Antioxidant Activity, Total Phenolic Contents, Total Flavonoid Contents and Cytotoxicity of Spent Coffee Ground Extracts from Different Extraction Methods

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Abstract

Spend coffee ground (SCG) composed of various active compounds. The aim of this work was to prepare spend coffee extracts by using different extraction methods including microwave assisted extraction and reflux. Different SCG/ethanol (w/v) ratios were 1:20, 1:30, 1:40. All extracts were evaluated for the antioxidant activity, total phenolic contents and total flavonoid contents. The results revealed that the antioxidant activity by DPPH assay (9.57±0.9 mmol Trolox eq/g dry weight) and total phenolic contents (14.34±0.55 mg gallic acid eq/g dry weight) of SCG extract prepared at the ratio of 1:30 w/v using microwave assisted extraction were higher than those of the SCG extracts. Interestingly, the total flavonoids of SCG extract performed at the ratio of 1:20 w/v (25.84±1.4 mg quercetin/g dry weight) using reflux for 2 hours was higher than those of SCG extracts. All SCG extracts were tested the cytotoxicity against the human breast cancer (MCF-7) and human small lung (NCl-H187). The results demonstrated that SCG extract using reflux for 2 hours displayed the strongest cytotoxicity, being more active against MCF-7 (IC_{50} 49.46±0.08 µM) and NCl-H187 (IC_{50} 34.05±0.12 µM).

Keywords: *Spent coffee ground, Cytotoxicity, Total phenolic contents, Antioxidant* activity

.1 Introduction

Nowadays, there is a great demand for the herbal industries such as pharmaceutical and cosmetic products. In this work, spent coffee ground (SCG) is used as the raw material for studying the bioactivities of SCG extract including antioxidant activity, total phenolic contents and total flavonoid contents and cytotoxicity. Fresh SCG, a dark colored waste, is the main residues from the coffee industry and also coffee shop. Although SCG is waste, it still has various active substances. For example, SCG contains 15.2–17.9% of lipids depending on the coffee species [1]. In addition, SCG was found to be rich in several polyphenol compounds with high antioxidant activity and anti-tumor activity [2]. Thus, the presence of bioactive compounds in SCG could be of great interest for the food, cosmetic and pharmaceutical industries. Moreover, SCG has been reported to be the material for biodiesel production [3] and adsorbents the cationic dyes [4].

Extraction is the first step to separate the desired natural products from the raw materials. Solvent extraction is one of the most widely used extraction methods. Many factors will affect and enhance the extraction efficiency in the solvent extraction for example the solvent-to-solid ratio, type and polarity of solvent, extraction temperature and extraction time etc [5]. Many organic solvents can be selected for extraction based on the law of similarity of a polarity value of solvent and solute. Moreover, selectivity, solubility, cost and safety should be considered in selection of solvents. For extraction methods, both the conventional extraction methods (maceration, percolation and reflux extraction) and novel extraction methods (microwave assisted extraction (MAE), super critical fluid extraction (SFC), pressurized liquid extraction (PLE) have also been applied in natural products extraction. In this work, two solvent extraction methods including reflux and MAE were performed to extract the SCG and compared the efficacy extraction methods from their bioactivities [5].

The objective of this work was to prepare SCG extracts by using reflux and MAE. All extracts were evaluated for the antioxidant activity, the total phenolic contents, the total flavonoids and also determined the 5-caffeoylquinic acid. Moreover, the anticancer activity of these SCG extracts were evaluate in two cell lines including human breast cancer (MCF-7) and human small lung (NCl-H187), respectively.

.2 Materials and Methods

The fresh SCG (Arabica) was obtained from coffee shop in Faculty of Science, Maejo University. The source of coffee beans has gotten the organic brand from U.S. department of agriculture (USDA). All chemical substances and solvents were purchased from Merck, Labscan and Sigma aldrich.

