Nutritional Composition, Phytochemical Properties, Soil Nutrient Profile, and Antibacterial Activity of Spent Coffee Grounds from Sakon Nakhon, Thailand, for Potential Utilization

Aekkaphon Thammayod[1,2,3], Wuttichai Roschat[1,2,3*], Nathaporn Jirawattanasomkul[4], Sunti Phewphong[1,2], Tappagorn Leelatam[1,2,5], Janeeya Khunchalee[6], Preecha Moonsin[6], and Prawit Nuengmatcha[7]

[1]*Biomass Energy Research Laboratory, Center of Excellence on Alternative Energy, Research and Development Institution, Sakon Nakhon Rajabhat University, 680 Nittayo Road, That Choeng Chum, Mueang District, Sakon Nakhon 47000, Thailand*

[2]*Innovation in Chemistry for Community Research Unit, Faculty of Science and Technology, Sakon Nakhon Rajabhat University, 680 Nittayo Road, That Choeng Chum, Mueang District, Sakon Nakhon 47000, Thailand*

[3]*Program of Chemistry, Faculty of Science and Technology, Sakon Nakhon Rajabhat University, 680 Nittayo Road, That Choeng Chum, Mueang District, Sakon Nakhon 47000, Thailand*

[4]*Program of Environment Science, Faculty of Science and Technology, Sakon Nakhon Rajabhat University, 680 Nittayo Road, That Choeng Chum, Mueang District, Sakon Nakhon 47000, Thailand*

[5]*Appropriated Technology Center, Faculty of Science and Technology, Sakon Nakhon Rajabhat University, 680 Nittayo Road, That Choeng Chum, Mueang District, Sakon Nakhon 47000, Thailand*

[6]*Program of Chemistry, Faculty of Science, Ubon Ratchathani Rajabhat University, 2 Ratcha Thani Road, Nai Mueang, Mueang District, Ubon Ratchathani 34000, Thailand*

[7]*Nanomaterials Chemistry Research Unit, Faculty of Science and Technology, Nakhon Si Thammarat Rajabhat University, 1 Moo 4, Tha Ngio, Mueang District, Nakhon Si Thammarat 80280, Thailand*

**** Corresponding author:***

tel: +66-876530534
email: roschat1@gmail.com

Received: July 30, 2025
Accepted: October 9, 2025

DOI: 10.22146/ijc.109907

Abstract: *This study explores the nutritional, phytochemical, and antimicrobial properties of spent coffee grounds (SCGs) collected from cafes in Sakon Nakhon Province, Thailand. Proximate analysis showed high contents of carbohydrates (48.23%), crude fiber (19.69%), and protein (10.10%). Fatty acid profiling revealed linoleic acid (41.06%) and palmitic acid (36.27%) as major components, along with essential omega-6 and omega-3 fatty acids. Phytochemical screening indicated the presence of flavonoids and phenolics (16.11 mg GAE/g DW), which may contribute to bioactivity. SCGs also exhibited favorable soil-enhancing properties, with high organic matter (66.89%) and organic carbon (38.81%), and moderate levels of N, P, K, Ca, and Mg. The extract demonstrated selective antibacterial activity against* Escherichia coli *(15.20 ± 0.98 mm inhibition zone) but not* Bacillus subtilis*, likely due to differences in cell wall structure. These findings suggest the potential use of SCGs in soil amendment, composting, and bioactive compound recovery. However, pretreatment is recommended due to their low pH and moderate phytotoxicity, underscoring their potential as a sustainable, value-added resource for agricultural, cosmetic, and chemical applications.*

Keywords: *spent coffee grounds; nutritional composition; phytochemicals; soil nutrients; antibacterial activity*

■ INTRODUCTION

Coffee is one of the most consumed beverages worldwide, appreciated for its distinctive aroma, taste, and stimulating effects. Global consumption exceeds 2.25 billion cups per day, and production in 2024 reached approximately 10.49 million tons, with Brazil, Vietnam, Colombia, Indonesia, and Ethiopia as the leading producers [1-4]. Beyond its popularity as a drink, coffee has attracted scientific attention for its diverse bioactive compounds, including caffeine, chlorogenic acids, diterpenes, and polyphenols, which exhibit strong antioxidant properties. These compounds are associated with various health benefits, including reducing oxidative stress, modulating inflammation, and potentially preventing chronic diseases such as cardiovascular disorders and certain cancers. Owing to its rich chemical composition and functional properties, coffee has become a focus of research not only for human health but also for the development of innovative applications in the food, pharmaceutical, and cosmetic industries [1-2,5-6]. In Thailand, coffee consumption has grown steadily in recent years. Data from 2024 indicate that the average Thai consumes over 340 cups of coffee per year—approximately one cup per day—totaling around 100,800 tons nationwide, a 3.1% increase from 2023 (97,800 tons). This upward trend reflects lifestyle changes and the expanding coffee culture among various age groups and regions. The domestic coffee market is valued at about 14 billion baht (≈383 million USD), underscoring its growing economic significance within Thailand's food and beverage sector. Key drivers of this growth include the rise of specialty coffee shops, improved access to premium coffee products, and increasing consumer interest in both traditional and innovative coffee beverages [7-8].

