# Antioxidant Compounds from Spent Coffee Grounds (SCGs)

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## Abstract

Spent coffee grounds (SCGs) are a rich source of several bioactive compounds. In this study, we applied bioassay-guided isolation and successed in identifying the active components. All 7 subfractions isolated from ethyl acetate extract were evaluated for antioxidants against DPPH and ABTS. Subfraction SCG-EA2 potentially inhibited against DPPH and ABTS with $IC_{50}$ values of 1.76 ± 0.14 and 1.56 ± 0.06 (mg/mL), tentative characterization of subfraction SCG-EA2 by $^{1}$H-NMR analysis suggested that terpenoids are the major content in this subfraction.

**Keywords:** Spent Coffee Grounds / Bioassay Guided Isolation / Antioxidant Potential / Bioactive Compounds/ Solvent Extraction

## 1. Introduction

Coffee is one of the most consumed beverages worldwide aside from water being only after tea, with over billions of cups being brewed every day. This no doubt increases the presence of waste with over 15

million tons of spent coffee grounds (SCGs) generated annually from the brewing process alone. The sheer volume of SCGs produced presents a significant waste stream that often ends up in landfills and further contributes to the influx of waste in the world [4],[5]. The occupancy of these SCGs in landfills leads to the production of the greenhouse gas, methane gas which is several times more potent than even carbon dioxide. The emission of greenhouse gases along with the increase in waste means that an effective method of waste management is absolutely crucial for the mitigation of any negative impacts given off by this abundant by-product [1],[5].

Aside from SCGs, the other by-products of coffee such as coffee skins, pulp, and chaff are already being progressively recycled into value-added and useful products such as green packaging, bio-composites, and even car parts. In contrast, the by-product SCGs remains largely underutilized by companies despite their similar amounts of abundance and high chemical potential. SCGs have been known to retain high levels of antioxidants, terpenoids as well as residual caffeine even after brewing; these bioactive compounds have a heightened potential for high-value applications [1],[2].

These compounds in SCGs can be used for eco-friendly biofuels, natural cosmetics, nutraceuticals along with food additives as well. Research on the topic of coffee recycling has predominantly focused on recycling coffee skins and pulp, resulting in there being a significant gap in the recovery of SCGs and its utilization in a formal scientific scenario [4],[5].

This study aims to develop and bridge the gap and optimize sustainable extraction methods to isolate, study and prove the potency of antioxidant compounds resting inside of these untapped SCGs sources to find out which specific chemical compounds are responsible for their antioxidant properties. The research seeks to reduce environmental waste, greenhouse gas emissions and to utilize specific compounds derived from SCGs in the pharmaceutical field while generating new revenue streams for companies that pile up a lot of SCG waste. The findings of this research will support the integration of SCG upcycling into a more modern bio economy further incentivizing collaboration between many spheres of economy as well.

## 2. Objective

1. To elucidate the bioactive components from the spent coffee grounds

## 3. Scope of study

1. To extract and isolate constituents from the spent coffee grounds to evaluate the antioxidants and anti-tyrosinase inhibition.

## 4. Experiment

### 4.1 Plant Material

The spent coffee grounds (SCGs) were obtained from Nestle Thailand. A voucher specimen was deposited at the Natural Products Research Laboratory of Center of Excellence in Natural Products, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand.

### 4.2 Extraction and Isolation

The spent coffee grounds (SCGs) (1.5 kg) were extracted with methanol (2 × 6 L, for 2 days) at room temperature and concentrated under reduced pressure to give an methanol extract (25.6g). The crude extract was subjected to liquid–liquid partition of the MeOH crude extract using hexane and ethyl acetate to give three fractions SCG-H, SCG-EA and SCG-MeOH, respectively. The silica gel column chromatographic fractionation of the SCG-EA fraction (18.2 g) was performed with hexanes/EtOAc (80: 20) to obtain fractions (EA1-EA7) Figure 1.