2.1 Extraction of spent coffee ground (SCG)

The fresh SCG was dried at 65°C and milled into flour by passing through a 60-mesh sieve (Figure 1). Extraction experiments were carried out by the reflux extraction, using aqueous ethanol (70%) as solvent, solid/solvent ratios (1:20, 1:30, and 1:40 w/v), and extraction times (0.5, 1, 1.5, 2.0, 3.0 hrs). Briefly, 1 g of spent coffee ground was extracted with 70% ethanol at 70-75°C at different extraction times (0.5-2.5 hrs). All spent coffee ground extracts were called SCG. All ethanolic SCG extracts were concentrated under reduced pressure by a rotary vacuum evaporator at 70°C and then freeze dry to obtain SCG extracts. The influence of these operational variables on the content of total phenolic content, antioxidant activity and total flavonoid content of all SCG extracts was evaluated. All experiments were run in triplicates. Moreover, 5-caffeoylquinic acid (5-CQA) was also quantified using high performance liquid chromatography.

Figure 1 The characteristic of spend coffee ground.

Fresh spent coffee ground was dried at 65°C and milled into flour by passing through a 60-mesh sieve. Microwave assisted extraction was conducted using modified Toshiba microwave oven, ER-SGS34 TH, output 1,000 W (Figure 2). The extraction variables were microwave power (200, 300, 400, 500, 600 W) and the extraction time was 180 seconds, sample mass (2.0 g of SCG) using 70% ethanol. Spent coffee ground was prepared by using MAE in different SCG/ ethanol (w/v) ratios 1:20, 1:30, and 1:40, respectively. Extracts were cooled to room temperature and were slowly transferred to the 15ml centrifuge tubes and centrifuged at 4000 rpm for 10 mins. Supernatant of the extracts were collected using whatman no.1 filter paper and concentrated under reduced pressure by a rotary vacuum evaporator at 65°C before freezing dry to obtain SCG extracts. The percentage of extraction yield and their bioactivities of all SCG extracted were evaluated and compared to the previous work [6-8].

Figure 2 The modified microwave, Toshiba microwave oven.

.22 Determination of antioxidant activity

The antioxidant activity of the extracts was evaluated on the basis of the scavenging activity of the DPPH (1,1-diphenyl 2-picrylhyorazyl free radical) stable radical, as described in Brand-Williams et al. [9-10]. Briefly, a total of 100 mg of dry extract was leached using 100 ml of ethanol and 0.1% HCl. The filtrate was filtered using filter paper. Aliquots of 0.5 mM DPPH in ethanol were mixed with 1.0 ml of the extracts. A blank was prepared by just adding DPPH, distilled water and ethanol 1.0 ml each. All the solutions were kept in dark for 10 mins. OD was measured at absorbance of 517 nm. Data were expressed as mmol trolox equivalents (eq) per gram of sample (dry weight SCG). All experiments were run in triplicates.

.23 Determination of total phenolic contents

The total phenolic contents were determined according to the Folin-Ciocalteau assay [11-12]. A total of 100 mg of dry extracts was leached using 100 ml of ethanol and 0.1% HCl. The filtrate was filtered using filter paper. A 0.5 ml of the extract or gallic acid was added to test tubes containing 1.5 mL of distilled deionized (DI) water. A reagent blank of DI water was prepared. Folin - Ciocalteu's phenol reagent (0.5 mL of 0.1 M) was added to the mixture and shaken. After 5 min, 2 mL of 15% sodium carbonate solution was added to the mixture. The solution was diluted with DI water and mixed. After incubation for 1 hour at 37 °C, OD was measured at absorbance of 750 nm. The total phenolic contents of all extracts were

expressed as milligrams of gallic acid equivalents per gram of sample. All experiments were run in triplicates.

.24 Determination of total flavonoid contents

A total of 100 mg of dry extract was leached using 100 ml of ethanol and 0.1% HCl. The filtrate was filtered using filter paper. For the assay of total flavonoid contents, 15 µl of extract was mixed with 125 µl DI water and 10 µl of 5% sodium nitrite. This mixture was incubated at room temperature for 6 min, after which 10 µl of 10% aluminum chloride was added and incubated for 5 min. Finally, 50 µl of 1M NaOH was added, followed by incubation at room temperature for 15 min. The absorbance of the well-mixed mixture was measured at 510 nm. The total flavonoid contents of all extracts were calibrated with a standard curve of catechin and expressed as milligrams of catechin equivalent per gram of sample (mg CE/g of sample) [13]. All experiments were run in triplicates.

