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ORIGINAL ARTICLE

A Green Method for Preparation of Curcuminoid-rich *Curcuma longa* Extract and Evaluation of its Anticancer Activity

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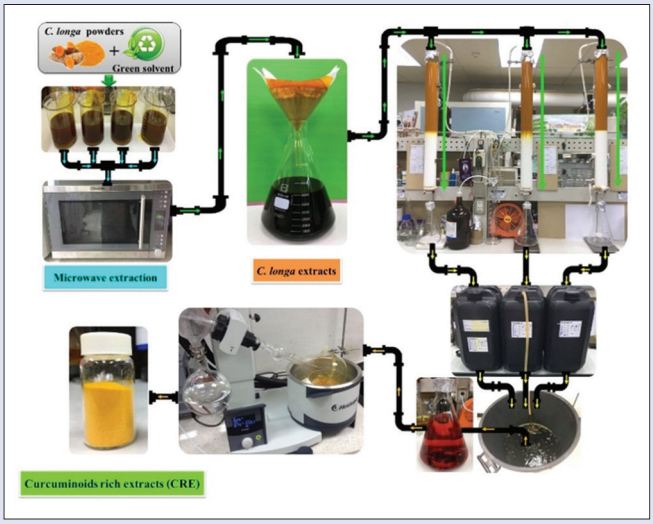
ABSTRACT

Background: Curcuminoids, i.e., curcumin, demethoxycurcumin, and bisdemethoxycurcumin are a major active constituent of *Curcuma longa* L., which possess antioxidant, anti-inflammatory, antitumor, anticancer, and various other biological activities. **Objective**: To establish a green method for preparation of curcuminoid-rich *C. longa* extracts (CRE) using microwave-assisted extraction (MAE) together with a simple one-step fractionation and to investigate the anticancer activity of CRE compared with the three marker curcuminoids. **Materials and Methods:** MAE was used as a green extraction method, and a macroporous resin (Diaion® HP-20) column was used for fractionation of *C. longa* extract to produce CRE. The sulforhodamine B assay was used to evaluate *in vitro* anticancer activity of the curcuminoids. **Results:** The optimal conditions of MAE for extraction of curcuminoids are employing ethanol as the solvent and using three irradiation cycles in a microwave powered at 900 W (one cycle is 3 min power-on and 30 s power-off). The curcuminoid extract was subsequently fractionated on a Diaion® HP-20 column eluted with 55% and 60% v/v ethanol, respectively, to obtain CRE that contained total curcuminoids of 88% w/w. CRE exhibited good anticancer activities against A549, MCF-7, HeLa, and HT-29 cells, with 50% inhibitory concentration values of 5.2, 4.5, 7.5, and 8.3 µg/mL, respectively, which almost equals those of the marker curcuminoids. **Conclusion:** This study indicated a potential use of CRE for anticancer purposes in food and nutraceutical applications. CRE has more advantages than pure curcuminoids for industrial applications in terms of using simple, low-cost, and environmentally friendly processes.

Key words: Anticancer, *Curcuma longa,* curcuminoid, green extraction, microwave

SUMMARY

- A low-cost green method has been established for curcuminoid-rich *Curcuma longa* extracts (CRE)
- CRE contains a high curcuminoid content of 88%w/w
- CRE exhibits good anticancer activities against A549, MCF-7, HeLa, and HT-29 cells
- CRE possesses good anticancer activities similar to the pure curcuminoids.



Abbreviations used: CRE: Curcuminoid-rich *Curcuma longa* extracts; Cu I: Curcumin; Cu II: Demethoxycurcumin; Cu III: Bisdemethoxycurcumin; DMEM: Dulbecco's modified eagle medium; FBS: Fetal bovine serum; HPLC: High performance liquid chromatography; IC_{50}: 50% inhibitory concentration; MAE: Microwave-assisted extraction; MHz: Megahertz; ODS: Octadecylsilane; PEG 400: Polyethylene glycol 400; PG: Propylene glycol; PBS: Phosphate buffer saline; SD: Standard deviation; SRB: Sulforhodamine B; W: Watt.