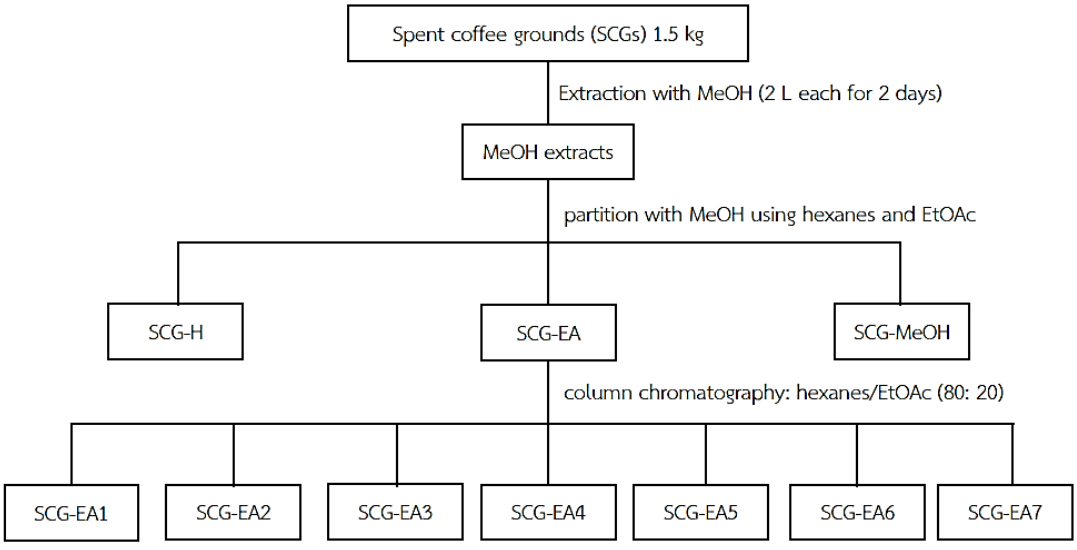


**Figure 1** Chromatographic separation of spent coffee grounds extract.

### 4.3 General Experimental section

$^{1}$H NMR spectra were recorded on 500 MHz NMR spectrometers. The chemical shifts were reported in ppm as referenced to solvent residues ($CDCl_3$, $\delta_H$ 7.26 ppm). Chromatography was performed on a Sephadex LH-20, Merck silica gel (70-230 mesh) and TLC was performed on precoated Merck silica gel 60 $F_{254}$ plates (0.25 mm thick layer).

### 4.4 Determination of the antioxidant capacity

#### 4.4.1 The DPPH free radical scavenging activity assay

Described the potential of the partitioned extracts was determined as previously (Khongkarat et al., 2022). The sample were prepared with DMSO and dilute five different concentrations, 20 µL of the sample was mixed with 80 µL of 0.15 mM DPPH in MeOH for each concentration and incubated at room temperature for 30 min. Then, the absorbance was measured at a wavelength of 517 nm (A517) using a microplate reader. Ascorbic acid (vitamin C) was used as the positive control. Each assay was performed in triplicate. The free radical scavenging activity was calculated from Equation (1):

$$\% \text{ DPPH radical scavenging activity} = [(A-B)/(A)] \times 100, \quad (1)$$

Where; A is the $A_{517}$ of the negative control and B is the $A_{517}$ of the treatment. The % inhibition (Y axis) was plotted against the extract concentrations (X axis) and the effective concentration at 50% ($EC_{50}$) was obtained from the graph.

#### 4.4.2 Determination of the trolox equivalent antioxidant capacity (TEAC)

The TEAC assay followed the described method [6]. The stock solution of $ABTS^{\bullet+}$ was prepared via reacting 7 mM ABTS solution mixed 2.45 mM potassium persulphate solution in distilled water at a 1:1 (v/v) ratio in the dark at room temperature for 16 h before use. The $ABTS^{\bullet+}$radical solution was prepared by diluting the stock $ABTS^{\bullet+}$ solution (1 mL) mixed ethanol (35 mL) to get an absorbance at 734 nm (A734) of 0.700 ± 0.025 and further performed with, 0.3 mL of each partitioned extract at different concentrations dissolved in DMSO was mixed with 2.7 mL of the prepared $ABTS^{\bullet+}$ solution, keep in the dark 6 min and then the A734 was read. The percentage of inhibition was calculated according to Equation (2),

$$\% \text{ ABTS radical scavenging activity} = [(A-B)/(A)] \times 100, \quad (2)$$

Where; A is the A734 of the negative control and B is the A734of the treatment. The % inhibition (X axis) and the $EC_{50}$ value was obtained from the graph and (Y axis) was plotted against the respective extract concentration the results were then compared with the Trolox standard curve (0-0.2 mM) and the results are expressed as mg/mL Trolox equivalents (TE)/g partitioned extract.