.25 5-Caffeoylquinic acid determinations

5-Caffeoylquinic acids was analysed by high performance liquid chromatography (HPLC) at room temperature. HPLC analysis was achieved with an analytical HPLC unit model 1100 (Agilent Technologies, Palo Alto, CA, USA) equipped with an automated sample injector. A reversed-phase Hypersil-ODS (5 µm particle size, 250 x 4.6 mm) column was used at 25 ºC and the sample injection volume was 100 µL. The chromatographic separation was performed using a gradient of methanol and Milli-Q water acidulated with phosphoric acid (pH 3.0) at a constant flow of 0.8 mL/min. The solvent mixture was degassed in an ultrasonic bath before to be used as mobile phase. Detection was accomplished with a diode-array detector, and chromatograms were recorded at 325 nm for caffeoylquinic acids. Identification of chlorogenic acid was performed by comparing the retention time and the UV/VIS spectra with that of the reference compounds. Results were expressed as milligrams of 5-caffeoylquinic acids per gram of spent coffee dry matter (spent coffee extracts) [14-15].

.26 Cytotoxicity

Cytotoxicity was determined by a tetrazolium dye-based microtitration assay [16]. All extracts were tested for cytotoxic activity against two cell lines including breast cancer (MCF-7) and human small lung (NCI-H187) for the evaluation of growth inhibition. Cell line growth was monitored using the MTT assay as reported and compared with doxorubicin as a positive control [17-19]. All experiments were run in triplicates.

3. Results and discussion

The present study was undertaken to evaluate the technical feasibility of recovering the active compound such as phenolic compounds contained in SCG by a cost-effective extraction process. SCG was extracted using two different extraction methods including reflux extraction and MAE using aqueous ethanol (70% v/v). Bioactivities of SCG extracts were investigated such as antioxidant activity, total phenolic contents, total flavonoid contents, quantity of 5-CQA and cytotoxicity.

3.1 Extraction of SCG

For reflux extraction, SCG extracts were extracted using different SCG/ethanol (w/v) ratios were 1:20, 1:30, 1:40. The extraction times were varied at 0.5, 1.0, 1.5, 2.0 and 3.0 hrs. The results of percentage yield of SCG extracts and their bioactivities were shown in Table 1.

Table 1 The percentage yields and bioactivities of SCG extracts from reflux extraction.

Extraction time (hrs)	SCG/ethanol (w/v) ratios	Yield and Bioactivities			
		%yield	Total phenolic content (mg gallic acid eq/g dry weight)	DPPH assay (mmol Trolox eq/g dry weight)	Total flavonoid content (mg quercetin/g dry weight)
0.5	1/20	8.41±0.10	6.10±0.05	6.65±0.20	17.72±1.10
	1/30	8.84±0.11	6.40±0.11	6.99±0.31	18.02±0.40
	1/40	9.09±0.05	7.51±0.32	7.10±0.45	18.93±1.15
1.0	1/20	8.45±0.20	7.33±0.06	7.43±0.76	19.09±1.50
	1/30	8.89±0.41	7.95±0.76	7.55±0.01	21.01±0.07
	1/40	9.13±0.45	8.33±1.33	7.58±0.41	21.88±0.11
1.5	1/20	8.89±0.22	8.59±0.11	7.64±0.72	22.10±0.23
	1/30	8.94±0.35	9.15±0.65	7.72±0.44	23.10±0.12
	1/40	9.19±0.04	10.00±0.10	7.78±0.11	23.91±0.09
2.0	1/20	9.23±0.11	10.02±0.02	7.89±0.15	25.84±1.40
	1/30	9.23±0.08	10.13±0.05	8.02±0.66	23.95±0.40
	1/40	9.25±0.11	10.19±0.20	8.11±0.32	22.09±0.17
3.0	1/20	8.95±0.72	10.03±0.55	7.81±0.50	21.87±0.23
	1/30	8.97±0.23	10.11±0.04	7.83±0.92	20.99±0.05

Extraction time (hrs)	SCG/ethanol (w/v) ratios	Yield and Bioactivities			
		%yield	Total phenolic content (mg gallic acid eq/g dry weight)	DPPH assay (mmol Trolox eq/g dry weight)	Total flavonoid content (mg quercetin/g dry weight)
	1/40	9.02±0.14	10.12±0.01	7.80±0.26	20.07±0.11

