were filtered, and the yields were recorded and then subjected to a quantitative HPLC analysis for chamuangone. The experiments were performed in triplicate.

Microwave powers

The powders of *G. cowa* leaves (10 g) were extracted with rice bran oil (100 mL) using an MAE with a microwave frequency of 2450 MHz at different microwave powers of 360, 600, and 800 W, with three irradiation cycles (one cycle was 60-s power-on and 30-s power-off).

Powder to solvent ratios

The powders of *G. cowa* leaves in different amounts, i.e., 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 g, were separately extracted with rice bran oil (100 mL) using the MAE at a power of 800 W, with three irradiation cycles (one cycle was 60-s power-on and 30-s power-off).

Irradiation cycles

The powders of *G. cowa* leaves (50 g) were extracted with rice bran oil (100 mL) using the MAE at a power of 800 W, with different irradiation cycles, including 2, 3, 4, 5, and 6 cycles (one cycle was 60-s power-on, and 30-s power-off).

Consecutive extraction times

The powders of *G. cowa* leaves (50 g) were extracted with rice bran oil (100 mL) using the MAE at a power of 800 W, with four irradiation cycles (one cycle was 60-s power-on and 30-s power-off). The extraction process was consecutively performed four times using the marc and fresh rice bran oil.

The extracts of all experiments were filtered through a filter paper. An aliquot of each sample was taken and subjected to the quantitative HPLC analysis of chamuangone. All of the experiments were performed in triplicate.

Scale-up for preparation of chamuangone-enriched *Garcinia cowa* leaf extract with rice bran oil

The powders of *G. cowa* leaves (500 g) were extracted with rice bran oil (1 L) using the MAE at a power of 900 W and four irradiation cycles (one cycle was 5-min power-on and 1-min power-off). The extracts were then filtered through a filter paper and subjected to the quantitative HPLC analysis of chamuangone as well as determinations of nutrition facts and anticancer activity.

Determination of nutrition facts

The nutrition facts of CEO were determined by a laboratory service of the Central Laboratory (Songkhla Branch) Co., Ltd (Songkhla, Thailand).

Determination of anticancer activity

Cell culture

The human lung adenocarcinoma (A549), human breast adenocarcinoma (MCF-7), human colorectal adenocarcinoma (HT-29), and HGF cell lines were maintained in DMEM medium supplemented with 10% heat-inactivated FBS, penicillin G sodium (50 units/mL), streptomycin sulfate (50 µg/mL), and amphotericin B (0.125 µg/mL) and incubated in a humidified 5% CO₂ incubator at 37°C.

Anticancer activity assay

The anticancer activity was determined using the SRB assay as described by Skehan *et al.*^[15] and assessed according to the protocol previously described by Keawpradub *et al.*^[16] Briefly, the cells were seeded into 96-well microplates at a density of 4×10^4 cells per well in 100 µL of complete medium and allowed to adhere for 24 h at 37°C in a 5% CO₂ incubator. The cells were treated with 100 µL of sample solutions at various concentrations (25-µg/mL crude extract or a fivefold diluted pure compound in medium) and incubated at 37°C for 72 h. After an incubation period, the cellular proteins were fixed with 100 µL of cold 10% (w/v) TCA to each well, and the plate was incubated at 4°C for at least 1 h. The plate was then washed with water for four washing cycles, dried completely at room temperature, and stained with 50-µL 0.4% SRB solution in 1% acetic acid in each well (allowed to stain for 30 min). The dye was then dissolved in Tris base solution (pH 10.5) and shaken for 5 min. The percentage of cell growth inhibition was determined by measuring the absorbance at 492 nm (PowerWave X plate reader: BioTEK Instruments, Inc.). The activities were reported as an IC₅₀ value. The IC₅₀ value (effective concentration of sample required to inhibit cell growth by 50%) was calculated from dose-response curves plotting between % inhibition and concentrations. Camptothecin was used as positive control.