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INTRODUCTION

In 2012, the International Agency for Research on Cancer estimated that the worldwide cancer incidence and mortality rate among young people aged 20–39 years included 14.1 million new cancer cases, 8.2 million cancer deaths, and 32.6 million people living with cancer. It is estimated that these numbers will be increased to 26 million new cancer cases and 17 million cancer deaths per year by 2030.[1] Although much time and effort has been spent, cancer remains an offensive killer worldwide. During the last decade, various novel synthetic chemotherapeutic agents currently used clinically have not succeeded in fulfilling expectations despite the considerable cost of their development. Likewise, evidence for potential plant-derived compounds as inhibitors of various stages of tumorigenesis and

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inflammation-associated processes have underlined the importance of such compounds in cancer prevention and therapy.[2]

Turmeric (*Curcuma longa* L.) is a rhizomatous herbaceous perennial plant the ginger family, Zingiberaceae. In Ayurvedic medicines, turmeric is primarily used for treatment of inflammatory conditions. In traditional Chinese medicine, it is used as stimulant, carminative, emmenagogue, astringent, and diuretic.[3] Three major curcuminoids, curcumin (Cu I), demethoxycurcumin (Cu II), and bisdemethoxycurcumin (Cu III), have been isolated from *C. longa* [Figure 1].[4] These curcuminoids possess anticancer, antioxidant, anti-inflammatory, antimutagenic, antifungal, and antiviral activities.[3] To date, there are many studies, both *in vitro* and *in vivo*, indicating the anticancer or cancer prevention activities of curcuminoids, especially curcumin.[4-9] Curcuminoids or *C. long* extracts are, therefore, considered promising natural-occurring cancer-preventing agents.

Reports have shown that extractions with ethanol produced the highest yield of *C. longa* extracts, but their curcuminoid contents were very low, due to the presence of oleoresins and other non-volatile oils.[10] Acetone and ethyl acetate have also been widely used for extraction of curcuminoids, but these organic solvents are dangerous and expensive. Generally, the use of a pure natural compound is considered a limitation for industrial application because its purification process requires multiple steps and is time-consuming and expensive.[11] The conventional techniques used for preparation of *C. longa* extracts, such as the heat reflux extraction and maceration, are time-consuming and require large amounts of toxic organic solvents.[12] Recent trends in extraction techniques have largely focused on finding methods that minimize the use of solvents and energy, reduce steps of production, and produce high-quality herbal extract. We are, therefore, interested in the preparation of curcuminoid-rich *C. longa* extracts (CRE) using green extraction concepts.[13] In this study, some alternative green solvents, i.e., ethanol, propylene glycol (PG), polyethylene glycol 400 (PEG 400), and glycerin, were determined for extraction of curcuminoids using microwave-assisted extraction (MAE). A simple one-step fractionation using a macroporous resin (Diaion® HP-20) column was determined for preparation of CRE, and the anticancer activity of CRE against human cancer cell lines was also evaluated compared with its three marker curcuminoids.

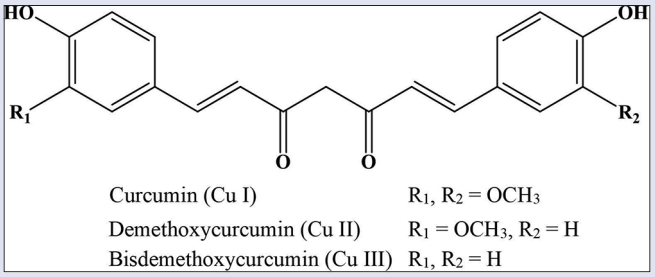
MATERIALS AND METHODS

Plant material

The dried powders of *C. longa* rhizomes were obtained from Bangkok Lab and Cosmetic Co, Ltd, Ratchaburi Province, Thailand, in October 2015. The rhizomes were identified by comparison with the herbarium specimen (Voucher No. 21.1.410.1.458), which was deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand.