Considering both global and domestic coffee consumption, spent coffee grounds (SCGs) represent a major by-product generated throughout the coffee supply chain. Globally, around 18 million tons of wet SCGs are produced each year from beverage preparation, most of which are disposed of in landfills or incinerated, contributing to greenhouse gas emissions [9-10]. However, SCGs contain valuable organic matter, lipids, carbohydrates, and antioxidants, making them a promising raw material for resource recovery through biofuel production, composting, biochar synthesis, and other value-added applications [1,6,11-13]. In agriculture, SCGs are increasingly recognized as eco-friendly soil amendments owing to their high nutrient content—particularly potassium, phosphorus, calcium, and magnesium—and low levels of heavy metals [14-17]. They help improve soil fertility, suppress weeds, and retain soil moisture, especially when co-composted with animal manure or organic wastes, enhancing organic matter and supporting plant growth [10,13,18-19]. Moreover, fermented SCGs (FSCGs) have demonstrated potential as a sustainable feed additive for ruminants. Studies report that microbial fermentation improves crude protein digestibility and nitrogen utilization, and reduces methane emissions, thereby enhancing both productivity and environmental sustainability [20-25]. Nonetheless, excessive inclusion may reduce digestibility due to the high fiber and lignin content, underscoring the need for further optimization.

Given the growing interest in valorizing SCGs for agricultural, environmental, and animal feed applications, a comprehensive understanding of their nutritional, chemical, and functional properties is essential, particularly in local contexts. This study focuses on SCGs collected from Sakon Nakhon Province, Thailand—an area with a rapidly growing coffee culture and substantial SCG generation from local cafés and production facilities. However, existing studies remain limited, often focusing on specific applications, such as composting or soil amendment, with little attention to their overall nutritional, phytochemical, and biological characteristics. An overview of the research framework, including the evaluation of nutritional composition, phytochemical properties, soil nutrient profile, and antibacterial activity of SCGs for potential applications, is illustrated in Fig. 1. Therefore, this study aims to comprehensively assess the nutritional composition, phytochemical properties, soil nutrient profile, and antibacterial activity of locally sourced SCGs to evaluate their potential for sustainable utilization. The findings will provide baseline data to support SCG valorization

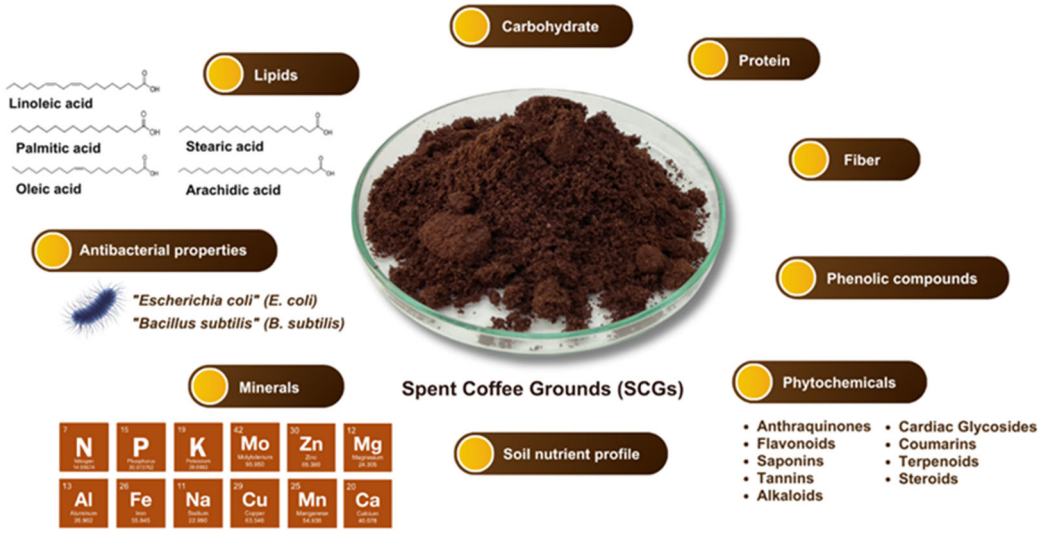


Fig 1. Overview of the study on nutritional, phytochemical, soil, and antibacterial properties of SCGs for potential applications

strategies in agriculture, soil improvement, and community-based enterprises, while contributing to circular economy practices and sustainable development in Thailand.

■ EXPERIMENTAL SECTION

Materials

All chemicals used in this study were of analytical reagent grade and were obtained from reputable suppliers. Examples of the substances used in this study include *n*-hexane (≥ 95%, Merck, Germany), copper(II) sulfate (≥ 98%, Sigma-Aldrich, USA), sodium hydroxide (≥ 97%, QRëC, New Zealand), and boric acid (≥ 99.5%, Fluka, Switzerland), which were used without further purification. Methylene blue (analytical grade, Carlo Erba, Italy) and methyl red (AR grade, Acros Organics, Belgium) were employed as indicators. Hydrochloric acid (37%, Lab Scan, Thailand) and sulfuric acid (98%, Merck, Germany) were used as mineral acids. Ethanol (95%, QRëC, New Zealand) and methanol (≥ 99.8%, Carlo Erba, Italy) were used as solvents. Folin–Ciocalteu reagent (Sigma-Aldrich, USA) and sodium carbonate (≥ 99%, Fluka, Switzerland) were used for total phenolic content determination. Gallic acid (≥ 98%, Sigma-Aldrich, USA) was used as the standard compound. Ammonium acetate (≥ 98%, Merck, Germany) was used to prepare the buffer. Dimethyl sulfoxide (DMSO, ≥ 99.5%, Sigma-Aldrich, USA) was used as a solvent for dissolving the crude SCG extract. SCGs used in this study were collected as mixed, unseparated samples from a café in Mueang District, Sakon Nakhon Province, Thailand, to provide a representative overview of locally produced SCGs. While this represents a single-source sampling, it aligns with the study's aim to comprehensively evaluate the nutritional, phytochemical, soil, and antibacterial properties of SCGs for potential applications in agriculture and community-based initiatives. The samples were then dried in a hot air oven at 60 °C overnight. After drying, they were stored in zip-lock bags and sealed in containers to preserve them for further analysis.