Statistical analysis

The results were expressed as the mean ± standard deviation. Within-group comparisons were performed by the analysis of variance using an ANOVA followed by Tukey's honestly significant difference test. Values of *P* < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Determination of an alternative solvent for extraction

Determination of vegetable oils as an alternative green solvent for extraction of chamuangone from *G. cowa* leaf powders using MAE found that when compared to the other solvents, palm oil produced the extract with the highest content of chamuangone as well as the highest total yield of chamuangone [Figure 1]. Although *n*-hexane gave the highest chamuangone content, the yield of extract was lowest due to its volatile property and led to a lower total yield of chamuangone [Table 1]. Palm oil contains a higher content of SFAs than the other vegetable oils used in this study. Thus, this may be suitable for extraction of chamuangone due to its nonpolarity that is similar to chamuangone. When compared to results from unsaturated fatty acids-rich vegetable oils, oils with higher levels of SFAs may result in a greater risk of cardiovascular diseases.^[17] Rice bran oil contains a lower level of SFAs but higher levels of MUFAs, PUFAs, and natural antioxidants than palm oil.^[18,19] This is more beneficial to human health than palm oil. Rice bran oil was therefore considered the most appropriate alternative green solvent for

preparation of the CEO used as a novel functional food. However, the concentration of chamuangone in the extract produced by rice bran oil was rather low. Further optimization of the MAE conditions for extraction of chamuangone using rice bran oil was performed in order to increase the concentration of chamuangone in the extract.

Optimization of extraction conditions

The variable factors for the MAE operating parameters included the microwave power, powder-to-solvent ratios, microwave irradiation

cycles, and extraction times; each factor was determined using the single-factor experiments.

Various microwave powers, i.e., 360, 600, and 800 W were examined for extraction of chamuangone from *G. cowa* leaf powders using rice bran oil as the alternative solvent for extraction. An increase in microwave powers resulted in an increase in the content and total yield of chamuangone [Table 2]. This may be due to an increase in temperature during the extraction process. Heat is generated directly from the moisture that is still contained in the plant cells after exposure to the microwave irradiation. The heat generated inside plant cells can accelerate the cell leakage, and therefore, facilitate extraction efficiency. In this study, the microwave power of 800 W, which is the maximum power of this instrument, produced the highest content of chamuangone in the extract. This implies that the heat generated by this condition (145°C–150°C) can facilitate the extraction efficiency and not affect the stability of chamuangone. Therefore, the microwave power of 800 W was used for further determination of the effect of the powder-to-solvent ratio.

The effect of the powder-to-solvent ratio was determined by increasing the amount of powdered leaves from 10 to 100 g/mL. An increase in the powder-to-solvent ratio resulted in increased concentration of chamuangone in the leaf extracts. However, the ratios higher than 50 g/100 mL resulted in a marked decrease in the yield of extracts, which led to a reduction of the total yield of chamuangone [Table 3]. The highest total yield of chamuangone was obtained from the ratio of 50 g/100 mL. This ratio was, therefore, selected as the most suitable ratio for preparation of chamuangone extract.

In determining MAE irradiation cycles, 2–6 cycles found that increasing irradiation up to 4 cycles resulted in enhanced chamuangone concentration of the extracts; following this increase, the concentrations of chamuangone did not significantly increase [Table 4]. Four irradiation cycles were sufficient for the plant cell rupture and chamuangone extraction. Hence, four irradiation cycles were used for further determination of consecutive extraction times.

The successive extractions of the sample residue, i.e., the number of consecutive extraction times, were determined. The sample residue was reextracted using fresh rice bran oil. After the first extraction, the chamuangone contents of the consecutive extracts were very low [Table 5]. It is, therefore, not worthwhile to perform the consecutive extractions using fresh solvent.

The optimal conditions of MAE for small-scale preparation of CEO are suggested as follows: use 50 g of dried powders of *G. cowa* leaves extracted with 100 mL of rice bran oil at a microwave power of 800 W, with four irradiation cycles (one cycle is 60-s power-on, and 30-s power-off), 145°C–150°C, and without consecutive extraction.

Scale-up for preparation of chamuangone-enriched *Garcinia cowa* leaf extract with rice bran oil

MAE conditions were determined for an extraction of 0.5-kg dried leaf powder of *G. cowa* with 1 L of rice bran oil using a 900-W microwave.