Chemicals and reagents

Cu I, Cu II, and Cu III were previously purified.[14] Chloroform, methanol, and ethanol (analytical or high performance liquid chromatography [HPLC] grades) were purchased from Labscan Asia (Bangkok, Thailand). PG, PEG 400 and glycerin (pharmaceutical grade) were purchased from Witthayasom (Songkhla, Thailand). Formic acid was from J. T. Baker (NJ, USA). Water was purified using a Milli-Q system (Millipore, Bedford, MA, USA). Diaion® HP-20 resin from Sigma-Aldrich (Merck, Germany) was used for column. Phosphate buffer saline and Dulbecco's modified eagle medium (DMEM) were from Invitrogen (MA, USA), fetal bovine serum (FBS) was from Gibco (BRL, USA), sulforhodamine B (SRB) was from Sigma-Aldrich (Merck, Germany), and trichloroacetic acid was from Carlo Erba (Cornaredo MI, Italy).



Curcumin (Cu I)	$R_1, R_2 = OCH_3$
Demethoxycurcumin (Cu II)	$R_1 = OCH_3, R_2 = H$
Bisdemethoxycurcumin (Cu III)	$R_1, R_2 = H$

Figure 1: Chemical structure of curcuminoids

Cell lines

The human lung adenocarcinoma (A549, CLS No. 300114), human breast adenocarcinoma (MCF-7, CLS No. 300273), human colorectal adenocarcinoma (HT-29, CLS No. 300215), and human cervical adenocarcinoma (HeLa, CLS No. 300194) cell lines were obtained from the National Cancer Institute (Bangkok, Thailand).

Quantitative HPLC analysis of curcuminoids

Quantitative HPLC analysis of curcuminoids was modified from the method previously reported.[15] The method was carried out using a Shimadzu LC-20AD series equipped with a photodiode array detector and auto sampler (Shimadzu Co., Kyoto Japan). Separation was achieved within 20 min on a 250 mm × 4.6 mm TSK-gel ODS-80Ts column (Tosho Co., Tokyo, Japan), with an isocratic elution of acetonitrile and 0.1% aqueous formic acid (50:50 v/v) at a flow rate of 1.5 mL/min. The injection volume was 20 µL, and the detection wavelength was 405 nm. The calibration curves were established from the standards Cu I, Cu II, and Cu III at the concentration between 3.59 and 62.5 µg/mL. The linear equations of Y = 68224X − 55754 (r^2 =0.9999), Y = 92480X + 49438 (r^2 = 0.9999), and Y = 115830X − 56084 (r^2 =0.9999) corresponded to Cu I, Cu II, and Cu III, respectively.

Extraction of curcuminoids

Determination of solvent

MAE was carried out in a microwave oven (LG Electronics Inc., Thailand), with a microwave frequency and power of 2450 MHz and 180 W, respectively. The green solvents, including ethanol, PG, PEG 400, and glycerin (20 mL) were separately added into a 125 mL-Erlenmeyer flask containing *C. longa* powders (2.0 g), then mixed well and placed in the microwave irradiation cavity. The extraction process was performed under microwave irradiation for 30 s. The extracts were then filtered and subjected to the HPLC analysis for curcuminoid content. The experiment was performed in triplicate. The solvent that gave the highest content of curcuminoids was used for further experiment.

Determination of powder-to-solvent ratio

The dried powders of *C. longa* (1, 1.5, 2.0, and 1.5 g) were separately extracted with ethanol (20 mL) using the MAE as described above. The extracts were then filtered and subjected to the HPLC analysis for curcuminoid content. The experiment was performed in triplicate. The powder-to-solvent ratio that gave the highest content of curcuminoids was used for further experiment.

Determination of microwave power

The dried powders of *C. longa* (2.0 g) were extracted with ethanol (20 mL) under the MAE conditions described above, but with different microwave powers (180, 360, and 600 W). The extracts were then filtered and subjected to the HPLC analysis for curcuminoid content. Each experiment was performed in triplicate. The microwave

power that gave the highest content of curcuminoids was used for further experiment.

Determination of microwave irradiation cycles

The dried powders of *C. longa* (2.0 g) were extracted with ethanol (20 mL) using MAE at a microwave power of 180 W and varied microwave irradiation cycles for 1, 2, 3, and 4 cycles (one cycle: 30 s power-on and 30 s power-off). The extracts were then filtered and subjected to the HPLC analysis for curcuminoid content. The experiment was performed in triplicate. The microwave irradiation cycles that gave the highest content of curcuminoids were used for further experiment.