Instrumentation

The experimental analyses were carried out using a range of laboratory instruments to ensure precise and reliable measurements. The equipment included a gas chromatograph (GC-7890A, Agilent, USA), a rotary evaporator (Hei-VAP Expert Control, Heidolph, Germany), a UV–visible spectrophotometer (UV-1800, Shimadzu, Japan), and a hot air oven (MC-2808, Memmert, Germany). For proximate and elemental analyses, an atomic absorption spectrometer (AAS, AA Duo 240FS/240Z, Agilent, Malaysia) was employed to determine mineral contents. Fat extraction was

performed using an automatic Soxhlet extraction apparatus (Soxtec 8000, FOSS, Denmark), while protein and nitrogen contents were analyzed with a protein and nitrogen analyzer (Kjeltec 8200, FOSS, Denmark). Fiber content was determined using a fiber extraction apparatus (Fibertec™ 800, FOSS, Denmark), and ash content analysis utilized a chamber furnace (ELF11/23, Carbolite, England). Microbiological and antibacterial assays were conducted under sterile conditions using a biosafety cabinet (1300 Series A2, Class II Type A2, Thermo Scientific, Germany). Sample sterilization was performed in an autoclave (Hiclave HV-II, Hirayama, Japan), and bacterial cultures were incubated in an incubator (311DS, Labnet, USA).

Procedure

Proximate analysis

The proximate composition of SCGs, including fat, protein, crude fiber, ash, moisture, and carbohydrates, as well as the energy value, was analyzed. The analysis followed the AOAC method [26] along with procedures adapted from previous studies by Preecharram et al. [27-29], Phewphong et al. [30], and Suwannatrai et al. [31-32]. All experiments were performed with at least three replicates, and the results were calculated as mean ± standard deviation (SD). All data were carefully controlled to ensure that the percent relative standard deviation (%RSD) did not exceed 5%.

To analyze the crude fat content, approximately 5 g of SCGs were weighed and placed in a Whatman filter paper thimble. The thimble was then positioned inside a Soxhlet extractor, which was connected to a 200 mL round-bottom flask containing hexane as the solvent. The system was heated to allow the hexane to evaporate, condense, and continuously wash over the sample for 4 h. Once the extraction process was complete, the thimble was removed, and the solvent was recovered, leaving the extracted oil in the flask. The flask was subsequently placed in an oven at 60 °C for 30 min to remove any residual solvent. After drying, the flask was cooled in a desiccator and weighed to determine the crude fat content. The fat yield was calculated based on the difference in weight between the flask before and after extraction, using Eq. (1).

$$\text{Fat } (\%) = \frac{\left[W_f - W_e \right]}{\text{Sample weight (g)}} \times 100\% \quad (1)$$

In this calculation, W_e refers to the weight of the empty extraction flask, while W_f represents the weight of the flask after the fat extract has been collected. The crude fat content was then determined by subtracting the second weight from the first. The result was expressed as a percentage of the sample's initial weight to indicate the fat content in the SCGs. In addition, the extracted SCGs oil samples were analyzed for their fatty acid composition using gas chromatography (GC-7890A), following the procedures outlined in ASTM D6751 and EN 14214 standards [33-34].

To determine the crude protein content, a 1.5 g sample of SCGs was digested with 15 mL of concentrated sulfuric acid in the presence of a catalyst tablet containing $CuSO_4$. The mixture was heated until a clear solution formed. After digestion, the solution was neutralized by adding an equal volume of 32% NaOH, followed by distillation with 4% boric acid containing methylene blue and methyl red indicators. The distillate was then titrated with 0.05 M HCl until a deep red color indicated the endpoint. A reagent blank was also prepared to ensure accuracy.

The nitrogen content (N%) was calculated using the titration data and then multiplied by a conversion factor of 6.25 to obtain the crude protein content. The calculations follow Eq. (2);

$$\text{Protein } (\%) = \text{N } (\%) \times 6.25 \quad (2)$$

where the nitrogen content N (%) is given by Eq. (3);

$$\text{N } (\%) = \frac{\left(V_s - V_b \right) \times N_{HCl} \times 1.4}{W} \quad (3)$$

where V_s is the volume of hydrochloric acid used for titration of the sample (mL), V_b is the volume of HCl used for titration of the blank (mL), and N_{HCl} represents the normality of the HCl solution, and W is the weight of the dried sample (g). The nitrogen content is calculated based on the difference between the sample and blank titration volumes and is expressed as a percentage relative to the original sample weight.

To determine the moisture content, approximately 5.0 g of the SCGs sample was weighed into a beaker. The sample was then dried in a hot air oven at 105 °C for 3 h.

After drying, the sample was cooled in a desiccator and weighed. The drying and weighing process was repeated until a constant weight was obtained. The moisture content was calculated based on the weight loss during drying, using Eq. (4).

$$\text{Moisture content } (\%) = \frac{\text{Initial weight}}{\text{Weight loss}} \times 100\% \quad (4)$$

In this calculation, weight loss refers to the difference between the weight of the beaker containing the SCGs sample before drying and the weight of the beaker with the sample after drying to a constant weight. The initial weight refers to the weight of the beaker with the SCGs sample before drying, minus the weight of the empty beaker. The resulting moisture content was expressed as a percentage of the initial sample weight.

To determine the total ash content, approximately 5.0 g of the sample was weighed into a pre-weighed crucible. The crucible containing the sample was then placed in a muffle furnace and heated to 550 °C until complete combustion was achieved, leaving only ash. After combustion, the crucible was cooled in a desiccator and weighed again to determine the total ash content. The ash content was calculated using Eq. (5).

$$\text{Ash content } (\%) = \frac{W_T - W_B}{W} \times 100\% \quad (5)$$

In this calculation, W_T refers to the weight of the crucible containing the ash (g), W_B is the weight of the empty crucible (g), and W is the weight of the sample (g). The result was expressed as a percentage of the initial sample weight.