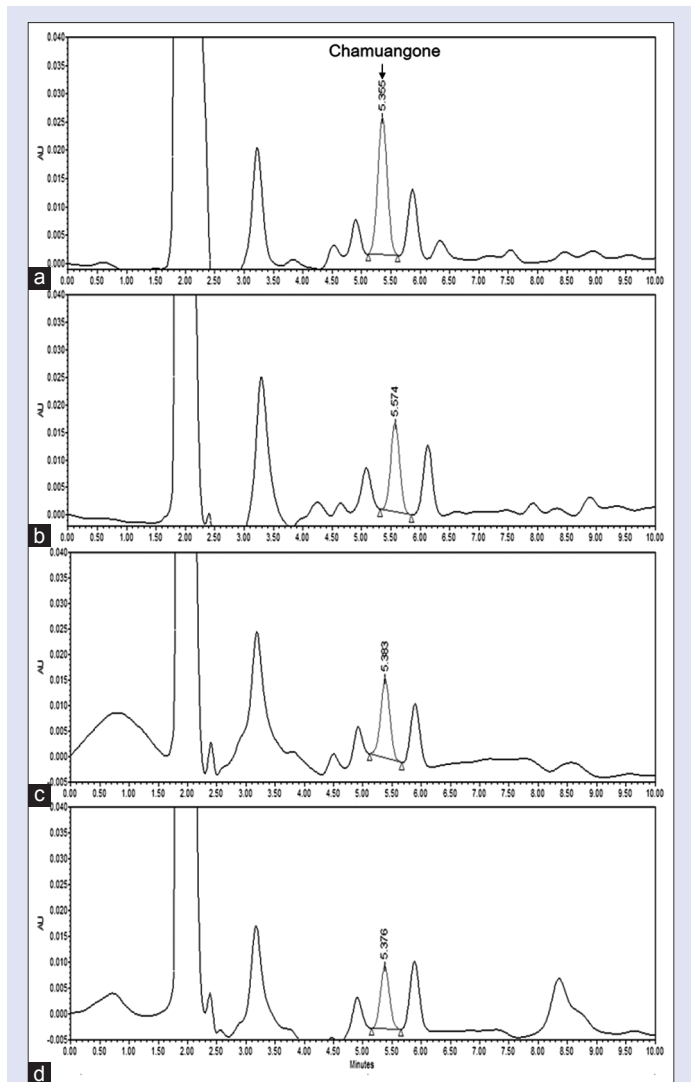


Figure 1: High-performance liquid chromatography chromatograms of chamuangone extracts using palm oil (a), rice bran oil (b), soybean oil (c), and sunflower oil (d) as the alternative green solvents

Table 1: Chamuangone content and yields of *Garcinia cowa* leaf extracts obtained from various extraction oils

Solvents	Mean±SD		
	Extraction yield (L/100 g dried powders)	Chamuangone content (mg/mL)	Total yield of chamuangone (mg/100 g dried powders)
<i>n</i> -Hexane	0.58±0.25	0.10±0.01	58.00±0.08
Palm oil	0.90±0.35*	0.08±0.01	72.00±0.15*
Rice bran oil	0.88±0.00*	0.06±0.00*	52.80±0.06
Soybean oil	0.86±0.35*	0.05±0.00*	43.00±0.06*
Sunflower oil	0.88±0.18*	0.04±0.00*	35.20±0.06*

*Significance difference ($P<0.05$) when compared with the data of *n*-hexane in the same column. SD: Standard deviation

Table 2: Effect of microwave powers on the chamuangone content and yields of chamuangone-enriched *Garcinia cowa* leaf extract with rice bran oil

Power (W)	Mean±SD		
	Extraction yield (L/100 g dried powders)	Chamuangone content (mg/mL)	Total yield of chamuangone (mg/100 g dried powders)
360	0.91±2.12	0.04±0.00	36.40±0.25
600	0.87±0.71*	0.05±0.00*	43.50±0.32*
800	0.74±1.77*	0.08±0.01*	59.20±0.06*

*Significance difference ($P<0.05$) when compared with the data of 360 W in the same column. SD: Standard deviation