#### 4.4.3 Determination of the total phenolic compounds (TPC)

The TPC of each partitioned extract was evaluated as previously reported [6]. First of all, Folin-Ciocalteu reagent was added 125 µL of 0.2 N with 25 µL of the diluted partitioned extract or gallic acid solution

in dimethyl sulfoxide (DMSO) and mixed for 5 min. The sodium carbonate solution 7.5% (w/v) 100 µL was added per well and incubated at room temperature for 2 h. in a 96-well plate.

**4.4.4 Determination of the total flavonoids compounds (TFC)**

The TFC of each partitioned extract was measured as previously described [6] based on the aluminium chloride ($AlCl_3$) colorimetric method but with adaptation for use in a 96 well microplate reader. In brief, the diluted partitioned extract solution dissolved with DMSO 25 µL was mixed with 100 µL of distilled water. Then, 7.5 µL of 5% (w/v) sodium nitrite solution was added and incubated at room temperature for 5 min. After that addition of 7.5 µL of 10% (w/v) $AlCl_3$. After 6 min of incubation at room temperature used 50 µL of 1 M sodium hydroxide was added and the absorbance at wavelength of 510 nm was measured. Each assay was performed in triplicate and The FC was expressed as mg of quercetin equivalents (QE)/g of partitioned extract using a standard graph for quercetin (0.1-1.0 mg/mL).

## 5. Result and discussion

### 5.1 The partitioned extracts of spent coffee grounds (SCGs)

After extraction spent coffee grounds (SCGs) with organic solvents from the lowest to the highest in polarity (hexane, ethyl acetate, and methanol) were obtained three crude extract and seven subfractions from ethyl acetate extract. These fractions were then under reduced pressure and weighed. The ethyl acetate extracts had the highest % inhibition of antioxidants and anti-tyrosinase. The ethyl acetate showed highest of TPC and TFC and exhibited strong antioxidants and ant-tyrosinase.

The fractions EA1-EA7 were obtained as dark brown gum. $^1$H NMR spectrum of (SCG-EA1)-(SCG-EA7) displayed signals range upfield 0.5-2.7 ppm Figure 2. The above $^1$H-NMR data suggested that the components of EA1 shown signals of olefinic proton at $\delta_H$ 4.2 (1H, d, J=2.0 Hz) $\delta_H$ 4.4 (1H, d, J=2.0 Hz) and $\delta_H$ 5.4 (m). The NMR spectrum showed that EA1 and EA2 were shown signals of significant components 0.5-5.4 ppm. Based on comparison with NMR spectrum EA1 compared with the spectrum EA2 observer the signals EA2 showed $\delta_H$ 5.6 (m) $\delta_H$ 6.0 (m), $\delta_H$ 6.1 (m), and $\delta_H$ 6.5 (m).

### 5.2 Determination of the TPC and FC

The TPC and TFC were determined from the calibration curves of gallic acid ($y = 7.5447x - 0.004$; $R^2 = 0.9975$) and quercetin ($y = 0.4651x + 0.0519$; $R^2 = 0.9781$), respectively, with the TPC and TFC of each extract shown in Tables 1 The effect of fractions on the TPC and TFC was significantly evident. The Fraction of ethyl acetate showed highest TPC and TFC of all fractions then followed extract of ethyl acetate, while the extract of hexanes had not showed amount of TPC and TFC. The fraction of EA reveals total phenolic compound between (273.55 ± 2.15 mg GAE/g extract) and total flavonoids displayed (374 ± 14.86 mg QE/g extract).