The results showed that the percentage yield of SCG extract from reflux extraction using SCG/ethanol ratio of 1:40 w/v and at extraction time 2.0 hrs exhibited the maximum values. For reflux extraction using 70% ethanol in a solvent/SCG ratio of 40 ml/g SCG, during 2 hrs, was the most suitable condition to produce a SCG extract with high content of phenolic compounds (10.19±0.20 mg gallic acid equivalents/g dry weight) and high antioxidant activity (DPPH assay of 8.11±0.32 mmol trolox eq/g dry weight), simultaneously. In addition, the highest total flavonoid contents was found in a solvent/solid ratio of 20 ml/g SCG, during 2 hrs. The previous literature since extraction of AS and SCG was generally performed in different such as water, ethanol or methanol as solvents.

For MAE, experimental conditions such as SCG/ethanol (w/v) ratios and microwave power were considered for microwave assisted extraction. SCG extracts were extracted using different SCG/ethanol (w/v) ratios were 1:20, 1:30, 1:40, respectively. Power of microwave was varied at 200, 300, 400, 500, 600 watts and the same extraction time for 180 seconds. The results of percentage yield of SCG extracts and their bioactivities were shown in Table 2.

Table 2 The percentage yields and bioactivities of SCG extracts from MAE.

SCG/ethanol (w/v) ratios	Power of microwave (watt)	Yield and Bioactivities			
		%yield	Total phenolic content (mg gallic acid eq/g dry weight)	DPPH assay (mmol trolox eq/g dry weight)	Total flavonoid content (mg quercetin/ g dry weight)
1/20	200	9.01±0.11	8.40±0.81	7.14±1.50	17.55±0.08
	300	9.04±0.04	8.99±1.20	7.82±0.20	17.73±1.11
	400	9.09±0.22	10.20±0.05	8.01±0.44	17.63±0.40
	500	9.10±0.09	9.87±0.50	7.53±0.82	17.56±0.42

SCG/ethanol (w/v) ratios	Power of microwave (watt)	Yield and Bioactivities			
		%yield	Total phenolic content (mg gallic acid eq/g dry weight)	DPPH assay (mmol trolox eq/g dry weight)	Total flavonoid content (mg quercetin/ g dry weight)
	600	9.11±0.42	9.58±0.11	7.02±0.11	17.33±0.15
1/30	200	9.10±0.13	12.20±0.04	7.84±0.33	17.53±0.33
	300	9.48±0.53	13.88±0.50	8.68±0.42	19.81±0.23
	400	9.37±0.41	14.34±0.55	9.57±0.90	19.74±0.15
	500	9.32±0.23	13.87±0.90	9.13±0.21	19.53±0.05
	600	9.42±0.22	12.72±1.22	8.68±0.64	19.40±0.11
1/40	200	9.15±0.05	11.57±0.72	8.42±0.93	17.58±0.82
	300	9.21±0.14	13.13±0.50	8.98±0.64	19.69±0.95
	400	9.28±0.82	13.52±0.41	9.03±0.10	19.57±0.01
	500	9.26±0.33	13.04±0.22	8.88±1.20	19.50±0.04
	600	9.23±0.03	12.21±1.05	8.71±1.20	19.39±0.67

The results showed that the highest percentage yield of SCG extract (9.48±0.53%) was obtained from the solvent/SCG ratio of 30 ml/g SCG and 300 W power within 180 seconds. Interesting, the highest values of total phenolic contents (14.34±0.55 mg gallic acid equivalents/g dry weight) and DPPH assay (9.57±0.90 mmol trolox eq/g dry weight) were carried out under the following combinations of solvent/SCG ratio of 30 ml/g SCG and 400 W power for 180 seconds. Similarly, for MAE the highest value of total flavonoid contents was recorded at same ratio of solvent/SCG ratio of 30 ml/g SCG, 300W for 180 seconds.