Determination of consecutive extraction times

The dried powders of *C. longa* (2.0 g) were extracted with ethanol (20 mL) using MAE at a microwave power of 180 W for 30 s. The extraction process was consecutively performed 3 times using the marc and fresh solvent. The obtained extracts were then subjected to the HPLC analysis for curcuminoid content. Each experiment was performed in triplicate.

Scale-up for preparation of curcuminoid extract

The dried powders of *C. longa* (240 g) were extracted with ethanol (2.4 L) using a MAE at 900 W and three irradiation cycles (one cycle: 3 min power-on; 30 s power-off). The extract was then filtered and subjected to further fractionation process.

Preparation of curcuminoid-rich Curcuma longa extracts

The curcuminoid extract was fractionated on a Diaion® HP-20 column to obtain the extracts enriched in curcuminoids. The Diaion® HP-20 (1 kg) was treated with ethanol and loaded into a column (8 × 100 cm). The column was washed twice with ethanol and then equilibrated with 55% v/v ethanol. The ethanol extract (1.5 L) was mixed with water (1.2 L) and loaded on the Diaion® HP-20 column, then eluted with 55% and 60% v/v ethanol, respectively. The fractions containing curcuminoids were pooled and dried under reduced pressure at 45°C to produce CRE.

In vitro anticancer assay

The cancer cell lines were cultured in DMEM supplemented with 10% heat-inactivated FBS and maintained at 37°C, 95% relative humidity, and CO_2 of <5%.

Anticancer activity was determined using the SRB assay previously described by Yuenyongsawad *et al.* (2014).[16] Briefly, the cells were seeded into 96-well microplates (4000 cells/well) and allowed to adhere for 24 h at 37°C in a 5% CO_2 incubator. Then, 100 µL of samples solution at various concentrations in medium were dispensed into the wells and incubated for 72 h. After removal of the medium, the bound cells were submerged in 200 µL of DMEM medium and further incubated for 72 h. The cells were then fixed with cold 40% trichloroacetic acid and kept at 4°C for 1 h and then washed with tap water. The cells were determined by the SRB assay. The absorbance was measured at 492 nm using a microplate reader (Power Wave X plate reader: Bio-TEK Instruments, Inc.). The activities were reported as 50% inhibitory concentration (IC_{50}) value. The IC_{50} value (effective concentration of the sample required to inhibit cell growth by 50%) was calculated from dose–response curves plotting between percentage inhibition and concentrations. Camptothecin was used as a positive control.

Statistics

Values were expressed as the mean ± standard deviation. The statistical significance was calculated by one-way analysis of variance, followed by Tukey's test. Values of $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Determination of green solvents for extraction

Although acetone and ethyl acetate have been previously described as the most appropriate solvent for extraction of curcuminoids from *C. longa* powders and most commonly used for preparation of curcuminoid extracts in the industrial production;[3] they are dangerous and expensive organic solvents. Therefore, the search for an alternative green solvent for preparation of curcuminoid extract is necessary. In the present study, some green solvents, i.e., ethanol, PG, PEG 400, and glycerin, were evaluated for their ability to extract curcuminoids from *C. longa* powders. The study found that ethanol produced the extract with the highest content of curcuminoids, followed by PG [Table 1]. This implies that besides the dielectric constant of solvents, viscosity of solvent and solubility of target compounds also play a major role in MAE. Therefore, ethanol was selected as a suitable green solvent for further studies on optimization of MAE conditions. However, using PG as an alternative green solvent, the obtained extract can be directly used for further drug formulations, for example, curcuminoid creams or curcuminoids in self-microemulsion systems, without solvent evaporation before use.

Optimization of microwave-assisted extraction conditions

The operating factors for MAE including the powder-to-solvent ratio, microwave irradiation power, microwave irradiation cycles, and consecutive extraction times were determined using single-factor experiments.

Different powder to solvent ratios of 1:20, 1.5:20, 2:20, and 2.5:20 g/mL were investigated for the extraction of curcuminoids using ethanol. An increase in the powder-to-solvent ratios resulted in an increase in the curcuminoid content of the extracts [Table 2]. However, the content of curcuminoids obtained from the ratio of 2.5 g/20 mL was not significantly different than that of 2 g/20 mL. This implied that a saturated capacity of ethanol for extraction of curcuminoids was found at the ratio of 2.5 g/20 mL. The powder-to-solvent ratio of 2 g/20 mL was therefore used for further study on the effect of microwave irradiation power.