To determine the crude fiber content, a 5.0 g sample was refluxed in 200 mL of 0.13 M H_2SO_4 for 30 min. After boiling, the sample was thoroughly washed with hot water. It was then reflux-boiled in 200 mL of 0.31 M NaOH solution for an additional 30 min. The sample was drained, rinsed, and dried before being transferred to a pre-weighed crucible. The crucible containing the sample was dried in an oven at 105 °C until a constant weight was obtained. Subsequently, it was burned in a muffle furnace to remove all organic matter, leaving only ash. The crude fiber content was calculated using Eq. (6).

$$\text{Crude fiber } (\%) = \frac{W_1 - W_2}{W} \times 100\% \quad (6)$$

In this equation, W_1 represents the weight of the crucible with the dried sample (g), W_2 is the weight of the crucible containing only ash (g), and W is the initial weight of the sample (g). The result was expressed as a percentage of the original sample weight.

The total carbohydrate content was calculated by subtracting the percentages of ash, crude fat, protein, and crude fiber from 100, according to Eq. (7).

$$\text{Total carbohydrate content } (\%) = 100 - (\%\text{moisture} + \%\text{ash} + \%\text{fat} + \%\text{protein} + \%\text{crude fiber}) \quad (7)$$

Additionally, the energy value of the sample was estimated using Atwater factors: protein and carbohydrates were multiplied by 4, and crude fat by 9, to calculate the total energy content.

Preliminary phytochemical screening

The SCGs extract was prepared using a soaking extraction method. Dried spent SCGs were soaked in 70% ethanol at a 1:100 (g/mL) ratio and left at room temperature for 24 h. After extraction, the mixture was filtered, and the filtrate was concentrated using a rotary evaporator to obtain the crude extract. The crude extract was stored in a tightly sealed bottle and refrigerated at 4 °C for further analysis. Subsequently, preliminary phytochemical screening was carried out on the SCGs extracts using chromophore reagents. For this analysis, 5 mL of the SCGs extract was placed in a glass test tube and subjected to qualitative phytochemical tests following the describes methods [35-37]. The tests aimed to identify and confirm the presence of phytochemical compounds, including anthraquinones, flavonoids, saponins, tannins, cardiac glycosides, coumarins, terpenoids, steroids, and alkaloids.

Determination of total phenolic content

The total phenolic content of the SCGs extract was determined using the Folin-Ciocalteu method. In this assay, 50 µL of the crude extract was added to each well of a 96-well microplate, followed by 80 µL of 10% Folin-Ciocalteu reagent and 150 µL of 7% Na_2CO_3 solution. The mixture was incubated in the dark at room temperature for 2 h. After incubation, the absorbance was measured at 765 nm using a microplate reader.

Gallic acid was used as the standard, and methanol served as the control. The total phenolic content was expressed as gallic acid equivalents (μg/mL), following the previously described methods [27-28,30,32].

Soil nutrient analysis of SCGs

The soil nutrient analysis of SCGs, including moisture content, pH, electrical conductivity (EC), organic matter (OM), Organic carbon (OC), total nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and germination index, was conducted following the methods described in detail by Cervera-Mata et al. [19], Thammayod et al. [38], and Jeon et al. [39]. The moisture content of SCGs was determined by drying approximately 10 g of sample in a hot air oven at 105 °C for 24 h until a constant weight was achieved. The pH and EC were measured by mixing SCGs with distilled water at a 1:10 (w/v) ratio and stirring for 30 min. The pH was measured with a pH meter, and EC was determined with a conductivity meter.

OM content was analyzed using the loss-on-ignition method, where dried SCGs were combusted in a muffle furnace at 550 °C for 4 h. OC content was estimated based on the measured OM content using the van Bemmelen factor (1.724), which assumes that organic matter contains approximately 58% organic carbon. Total N was determined using the Kjeldahl digestion method. Available P was analyzed using the Olsen method, involving $NaHCO_3$ extraction followed by colorimetric determination. K, Ca, and Mg were extracted with ammonium acetate solution (pH 7.0) and quantified using AAS. The phytotoxicity of SCGs was evaluated through the germination index (GI) test using lettuce seeds (*Lactuca sativa*). Aqueous extracts were prepared by mixing 10 g of SCGs with 100 mL of distilled water, shaking for 24 h, and filtering the mixture. Ten lettuce seeds were placed on filter paper in Petri dishes, and 5 mL of the SCGs extract was added. The dishes were incubated at 25 °C for 72 h. The GI was then calculated based on the percentage of seed germination and root elongation relative to the control. Additionally, the mineral elements present in the SCGs samples, including Na, Al, Fe, Mn, Cu, Zn, and Mo, which are commonly found and beneficial to plants, were also analyzed using AAS technique.

Evaluation of antibacterial properties of SCGs extract

The antibacterial activity of the SCGs extract was evaluated using the paper disk diffusion assay, a standard method widely applied in microbiological research to assess antimicrobial properties [3,7]. In this study, the inhibitory effects of the SCG extract were evaluated against selected bacteria, including the Gram-positive *Bacillus subtilis* TISTR 1248 and the Gram-negative *Escherichia coli* TISTR 527. The crude SCG extract was diluted to 100 mg/mL in DMSO. The experimental procedure followed the described methods [40-43] with minor modifications. Briefly, bacterial suspensions were prepared and adjusted to the 0.5 McFarland turbidity standard. The suspensions were then evenly spread onto agar plates to create a consistent bacterial lawn. Sterile 6 mm filter paper disks were carefully placed on the agar surfaces, and 10 μL of the SCG extract, along with the positive and negative controls, were applied onto each disk. Ampicillin at 100 mg/mL was used as the positive control, and DMSO as the negative control. The plates were incubated at 37 °C for 24 h. Following incubation, the antibacterial activity was assessed by measuring the diameter of the inhibition zones surrounding the disks, with results reported in mm.