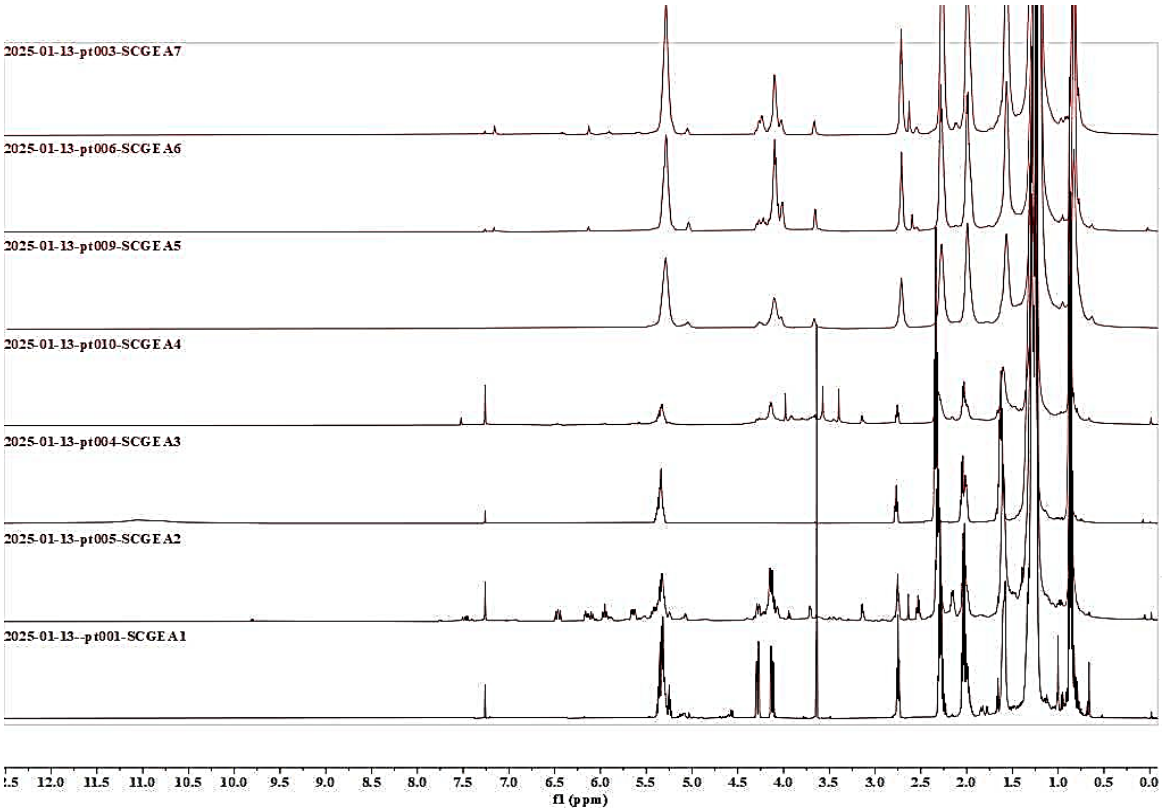


**Figure 2** $^{1}$H NMR spectra (500 MHz, $CDCl_3$) of fractions (SCG-EA1) –(SCG-EA7)

**Table 1** The TPC and FC of the spent coffee grounds extracts

| Sample | Total phenolic (mg GAE/ g extract) | Total flavonoids (mg QE/ g extract) |
|---|---|---|
| Hexanes extract from SCGs | ND | ND |
| EtOAc extract from SCGs | 273.55 ± 2.15 | 374 ± 14.86 |
| MeOH extract from SCGs | 72.58 ± 0.60 | 248.41 ± 6.76 |

Notes. Data are shown as the mean ± 1SD derived from three replicates.

### 5.3 Antioxidant activity of the fractionation

The results of three fractions from hexanes, ethyl acetate and methanol with antioxidant assays shown in Table 2 along with that for ascorbic acid as the positive control for the DPPH and Trolox as the standard reference for the ABTS assays. The $EC_{50}$ values ranged from 2.15 ± 1.00 to 0.14 ± 0.02 mg/mL. The fraction ethyl acetate showed a significantly strong DPPH radical scavenging ability ($EC_{50}$ of 0.14 ± 0.02 mg/mL), while faction of ethyl acetate had the weakest DPPH scavenging capacity. Subfractions SCG-EA1 and SCG-EA2 of ethyl acetate extract reveals with $EC_{50}$ values 1.87 ± 0.11 and 1.76 ± 0.14 mg/mL, respectively Figure 3.