Various microwave powers, i.e., 180, 360, and 600 W were examined for extraction of curcuminoids. The curcuminoid content of the ethanol extracts was not significantly increased by increasing the irradiation power from 180 W (70 ± 1°C) up to 600 W (75 ± 1°C) [Table 3]. The microwave irradiation power of 180 W was selected for further studies on the effects of microwave irradiation cycle.

The microwave irradiation cycles (one cycle: 30 s power-on and 30 s power-off) including 1, 2, 3, and 4 cycles were determined for increased curcuminoids content of the ethanol extract. However, the increased number of irradiation cycles did not increase the curcuminoid content [Table 4]. The temperatures during extraction were increased to 74 ± 1, 77 ± 1, and 79 ± 1°C when we increased radiation cycles to 2, 3, and 4 cycles, respectively. One cycle was enough for extracting curcuminoids by MAE. The results implied that neither the increased temperature (up to 79°C) nor time of extraction was capable of increasing curcuminoid extraction using the MAE. Therefore, the suitable temperatures for MAE extraction of curcuminoids using ethanol should be between 70°C and the boiling point of ethanol (78°C), and one cycle of irradiation is enough for extraction.

The number of consecutive extraction times was determined to check a successive extraction of the plant powders. The marc from the extraction was re-extracted twice using fresh ethanol. After the first extraction, the contents of curcuminoids were very low [Table 5]. Thus, consecutive extractions using fresh solvents are not worthwhile.

The optimal conditions of MAE for small-scale preparation of curcuminoid extracts are as follows: A single extraction of 2 g *C. longa* powders extracted with 20 mL ethanol at a microwave power of 180 W for 30 s at a temperature of 70 ± 1°C. These findings support MAE as a green extraction method that reduces energy and time consumption and obtains a high quality of extract.

Scale-up for preparation of curcuminoid extract

MAE conditions were optimized for an extraction of 240 g *C. longa* powder with 2.4 L ethanol using a 900 W microwave. The extraction conditions were optimized based on the suitable small-scale MAE conditions described above using the extraction temperature as a key condition. The present study revealed that the MAE conditions needed three irradiation cycles (one cycle: 3 min power-on and 30 s power-off) at 75 ± 1°C to obtain curcuminoid extracts with a total curcuminoid content of 7.78 ± 0.74 mg/mL (Cu I, Cu II, and Cu III were 4.93 ± 0.48, 1.51 ± 0.13, and 1.34 ± 0.01 mg/mL, respectively) that was not significantly different to those of small-scale extraction. This curcuminoid extract in the solution form was used for preparation of CRE. After evaporation, the crude ethanol extract contained a total curcuminoid content of

Table 1: Curcuminoid content of *Curcuma longa* extracts, extracted with the microwave-assisted extraction using various solvents

Solvents	Curcuminoid content (mg/mL)				Volume of extract (mL)	Total curcuminoids (mg)
	Cu I	Cu II	Cu III	Total		
Ethanol	3.58±0.19	1.10±0.04	1.01±0.01	5.68±0.24[a]	15	85.2±3.6
PG	2.59±0.19	0.83±0.06	0.77±0.05	4.18±0.29[b]	15	62.7±4.4
PEG 400	2.81±0.15	0.66±0.04	0.58±0.04	3.42±0.22[c]	14	47.9±3.1
Glycerin	ND	ND	0.11±0.01	0.11±0.01[d]	9	0.99±0.09

Means followed by the same letter within a column are not significantly different according to Tukey's test. Values of $P<0.05$ were considered statistically significant. ND: Not detected; PM: Propylene glycol; PEG 400: Polyethylene glycol 400; Cu I: Curcumin; Cu II: Demethoxycurcumin; Cu III: Bisdemethoxycurcumin

Table 2: Curcuminoid content of *Curcuma longa* extracts, extracted with the microwave-assisted extraction using various powders to solvent ratios