■ RESULTS AND DISCUSSION

The Proximate Nutrient Composition of SCGs

The proximate composition of SCGs analyzed in this study is summarized in Table 1. The results were compared with values reported in previous literature [44-45], revealing both consistencies and notable variations. The moisture content of the SCGs was 12.74 ± 0.09%, which is substantially higher than previously reported values (2.65–2.84%). This difference may be attributed to variations in drying techniques, storage conditions, or coffee processing methods. The crude fat content was 8.17 ± 0.06%, which falls within the reported range (8.58–18.55%) but is lower than specific values, potentially due to differences in oil extraction

Table 1. Percentage of proximate composition and energy value of SCGs

Component (%)	Current study	Previous studies	
		Bijla et al. [44]	Panpraneecharoen et al. [45]
Moisture content	12.74 ± 0.09	2.65 ± 0.01	2.84 ± 0.17
Crude fat	8.17 ± 0.06	8.58 ± 0.01–18.55 ± 0.01	14.42 ± 0.43
Crude protein	10.10 ± 0.03	13.87 ± 1.12–16.12 ± 1.30	10.93 ± 0.03
Crude fiber	19.69 ± 0.23	NR	NR
Ash	1.07 ± 0.01	2.16 ± 0.01–3.61 ± 0.01	14.93 ± 0.55
Total carbohydrate	48.23 ± 0.26	61.76 ± 1.21–70.37 ± 1.21	56.88[a]
Energy value (kcal/100g)	306.85 ± 1.16	NR	NR

Note: The carbohydrate content was calculated by difference using the formula: 100 − (moisture + ash + protein + fat); NR = Not Reported

efficiency, coffee bean variety, and roasting conditions. Crude protein was measured at 10.10 ± 0.03%, slightly below previously published ranges (10.93–16.12%), possibly influenced by the degree of defatting and brewing method. The crude fiber content was relatively high at 19.69 ± 0.23%, although comparable data were not available in the referenced studies. This substantial fiber fraction suggests SCGs may serve as a viable source of dietary fiber or act as a bulking agent in agricultural or compost applications.

Ash content was low (1.07 ± 0.01%) compared to previous reports (2.16–14.93%), indicating a reduced presence of inorganic matter, which may result from effective removal of soluble minerals during coffee brewing. Total carbohydrate content, calculated by difference, was 48.23 ± 0.26%, which is lower than reported values (56.88–70.37%). These variations may reflect differences in proximate composition arising from processing techniques or the relative distribution of macronutrients. The estimated energy value was 306.85 ± 1.16 kcal/100 g, reflecting the cumulative contribution of proteins, lipids, and carbohydrates. Although not directly reported in earlier studies, this caloric value suggests that SCGs retain substantial energy content even after coffee extraction. Overall, the findings confirm that SCGs possess considerable nutritional and calorific potential, supporting their suitability for applications in food, animal feed, and bio-based product development.

Fatty Acids Composition of Oil Extracted from SCGs

The fatty acid profile of oil extracted from SCGs, as illustrated in Fig. 2 and summarized in Table 2, revealed the presence of 18 identifiable fatty acid compounds comprising both saturated and unsaturated types. The predominant fatty acids were linoleic acid ($C_{18:2n6c}$, 41.06 ± 0.560 wt.%), palmitic acid ($C_{16:0}$, 36.27 ± 0.860 wt.%), oleic acid ($C_{18:1n9c}$, 9.47 ± 0.120 wt.%), and stearic acid ($C_{18:0}$, 7.29 ± 0.160 wt.%), which collectively accounted for over 94% of the total fatty acid content. Linoleic acid (omega-6) was the most abundant unsaturated fatty acid. Its concentration in this study was slightly lower than values reported in previous literature, while palmitic acid, the principal saturated fatty acid, was comparatively higher. Oleic acid (omega-9) and stearic acid ($C_{18:0}$) were found in moderate amounts, with stearic acid aligning closely with earlier reports and oleic acid slightly lower. Linolenic acid ($C_{18:3n3}$), an omega-3 fatty acid, was detected in minor quantities (0.93 wt.%), consistent with previously observed ranges. In addition to the major components, several long-chain saturated fatty acids, including arachidic ($C_{20:0}$), behenic ($C_{22:0}$), and lignoceric acid ($C_{24:0}$), were present in trace amounts. The total saturated fatty acid content (SFA) was 48.19 wt.%, which exceeds values documented in earlier studies (35.79–44.5 wt.%), indicating a relatively higher proportion of saturated lipids. Conversely, the total unsaturated fatty acid content (UFA) was 51.81 wt.%, slightly below previously reported values (55.4–64.21 wt.%).