The SCG extraction methods have reported in many techniques sometimes, conventional methods, microwave or ultrasound extractions in order to improve the extraction process [10, 13]. Each methods have been evaluated both total phenolic contents (mg gallic acid equivalents/g SCG) and total flavonoid contents (mg quercetin/g SCG) and also determined the antioxidant activities by *in vitro* assays including, ferric reducing antioxidant power (FRAP), DPPH and 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assays. The extraction methods have been reported for SCG extracts for example soxhlet, solid liquid extraction, solid state cultivation of microorganisms and mild hydrothermal pre-treatment. The variable conditions were considered including solvents (water, aqueous methanol and aqueous ethanol), extraction times and temperatures, ratios of solvent to solid (w/v) and microorganisms. The value of total phenolic contents ranged from 6.50 to 28.26 mg gallic acid equivalents/g SCG [20]. Concerning antioxidant activity of SCG extracted

measured through the ABTS assay (1.9-16.6 mg trolox eq/g) and DPPH assay (2.0-4.0 mg trolox eq/g), the results indicated that antioxidant activity detected by ABTS assay was significantly higher if compared to that by DPPH assay and, in agreement with the findings of other authors [21-22]. The total flavonoid contents of SCG extracts were evaluated in all SCG extracts. The highest total flavonoid contents were investigated under the adopted optimal conditions of reflux extraction for 2 hrs and the ratio of solvent to solid at 1/20 w/v which were higher than that extracted using MAE. The total flavonoid contents declined with longer time, similarly as in the case of total phenolic contents for reflux extraction.

In this work, conventional extraction techniques like reflux extraction and novel extraction techniques like MAE were used for the extraction of SCG. The conventional and novel extraction techniques were compared based on various parameters like extraction times, ratio of solid to solvent and microwave power and observed the results of extraction yields and bioactivities. The results revealed that MAE was found to be better than reflux extraction as MAE requires less time, consume less solvent and give higher percentage yields of SCG extracts. In addition, the amount of total phenolic contents and antioxidant activity using DPPH assay of SCG extracts were higher in MAE than for the reflux extraction except total flavonoid contents.

.32 Optimization of the extraction of SCG

In order to achieve maximum yield and bioactivities of the SCG extract from reflux extraction and MAE, several factors were investigated using 70% ethanol as a solvent. For reflux extraction, the higher value of percentage yield, total phenolic contents, antioxidant activity were found when increasing the extraction time from 0.5-2.0 hrs and solvent/SCG ratio of 20-40 ml/g SCG. Applying extraction times longer than 2.0 hrs a constant decline in the extraction yield and bioactivities of SCG extract could be notice. This effect can be explained that longer exposure to high temperature probably resulted in degradation of the active or desired compounds [23-24].

The influence of the microwave power on the total phenolic contents and antioxidant activity of SCG extract was examined by varying two factors including power of microwave from 200 W to 600 W and ratio of SCG to solvent (w/v) as shown in Figure 3 and Figure 4, respectively. The results indicated that the highest total phenolic contents and antioxidant activity were observed in the same microwave power at 400 W using SCG to solvent (1:30 w/v). Applying microwave power higher than 400 W decrease in the total phenolic contents and antioxidant activity could be notice. These results were difference from the previous literature which mentioned that in general, it is considered that microwave power has a minor influence on the extraction yield, total phenolic contents and antioxidant activity [23, 25].

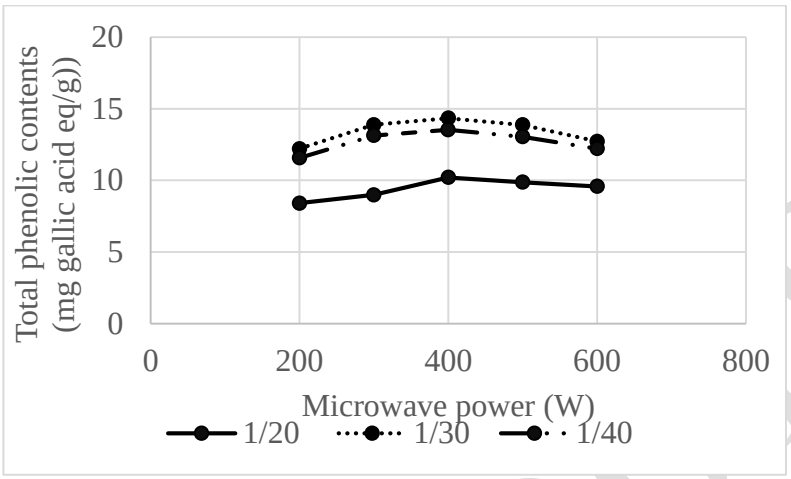

Figure 3 Total phenolic contents of SCG extract using MAE in different ratios of SCG to solvent (w/v) and microwave power.