Ratio (g/20 mL)	Curcuminoid content (mg/mL)				Volume of extract (mL)	Total curcuminoids (mg)
	Cu I	Cu II	Cu III	Total		
1.0	2.42±0.08	0.71±0.02	0.65±0.02	3.78±0.12[a]	15	56.7±1.8
1.5	3.57±0.07	1.03±0.02	0.96±0.02	5.56±0.11[b]	15	83.4±1.6
2.0	4.71±0.13	1.38±0.07	1.26±0.07	7.35±0.19[c]	14	102.9±2.7
2.5	4.76±0.06	1.44±0.02	1.33±0.04	7.52±0.13[c]	14	105.3±1.8

Means followed by the same letter within a column are not significantly different according to Tukey's test. Values of $P<0.05$ were considered statistically significant. Cu I: Curcumin; Cu II: Demethoxycurcumin; Cu III: Bisdemethoxycurcumin

Table 3: Curcuminoid content of *Curcuma longa* extracts, extracted with the microwave-assisted extraction using various irradiation powers

Irradiation power	Curcuminoid content (mg/mL)				Volume of extract (mL)	Total curcuminoids (mg)
	Cu I	Cu II	Cu III	Total		
180 W	4.43±0.13	1.30±0.03	1.16±0.01	6.89±0.17[a]	14	96.5±2.4
360 W	4.29±0.32	1.26±0.09	1.14±0.09	6.69±0.50[a]	14	93.7±7.0
600 W	4.40±0.30	1.33±0.03	1.16±0.10	6.89±0.42[a]	14	96.5±5.9

Means followed by the same letter within a column are not significantly different according to Tukey's test. Values of $P<0.05$ were considered statistically significant. Cu I: Curcumin; Cu II: Demethoxycurcumin; Cu III: Bisdemethoxycurcumin

Table 4: Curcuminoid content of *Curcuma longa* extracts, extracted with the microwave-assisted extraction using various irradiation cycles

Irradiation cycles	Curcuminoid content (mg/mL)				Volume of extract (mL)	Total curcuminoids (mg)
	Cu I	Cu II	Cu III	Total		
1	4.27±0.06	1.27±0.02	1.14±0.02	6.68±0.09[a]	14	93.5±1.3
2	4.37±0.03	1.30±0.01	1.17±0.01	6.84±0.04[a]	14	95.8±0.6
3	4.41±0.09	1.31±0.02	1.19±0.02	6.91±0.13[a]	14	96.7±1.8
4	4.41±0.18	1.24±0.05	1.14±0.05	6.56±0.28[a]	14	91.8±3.9

Means followed by the same letter within a column are not significantly different according to Tukey's test. Values of $P<0.05$ were considered statistically significant. Cu I: Curcumin; Cu II: Demethoxycurcumin; Cu III: Bisdemethoxycurcumin

Table 5: Curcuminoid content of *Curcuma longa* extracts, extracted with the microwave-assisted extraction using various consecutive extraction times

Extraction times	Curcuminoid content (mg/mL)				Volume of extract (mL)	Total curcuminoids (mg)
	Cu I	Cu II	Cu III	Total		
1	4.48±0.21	1.32±0.06	1.17±0.05	6.97±0.32[a]	14	97.6±4.5
2	0.29±0.01	ND	ND	0.29±0.01[b]	15	4.4±0.2
3	ND	ND	ND	ND	15	ND

Means followed by the same letter within a column are not significantly different according to Tukey's test. Values of $P<0.05$ were considered statistically significant. ND: Not *detected*; Cu I: Curcumin; Cu II: Demethoxycurcumin; Cu III: Bisdemethoxycurcumin

27.60% w/w DW, in which the amount of Cu I, Cu II, and Cu III were 17.56%, 5.34%, and 4.70% w/w, respectively [Table 6].

Preparation of curcuminoid-rich *Curcuma longa* extracts

The curcuminoid extracts were fractionated on a Diaion® HP20 column eluted with 55%–60% v/v ethanol in water to produce the CRE. This method can increase the total content of curcuminoids from 27.60% w/w of the crude ethanol extract to be 88% w/w of CRE, with a markedly increased content of curcumin up to 72.81% w/w [Table 6]. The other compounds including oleoresins and other pigments were noticeably excluded. Diaion® HP20 resin is a polyaromatic adsorbent resin for isolation of hydrophobic compounds. This is the first report using a Diaion® HP20 column chromatography as a simple one-step for purifying curcuminoids from *C. longa* extract. This method could be considered as a green, low-cost method due to the use of only green solvents for column elution and the reuse of Diaion® HP20 resin.