Compared with previous studies by Bijla et al. [44] and Vu et al. [46], the fatty acid distribution in the present study showed similar qualitative profiles. Still, it differed

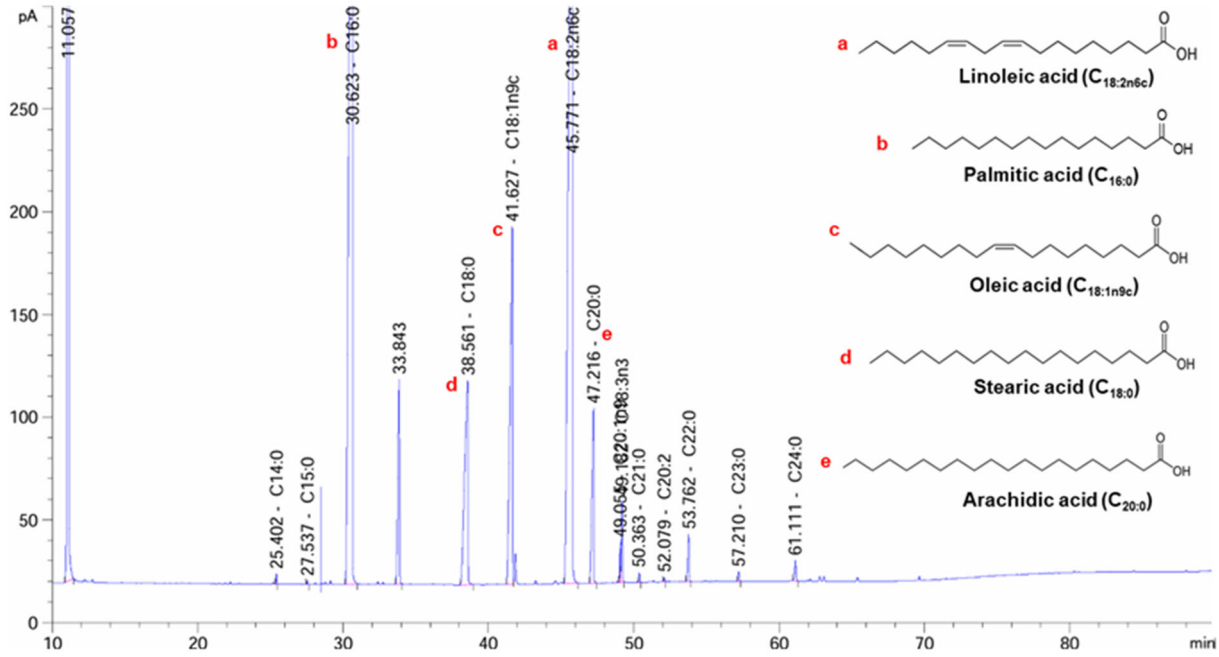


Fig 2. GC chromatogram of fatty acid composition in oil extracted from SCGs

Table 2. Fatty acid compositions of oil extracted from SCGs

Fatty acid	Composition (wt.%)		
	Current study	Bijla et al. [44][a]	Vu et al. [46]
Octanoic acid ($C_{8:0}$)	-	-	-
Decanoic acid ($C_{10:0}$)	-	-	-
Lauric acid ($C_{12:0}$)	-		-
Myristic acid ($C_{14:0}$)	0.08 ± 0.001	0.10	-
Pentadecylic acid ($C_{15:0}$)	0.03 ± 0.001	-	-
Palmitic acid ($C_{16:0}$)	36.27 ± 0.860	27.16	33.4
Palmitoleic acid ($C_{16:1}$)	-	0.12	-
Heptadecanoic acid ($C_{17:1}$)	-	0.07	-
Stearic acid ($C_{18:0}$)	7.29 ± 0.160	5.76	7.2
Oleic acid ($C_{18:1n9c}$)[b]	9.47 ± 0.120	13.33	11.0
Linoleic acid ($C_{18:2n6c}$)[c]	41.06 ± 0.560	49.39	43.0
Linolenic acid ($C_{18:3n3}$)[d]	0.93 ± 0.001	0.90	1.0
Arachidic acid ($C_{20:0}$)	3.35 ± 0.045	2.67	3.0
Eicosenoic acid ($C_{20:1n-9}$)	0.35 ± 0.001	0.37	0.4
Eicosadienoic acid ($C_{20:2}$)	0.05 ± 0.001	-	-
Heneicosanoic acid ($C_{21:0}$)	0.09 ± 0.001	-	-
Behenic acid ($C_{22:0}$)	0.61 ± 0.022	-	0.6
Erucic acid ($C_{22:1}$)	-	-	-
Tricosanoic acid ($C_{23:0}$)	0.11 ± 0.001	-	-
Ligniceric acid ($C_{24:0}$)	0.31 ± 0.001	-	0.3
Total saturated fatty acid compound	48.19 ± 0.755	35.79	44.5
Total unsaturated fatty acid compound	51.81 ± 0.550	64.21	55.4

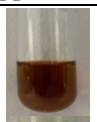
Note: a = refers to sample code SCG1, b = refers to omega 9, c = refers to omega 6, and d = refers to omega 3

quantitatively, particularly in the relative proportions of palmitic, linoleic, and oleic acids. These differences may stem from variations in coffee origin, roasting conditions, and oil extraction methods. The GC chromatogram (Fig. 2) confirmed these findings, showing dominant peaks for linoleic, palmitic, and oleic acids. The significant levels of polyunsaturated and monounsaturated fatty acids, particularly linoleic and oleic acids, suggest that SCG-derived oil retains nutritional and functional properties suitable for various bio-based applications, including food, cosmetics, and biodiesel production. Therefore, the SCG oil exhibited a balanced fatty acid composition, characterized by essential fatty acids and a notable ratio of unsaturated to saturated compounds. These attributes reinforce SCGs' potential as a sustainable source of functional lipids. Such fatty acid profiles are desirable not only for nutritional applications but also for incorporation into cosmetic formulations. SCG-derived oil can be used to produce moisturizing soaps, body lotions, skin creams, and other personal care products due to its emollient properties, oxidative stability, and natural origin. Furthermore, the presence of unsaturated fatty acids enhances skin absorption and contributes to the formulation of environmentally friendly, bio-based cosmetic products.