Table 3: Effect of powder-to-solvent ratios on the chamuangone content and yields of chamuangone-enriched *Garcinia cowa* leaf extract with rice bran oil

Powder amount (g)	Mean±SD		Total yield of chamuangone content (mg)
	Extraction yield (mL)	Chamuangone content (mg/mL)	
10	73.75±1.77 ^a	0.08±0.01 ^a	6.08±0.60 ^a
20	74.00±0.71 ^a	0.13±0.00 ^a	9.69±0.15 ^b
30	62.00±0.71 ^b	0.20±0.00 ^b	12.40±0.17 ^c
40	49.25±0.35 ^c	0.28±0.00 ^c	13.71±0.22 ^{c,e}
50	59.25±0.63 ^d	0.35±0.00 ^d	20.35±0.26 ^d
60	39.50±0.71 ^e	0.40±0.04 ^d	15.78±1.41 ^e
70	26.25±1.06 ^f	0.49±0.03 ^e	12.79±0.87 ^{c,e}
80	18.50±0.71 ^g	0.53±0.01 ^{e,f}	9.76±0.16 ^b
90	11.50±0.71 ^h	0.60±0.04 ^f	6.85±0.46 ^a
100	6.50±0.58 ⁱ	0.67±0.05 ^g	4.38±0.33 ^a

The results were expressed as mean±SD of three determinations for each sample. Means followed by the same letter in the same column are not significantly different according to one-way, followed by Tukey's HSD test. The term significant has been used to denote the differences for which $P<0.05$. HSD: Honestly significant difference; ANOVA: Analysis of variance; SD: Standard deviation

Table 4: Effect of irradiation cycles on the chamuangone content and yields of chamuangone-enriched *Garcinia cowa* leaf extract with rice bran oil

Irradiation cycles	Mean±SD		Total yield of chamuangone (mg/100 g dried powders)
	Extraction yield (L/100 g dried powders)	Chamuangone content (mg/mL)	
2	0.12±0.14 ^a	0.34±0.00 ^a	40.80±0.07 ^a
3	0.12±0.57 ^a	0.36±0.01 ^{a,b}	43.20±0.59 ^b
4	0.12±0.99 ^a	0.37±0.00 ^{b,c}	44.40±0.03 ^{b,c}
5	0.12±0.41 ^a	0.38±0.01 ^c	45.60±0.32 ^c
6	0.11±0.28 ^a	0.39±0.02 ^c	42.90±0.97 ^b

The results were expressed as mean±SD of three determinations for each sample. Means followed by the same letter in the same column are not significantly different according to one-way ANOVA, followed by Tukey's HSD test. The term significant has been used to denote the differences for which $P<0.05$. HSD: Honestly significant difference; ANOVA: Analysis of variance; SD: Standard deviation

The extraction conditions were modified based on the small-scale MAE conditions described above. An extraction efficiency of MAE is usually dependent on the temperature of extraction, which relates to the radiation power and time. The present study showed that the extraction conditions needed four irradiation cycles (one cycle is 5-min power-on and 1-min power-off), at 150°C to obtain the CEO containing chamuangone concentration up to 1.97 ± 0.01 mg/mL. This CEO was used for further studies on nutrition facts and anticancer activity.

Determination of nutrient facts

There are several nutrients derived from plants, such as vitamins, antioxidants, and phytochemicals that provided health benefits as well as cancer prevention. The CEO was enriched in MUFAs (41.89 g/100 g) and PUFAs (33.99 g/100 g), contained a low content of saturated fat (20.32 g/100 g) and no trans fat. In addition, it contained high levels of several natural antioxidants, including α -tocopherol (76.73 mg/100 g), γ -oryzanol (cycloartenol ferulate: 67.1 μ g/mL and 24-methylenecycloartenol ferulate: 85.6 μ g/mL), chlorophyll a (149.30 mg/100 g), chlorophyll b (2.65 mg/100 g), and antioxidant compounds determined as ascorbic acid (258.72-mM ascorbic acid equivalent per gram). Therefore, CEO does not only lower the risk of cancer but it also lowers the risk of cardiovascular diseases.