For the ABTS assay, shown $EC_{50}$ values Figure 3 ranged from > 1 to 0.20±0.01 mg/mL. The fraction of ethyl acetate showed a significantly strong ABTS radical scavenging ability ($EC_{50}$ of 0.20±0.01 mg/mL). The subfractions SCG-EA1 and SCG-EA2 of ethyl acetate extract reveals with $EC_{50}$ values 1.25 ± 0.25 and 1.56 ± 0.06, respectively.

**Table 2** Antioxidant activity of the spent coffee grounds extracts.

| Sample | DPPH $EC_{50}$ (mg/mL) | ABTS $EC_{50}$ (mg/mL) |
|---|---|---|
| Hexanes extract from SCGs | 1.25 ± 1.00 | > 1 |
| EtOAc extract from SCGs | 0.14 ± 0.02 | 0.20 ± 0.01 |
| MeOH extract from SCGs | 0.48 ± 0.05 | 0.63 ± 0.06 |

Notes. Data are shown as the mean ± 1SD derived from three replicates.

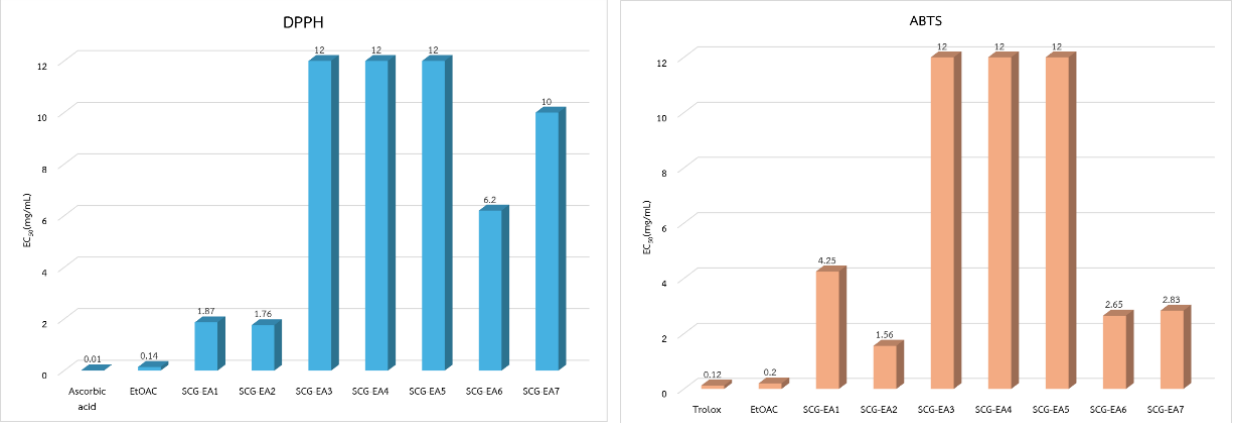


**Figure 3** Antioxidant activity of the EtOAc and subfractions

### 5.4 Determination of chemical components by NMR

To gain insight into chemical components in 7 subfractions obtained after chromatographic separation of EtOAc extract, $^1$H NMR spectra of (SCG-EA1)-(SCG-EA7) were recorded (Figure 2). All subfractions showed similar spectra pattern, except for subfractions SCG-EA1 and SCG-EA2. $^1$H NMR spectra of SCG-EA1 and SCG-EA2 revealed the signals essentially typical to fatty acid containing unsaturations ($\delta_H$ 5.3-5.5 ppm). In addition to unsaturated fatty acid, subfraction SCG-EA2 also contained phenolic compounds as minor components, which can be observed from a series of multiple signals in range of 5.6-6.5 ppm.

## 6. Conclusion

Spent coffee grounds (SCGs) had promising antioxidant activity, which were correlated to the highest TPC and TFC. Bioassay-guided fractionation of ethyl acetate extract, the highest active extract, led to 7 subfractions (SCG-EA1) -(SCG-EA7); two of them SCG-EA1 and SCG-EA2 were identified as the most active antioxidant subfractions. 1H NMR analysis demonstrated that unsaturated fatty acids are likely to be the major components.

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