Preliminary Phytochemical Screening of SCGs Extracts

The preliminary phytochemical screening of the SCG extract, as summarized in Table 3, revealed the presence of various bioactive compounds. Among the tested compounds, terpenoids were highly detected (+++) using the Salkowski test, indicating a strong presence in the SCG extract. Flavonoids, saponins, tannins, cardiac

Table 3. Results of preliminary phytochemical screening of the SCGs extract

Phytochemical compounds	Test method	Sample extract		Test results[1]	References[2]	
		Test result appearance	Original extract appearance		El-Desouky et al. [47]	Tinrat [48]
Anthraquinones	Bornträger reaction			-	No result	-
Flavonoids	Lead acetate test			++	+	+
Saponins	Froth test			++	+	+
Tannins	Braymer test			++	+++	+
Cardiac Glycosides	Keller Killani test			++	+	-
Coumarins	NaOH test			+	No result	No result
Terpenoids	Salkowski test			+++	No result	+
Steroids	Color test			-	-	+

Phytochemical compounds	Test method	Sample extract		Test results[1]	References[2]	
		Test result appearance	Original extract appearance		El-Desouky et al. [47]	Tinrat [48]
Alkaloids	Wagner reagent			++	+	+

Note: [1] Test results: +++ (highly detected), ++ (moderately detected), + (slightly detected), - (not detected); [2] References indicate detection results from El-Desouky et al. [47] and Tinrat [48]

glycosides, and alkaloids were moderately detected (++), suggesting these compounds are also present in considerable amounts. Meanwhile, coumarins were slightly detected (+) based on the NaOH test. In contrast, anthraquinones and steroids were not detected (−) in the crude SCG extract under the conditions used. The absence of steroids is noteworthy and aligns partially with previous findings. Compared with earlier studies by El-Desouky et al. [47] and Tinrat [48], this study's results were largely consistent, particularly in the detection of flavonoids, saponins, terpenoids, and alkaloids. However, some discrepancies were observed, such as the absence of anthraquinones and steroids in this study, whereas El-Desouky et al. [47] reported their presence. The observed differences in phytochemical profiles may be attributed to various factors, including differences in extraction methods, solvents used, coffee bean origin, roasting conditions, and storage duration. The presence of these secondary metabolites, especially terpenoids and flavonoids, supports the potential bioactivity of SCGs extract, including its antioxidant and antimicrobial properties reported in related studies.

Total Phenolic Content

The total phenolic content of the SCGs in this study was 16.11 ± 0.65 mg GAE/g DW. The SCGs used were mixed, unseparated samples collected from a café, representing a combination of various coffee types commonly found in the area. When compared with previous studies, Zainol et al. [49] reported the total phenolic content of SCGs from Arabica, Robusta, and Liberica varieties, ranging from 18.94 ± 0.06 to 26.23 ± 0.86 mg GAE/g, with Robusta SCGs exhibiting the highest value among the three species. Similarly, Arauzo et al. [50] reported a total phenolic content of 17.9 mg GAE/g, while Maiyah et al. [51] reported 6.80 to 31.31 mg GAE/g DW in SCGs. Although the value obtained in this study is slightly lower than those reported in the aforementioned studies, the difference is relatively minor. Such variations may arise due to multiple factors, including the origin of the coffee beans, cultivation conditions, degree of roasting, grinding size, and extraction procedures (solvent concentration, extraction time, and temperature). The type of coffee served at each café and the brewing method (e.g., espresso or drip) could also influence the residual phenolic content in SCGs. Nevertheless, these results confirm that SCGs consistently retain significant amounts of phenolic compounds, regardless of their source or processing conditions. These bioactive compounds possess strong antioxidant, antimicrobial, and anti-inflammatory properties, making SCGs a promising low-cost raw material for the development of value-added products in food, cosmetics, and pharmaceutical industries. Furthermore, utilizing SCGs for phenolic compound extraction could contribute to waste valorization and environmental sustainability by reducing the amount of organic waste sent to landfills.

Physicochemical and Nutrient Properties of SCGs

The physicochemical characteristics and nutrient composition of SCGs used in this study are presented in Table 4. The pH of the SCG extract was slightly acidic at 4.47 ± 0.02, which is lower than the pH range (5.0–6.0) reported in previous studies. The EC was measured at 0.81 ± 0.002 mS/cm, indicating a relatively low salinity compared to prior reports (1.47–2.17 mS/cm), which may reflect differences in the coffee type, extraction method, or geographical origin. The SCGs contained a high OM content of 66.89 ± 1.20% and OC content of

Table 4. Physicochemical properties and soil nutrient analysis of SCGs

Parameter	SCGs	
	This work	Previous reports
pH (1:10)	4.47 ± 0.02	5.0–6.0
Electrical conductivity (EC)	0.81 ± 0.002 mS/cm	1.47–2.17 mS/cm
Organic matter (OM)	66.89 ± 1.20%	~62%
Organic carbon (OC)	38.81 ± 0.70%	~36%
Total nitrogen (Total N)	1.47 ± 0.005%	1.8–2.5%
Phosphorus (P)	0.08 ± 0.001%	1.47%
Potassium (K)	0.23 ± 0.005%	3.70%
Calcium (Ca)	1.85 ± 0.04%	1.38%
Magnesium (Mg)	0.19 ± 0.003%	1.29%
Germination index (GI)	52 ± 1.5%	-
Sodium (Na)	280.00 ± 5.44 mg kg^{-1}	349.18 ± 28.31 mg kg^{-1}
Aluminum (Al)	76.32 ± 2.79 mg kg^{-1}	22.30 ± 3.50 mg kg^{-1}
Iron (Fe)	152.66 ± 5.82 mg kg^{-1}	45.27 ± 3.66 mg kg^{-1}
Manganese (Mn)	21.02 ± 1.51 mg kg^{-1}	16.09 ± 1.31 mg kg^{-1}
Copper (Cu)	15.38 ± 0.65 mg kg^{-1}	23.97 ± 1.95 mg kg^{-1}
Zinc (Zn)	38.40 ± 2.63 mg kg^{-1}	9.72 ± 0.74 mg kg^{-1}
Molybdenum (Mo)	1.49 ± 0.15 mg kg^{-1}	< 0.08 mg kg^{-1}