Determination of anticancer activity

The extracts giving IC_{50} values lower than 30 μ g/mL were considered as having good cytotoxic activity.^[20] On the basis of SRB assay, chamuangone exhibited strong cytotoxic activity against HT-29 and good cytotoxic activity against MCF-7 and A549 with IC_{50} values of 0.02, 2.03, and 4.23 μ g/mL, respectively, and no toxicity to HGF at a concentration of 25 μ g/mL [Table 6]. Therefore, chamuangone was shown as selectively inhibiting the growth of the colorectal cancer cell. A similar, previously reported result showed that some polyisoprenylated benzophenones exhibited potential cytotoxic activity against several cancer cells, including breast, colon, pancreatic, and leukemia, with no cytotoxic effect against normal cells.^[21] This finding also agrees with a previous report on a selective antitumor activity of a polyisoprenylated compound that isolated from *G. cowa*.^[22] Although the CEO contained only 1.97 mg/mL chamuangone, it also showed satisfactory cytotoxicity to the cancer cells, with IC_{50} values of 12.82, 15.95, and 15.26 μ g/mL, respectively, and had no toxicity on HGF at a concentration of 50 μ g/mL. Thus, the anticancer effect of CEO might be enhanced by rice bran oil, which exhibited its own anticancer activity. Unlike chamuangone, the anticancer activity of CEO was not selective to HT-29. This may be due to a low concentration of chamuangone in CEO.

Table 5: Effect of consecutive extraction times on the chamuangone content and yields of chamuangone-enriched *Garcinia cowa* leaf extract with rice bran oil

Extraction times	Mean±SD		
	Extraction yield (L/100 g dried powders)	Chamuangone content (mg/mL)	Total yield of chamuangone (mg/100 g dried powders)
1	0.12±0.71 ^a	0.36±0.01 ^a	43.20±0.65 ^a
2	0.12±0.71 ^a	0.03±0.00 ^b	3.60±0.25 ^b
3	0.12±0.71 ^a	0.02±0.00 ^b	2.40±0.32 ^b
4	0.12±0.00 ^a	0.01±0.00 ^b	1.20±0.18 ^b

The results were expressed as mean±SD of three determinations for each sample. Means followed by the same letter in the same column are not significantly different according to one-way ANOVA, followed by Tukey's HSD test. The term significant has been used to denote the differences for which $P < 0.05$. HSD: Honestly significant difference; ANOVA: Analysis of variance; SD: Standard deviation

Table 6: Cytotoxic activities of chamuangone and chamuangone enriched against cancer and normal cell lines

Compounds	IC ₅₀ ±SD (µg/mL)			
	Cancer cell lines			Normal cell
	HT-29	MCF-7	A549	HGF
Chamuangone	0.02±2.48	2.03±0.46	4.23±2.53	NA
CEO	12.82±2.48	15.95±1.94	15.26±2.67	NA
Rice bran oil	34.04±3.17	29.57±1.96	30.06±1.48	NA
Camptothecin	<0.02	<0.02	<0.02	0.68±0.13

NA: Not active at 25 µg/mL for chamuangone, and 50 µg/mL for CEO contained 1.97 mg/mL chamuangone; HT-29: Human colorectal adenocarcinoma cell line; MCF-7: Human breast adenocarcinoma cell line; A549: Human lung adenocarcinoma cell line; HGF: Human gingival fibroblasts; CEO: Chamuangone-enriched *Garcinia cowa* leaf extract with rice bran oil; IC₅₀: 50% Inhibitory concentration

CONCLUSION

Rice bran oil can be used as an alternative green solvent for extraction of chamuangone from *G. cowa* leaf powders using MAE. The CEO can be directly used as a functional food without the step of solvent evaporation. This extraction method is a simple and low-cost production. The standardized CEO containing 1.97 mg/mL chamuangone exhibited strong cytotoxicity against HT-29, MCF-7, and A549 cells and no toxicity to normal cells HGF. It also showed satisfactory antioxidant capacity and fatty acid balance. Therefore, the CEO may be used as a novel nutraceutical.

Acknowledgements

We wish to thank Ms. Maria Mullet for help with English language editing.

Financial support and sponsorship

This research was financially supported by the Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program (grant number PHD/0129/2556).

Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin* 2016;66:7-30.
- Smith RA, Andrews K, Brooks D, DeSantis CE, Fedewa SA, Lortet-Tieulent J, *et al.* Cancer screening in the United States, 2016: A review of current American Cancer Society Guidelines and current issues in cancer screening. *CA Cancer J Clin* 2016;66:96-114.
- Newman DJ, Cragg GM. Natural products as sources of new drugs over the last 25 years. *J Nat Prod* 2007;70:461-77.

- Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. *CA Cancer J Clin* 2017;67:7-30.
- GBD 2016 Causes of Death Collaborators. Global, regional, and national age-sex specific mortality for 264 causes of death, 1980-2016: A systematic analysis for the global burden of disease study 2016. *Lancet* 2017;390:1151-210.
- Park EJ, Pezzuto JM. Botanicals in cancer chemoprevention. *Cancer Metastasis Rev* 2002;21:231-55.
- Subcharoen P. Handbook of Anticancer: Thai Traditional Medicine: New Concept for Treated Cancer. 1st ed. Bangkok: Thai Traditional Medicine Institute; 1998.
- Liu RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *Am J Clin Nutr* 2003;78:517S-20S.
- Sakunpak A, Matsunami K, Otsuka H, Panichayupakaranant P. Isolation of Chamuangone, a cytotoxic compound against *Leishmania major* and cancer cells from *Garcinia cowa* leaves and its HPLC quantitative determination method. *J Cancer Res Updat* 2017;6:38-45.
- Panichayupakaranant P, Sakunpak A. Quantitative HPLC analysis of chamuangone in *Garcinia cowa* leaf extracts. *Planta Med* 2013;79:PK29.
- Sakunpak A, Panichayupakaranant P. Antibacterial activity of Thai edible plants against gastrointestinal pathogenic bacteria and isolation of a new broad spectrum antibacterial polyisoprenylated benzophenone, chamuangone. *Food Chem* 2012;130:826-31.
- Agency for Toxic Substances and Disease Registry. Toxicological Profile for Hexane. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; 1999.
- Zayats MF, Leschew SM, Zayats MA. A novel method for the determination of some pesticides in vegetable oils based on dissociation extraction followed by gas chromatography-mass spectrometry. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* 2016;33:1337-45.
- Laupheimer M. Gelled Bicontinuous Microemulsions: A New Type of Orthogonal Self-Assembled Systems. New York: Springer International Publishing; 2014.
- Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, *et al.* New colorimetric cytotoxicity assay for anticancer-drug screening. *J Natl Cancer Inst* 1990;82:1107-12.
- Keawpradub N, Houghton PJ, Eno-Amooquaye E, Burke PJ. Activity of extracts and alkaloids of Thai *Alstonia* species against human lung cancer cell lines. *Planta Med* 1997;63:97-101.
- Wang DD, Hu FB. Dietary fat and risk of cardiovascular disease: Recent controversies and advances. *Annu Rev Nutr* 2017;37:423-46.
- Kusum R, Bommayya H, Fayaz PP, Ramachan-Dran HD. Palm oil and rice bran oil: Current status and future prospects. *Int J Plant Physiol Biochem* 2011;3:125-32.
- Wilson TA, Nicolosi RJ, Woolfrey B, Kritchevsky D. Rice bran oil and oryzanol reduce plasma lipid and lipoprotein cholesterol concentrations and aortic cholesterol ester accumulation to a greater extent than ferulic acid in hypercholesterolemic hamsters. *J Nutr Biochem* 2007;18:105-12.
- Vijayarathna S, Sasidharan S. Cytotoxicity of methanol extracts of *Elaeis guineensis* on MCF-7 and vero cell lines. *Asian Pac J Trop Biomed* 2012;2:826-9.
- Saadat N, Gupta SV. Potential role of garcinol as an anticancer agent. *J Oncol* 2012;2012:647206.
- Kan WL, Yin C, Xu HX, Xu G, To KK, Cho CH, *et al.* Antitumor effects of novel compound, guttiferone K, on colon cancer by p21Waf1/Cip1-mediated G (0)/G (1) cell cycle arrest and apoptosis. *Int J Cancer* 2013;132:707-16.