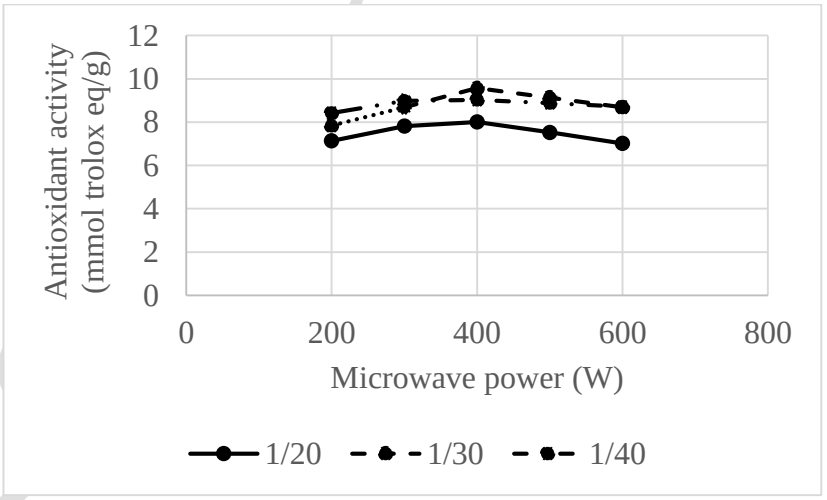

Figure 4 Antioxidant activity of SCG extract using MAE in different ratios of SCG to solvent (w/v) and microwave power.

.33 5-Caffeoylquinic acid determinations

Caffeoylquinic acids are the most abundant phenolic compounds in coffee and also SCG. These chlorogenic acids are water soluble esters formed between quinic acid and one or two moieties of caffeic acid. 5-Caffeoylquinic acid, one of monocaffeoylquinic acids (3-CQA, 4-CQA, 5-CQA) is the most phenolic compounds found in SCG extract. For determination of

5-caffeoylquinic acids or chlorogenic acid in SCG extracts, the results of 5-caffeoylquinic acids (5-CQA) in SCG extracts from MAE ranged between 1.66-1.91 mg/g whereas SCG extracts from reflux extraction gave the value of 1.44-1.81 mg/g (Table 3 and Table 4). The optimized 5-CQA values showed that the condition of 2.0 hrs and the solvent/SCG ratio of 40 ml/g gave maximum results with 1.81 mg/g for reflux extraction whereas, 400 W power, and the solvent/SCG ratio of 40 ml/g achieved 1.91 mg/g for MAE. In previous work have reported that all spent coffee grounds had relevant amounts of mono caffeoylquinic acids ranging from 11.05 to 13.24 mg per g of Arabica spent coffee [26]. Cruz et al. reported similar high amounts of 5-CQA [27]. However, in the present study the 5-caffeoylquinic acids range values reach lower results than that reported in Cruz et al. study. This discrepancy could be probably due to the source and type of Arabica in the present study but also to other technological factors, such as roasting degree, extraction methodology, etc [26].

Table 3 5-Caffeoylquinic acids (mg/g) in spent coffee ground from reflux extraction.

Extraction time (hrs)	SCG/ethanol (w/v) ratios	5-CQA (mg/g)
0.5	1/20	1.44
	1/30	1.49
	1/40	1.48
1.0	1/20	1.52
	1/30	1.59
	1/40	1.61
1.5	1/20	1.71
	1/30	1.69
	1/40	1.66
2.0	1/20	1.73
	1/30	1.77
	1/40	1.81
3.0	1/20	1.75
	1/30	1.77
	1/40	1.76

Table 4 5-Caffeoylquinic acids (mg/g) in spent coffee ground from MAE.