Anticancer activity of curcuminoid-rich *Curcuma longa* extracts

The cytotoxic activity of CRE were evaluated against four human cancer cell lines, A549, MCF-7, HeLa, and HT-29 cells compared with the crude ethanol extract and three marker curcuminoids. CRE exhibited good anticancer activity against A549, MCF-7, HeLa, and HT-29 cells, with the IC_{50} values of 5.18, 3.46, 2.73, and 7.66 μg/mL, which is better activity than that of the crude ethanol extract, but similar or a bit lower than those of the three marker curcuminoids [Table 7]. This implies that an increase in curcuminoid content of the extract results in increased cytotoxicity against cancer cells. However, each curcuminoid exhibited different potency to the anticancer activity. All three pure curcuminoids exhibited good anticancer activity against all tested cancer cells, but Cu II and Cu III exhibited their strongest potency against HT-29 and MCF-7 cells, respectively. In addition, CRE also showed the strongest anticancer activity against HT-29 cells, like Cu II. This may be due to an increased Cu II content in CRE when compared to the crude ethanol extract. It has been reported that a commixture of Cu I and Cu II is more stable than pure Cu I.[17] Thus, preparation of CRE may improve the stability of Cu I in the extract. In addition, Cu II induced apoptosis of human lung cancer (NCI-H460),[18] and inhibited cell proliferation and invasion of human breast cancer (MDA-MB-231)[19] and human prostate cancer (PC-3)[20] cells. The anticancer activities of curcuminoids have been reported through multiple mechanisms, for example, induced apoptosis by downregulated Bcl-2, BCL-xl, and miR-21expression as well as upregulating the caspase-3 and PTEN/Akt signaling pathways[21-24] and inhibition of migration and invasion of cancer cells through the inhibition of matrix metalloproteinase-2 and -9.[25]

Table 6: Curcuminoid content of crude ethanol extract and curcuminoid-rich *Curcuma longa* extracts

Extracts	Yield (%w/w)	Curcuminoid content (% w/w dry weight)			
		Cu I	Cu II	Cu III	Total
Ethanol extract	19.0	17.56±0.33	5.34±0.07	4.70±0.14	27.60±0.36[a]
CRE	3.4	72.81±0.83	12.49±0.57	4.24±0.16	88.92±0.70[b]

Means followed by the same letter within a column are not significantly different according to Tukey's test. Values of $P<0.05$ were considered statistically significant. CRE: Curcuminoid-rich *Curcuma longa* extracts; Cu I: Curcumin; Cu II: Demethoxycurcumin; Cu III: Bisdemethoxycurcumin

Table 7: Cytotoxic activity of pure curcuminoids and extracts against human cancer cells

Extract/ compounds	IC_{50} (μg/mL)			
	A549	MCF-7	HT-29	HeLa
Cu I	2.96±0.43[a]	3.39±0.13[a]	4.36±0.18[a]	7.45±0.42[a]
Cu II	2.62±0.15[a]	3.06±0.14[b]	2.77±0.13[b]	6.21±0.22[b]
Cu III	2.79±0.09[a]	2.37±0.17[c]	3.06±0.22[c]	5.95±0.07[c]
CRE	5.18±0.39[c]	3.46±0.37[a]	2.73±0.25[b]	7.66±0.13[a]
Crude extract	12.62±0.51[b]	12.95±0.64[d]	7.57±0.30[d]	12.23±0.39[d]
Camptothecin	<0.02	<0.02	<0.02	<0.02

Means followed by the same letter within a column are not significantly different according to Tukey's test. Values of $P<0.05$ were considered statistically significant. CRE: Curcuminoid-rich *Curcuma longa* extracts

CONCLUSION

The present study has established a low-cost green extraction and fractionation method for preparation of CRE containing a curcuminoid content of 88% w/w. The method requires MAE coupled with the simple one-step fractionation method using a macroporous resin (Diaion® HP-20) column. The standardized CRE possessed good anticancer activities, similar to the pure curcuminoids. Although additional toxicity and clinical studies are required, this method should be considered as a promising candidate for anticancer agent commercialization.

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Conflicts of interest

There are no conflicts of interest.

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