SCG/ethanol (w/v) ratios	Power of microwave (watt)	5-CQA (mg/g)
1/20	200	1.66
	300	1.69
	400	1.66
	500	1.63
	600	1.45
1/30	200	1.79
	300	1.82
	400	1.88
	500	1.85
	600	1.79
1/40	200	1.82
	300	1.85
	400	1.91
	500	1.74
	600	1.71

.34 Cytotoxicity

This is the first report of cytotoxicity of SCG extracts against MCF-7 and NCI-H187. All results showed in Table 5 and Table 6. For reflux extraction, extraction time and solvent to solid were varied from 0.5 to 3.0 hrs and 1:20, 1:30 and 1:40 w/v in order to establish the optimal factors influence on the cytotoxicity of all SCGs. The results demonstrated that SCG extract using reflux for 2 hrs and ratio of solvent to solid at 1/30 w/v displayed the strongest cytotoxicity, being more active against MCF-7 (IC_{50} 49.46±0.08 µM) and NCl-H187 (IC_{50} 34.05±0.12 µM). For MAE, the cytotoxicity of SCG extracts was determined and found that the IC_{50} ranged from 53.22±0.18 to 64.47±0.87 against MCF-7 and from 39.19±0.96 to 49.66±0.14 against NCl-H187. These values indicated that all SCG extracts exhibited higher cytotoxicity value against NCl-H187 than MCF-7.

Table 5 Cytotoxicity for all SCG extracts from reflux extraction.

Extraction time (hrs)	SCG/ethanol (w/v) ratios	Cytotoxicity (IC_{50}, µM)	
		MCF-7	NCl-H187
0.5	1/20	inactive	inactive
	1/30	inactive	inactive
	1/40	inactive	inactive
1.0	1/20	inactive	inactive
	1/30	inactive	inactive

Extraction time (hrs)	SCG/ethanol (w/v) ratios	Cytotoxicity (IC_{50}, µM)	
		MCF-7	NCl-H187
	1/40	inactive	inactive
1.5	1/20	51.09±0.11	36.98±0.01
	1/30	52.40±0.25	37.51±0.03
	1/40	52.52±0.34	39.56±0.12
2.0	1/20	52.19±0.05	39.19±0.02
	1/30	49.46±0.08	34.05±0.12
	1/40	53.22±0.12	42.05±0.55
3.0	1/20	59.03±0.23	54.23±0.76
	1/30	62.23±0.51	59.89±0.07
	1/40	63.89±0.49	62.08±0.52

Table 6 Cytotoxicity for all SCG extracts from MAE.

SCG/ethanol (w/v) ratios	Power of microwave (watt)	Cytotoxicity (IC_{50})	
		MCF-7	NCl-H187
1/20	200	inactive	inactive
	300	inactive	inactive
	400	54.67±0.02	39.19±0.96
	500	58.02±0.23	42.12±0.02
	600	62.45±0.14	45.09±0.33
1/30	200	inactive	inactive
	300	55.07±0.09	41.67±0.12
	400	53.22±0.18	39.06±0.08
	500	56.13±0.20	41.98±0.09
	600	58.55±0.34	43.99±0.76
1/40	200	inactive	inactive
	300	59.50±0.43	49.66±0.14
	400	58.12±0.09	48.12±0.05
	500	56.24±0.27	44.95±0.11
	600	64.47±0.87	47.04±0.64

4. Conclusions

In this work, SCG was extracted using two extraction methods including reflux and MAE. For reflux extraction, different SCG/ethanol (w/v) ratios were 1:20, 1:30, 1:40 and extraction time (0.5-3.0 hrs) were optimized and evaluated the antioxidant activity, total phenolic contents, total flavonoid contents and 5-caffeoylquinic acids of all SCG extracts. For MAE, different SCG/ethanol (w/v) ratios were 1:20, 1:30, 1:40 and microwave power (200-600 w) were varied in order to evaluate the bioactivities as well as those value obtained from reflux extraction. All SCG extracts were tested the cytotoxicity against the human breast cancer (MCF-7) and human small lung (NCl-H187). The results indicated that SCG had high total phenolic contents and antioxidant activity and could be of great interest for the food, cosmetic and pharmaceutical industries, giving added value to a residue generated from coffee industry.

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