Note: Previous studies reported by de Bomfim et al. [11], Bijla et al. [44], Alghamdi et al. [52], Afriliana et al. [53], and Ballesteros et al. [54] were also referenced

38.81 ± 0.70%, consistent with values reported in previous literature (~62% OM and ~36% OC). These values highlight the potential of SCGs as an organic soil amendment. The total N content was 1.47 ± 0.005%, which is slightly lower than previous reports (1.8–2.5%). In terms of primary macronutrients, P and K contents were relatively low at 0.08 ± 0.001% and 0.23 ± 0.005%, respectively, when compared to previous findings [11,44,52-54].

Ca and Mg contents were found to be 1.85 ± 0.04% and 0.19 ± 0.003%, respectively. Notably, the Ca content in this study was higher than previously reported values (1.38%), while Mg was lower (1.29%). The GI of SCGs was 52 ± 1.5%, indicating moderate phytotoxicity, as values below 80% generally suggest the need for further composting or dilution prior to direct soil application. For trace elements, Na was detected at 280 ± 5.44 mg/kg, slightly lower than the 349.18 ± 28.31 mg/kg reported in earlier studies. Al, Fe, Mn, Cu, Zn, and Mo were all present in varying amounts, with Fe and Zn levels (152.66 ± 5.82 and 38.40 ± 2.63 mg/kg, respectively) being notably higher than previously reported values [11,44,52-54]. This variation may be influenced by factors such as soil contamination, roasting process, and extraction conditions. Overall, the results demonstrate that SCGs possess favorable organic matter and carbon contents, along with a rich micronutrient profile, making them a promising candidate for use as a soil amendment or in composting applications. However, the low pH and moderate phytotoxicity suggest that pretreatment or co-composting with other organic materials may be required for optimal agronomic performance.

Evaluation of Antibacterial Properties of SCGs Extract

The evaluation of the antibacterial properties of SCGs extract is presented in Fig. 3, which shows photographs illustrating the antibacterial activity of the samples against Gram-positive *B. subtilis* and Gram-negative *E. coli* at a concentration of 100 mg/mL of crude SCG extract. Ampicillin at the same concentration was used as the positive control, while DMSO served as the negative control. The results indicated that the SCGs extract exhibited antibacterial activity against *E. coli*, with a clear zone measuring 15.20 ± 0.98 mm, which was

smaller than that produced by the positive control, ampicillin (33.00 ± 1.79 mm) as shown in Fig. 3(a). In contrast, no inhibition zone was observed for *B. subtilis* when treated with the SCGs extract, whereas ampicillin produced a clear inhibition zone of 46.80 ± 2.32 mm, as shown in Fig. 3(b). Selective inhibition of *E. coli* by the SCG extract can be traced to fundamental differences in cell-envelope architecture.

The lipopolysaccharide-rich outer membrane of Gram-negative bacteria facilitates the binding of phenolic and flavonoid compounds, compromising membrane integrity and leading to cell death. In contrast, the thick peptidoglycan layer of the Gram-positive *B. subtilis* limits the penetration of these bioactive compounds, thereby reducing antibacterial efficacy [40,55-56]. The presence of phenolic compounds, flavonoids, and terpenoids in the SCG extract, as confirmed by phytochemical screening, likely contributes to its antibacterial activity. Phenolics are known to cause protein precipitation and membrane disruption, while flavonoids may inhibit nucleic acid synthesis and interfere with bacterial energy metabolism. Moreover, the findings of Canci et al. [55] align with this, as their study demonstrated selective inhibition of Gram-negative pathogens *Salmonella typhimurium* and *E. coli* by aqueous extracts of roasted and green coffee (*C. arabica* and *C. canephora*). In contrast, no inhibitory effect was observed against Gram-positive probiotic bacteria *Lactobacillus plantarum* and *L. rhamnosus*. This selective

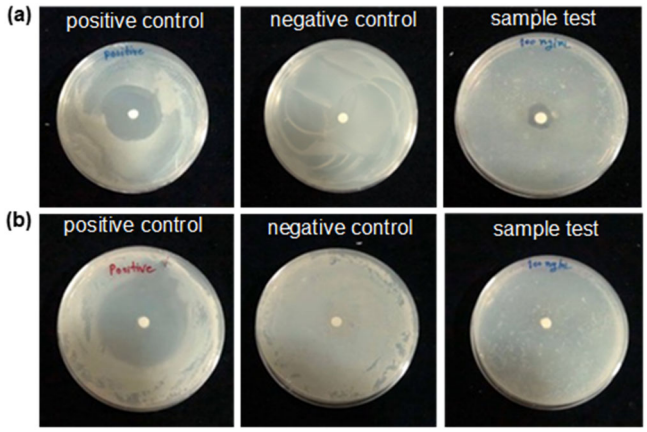


Fig 3. Photographs showing the antibacterial activity of the samples against (a) the Gram-negative *E. coli* TISTR 527 and (b) the Gram-positive *B. subtilis* TISTR 1248 at a concentration of 100 mg/mL of crude SCG extract

antibacterial action may be attributed to bioactive compounds such as chlorogenic and caffeic acids, which inhibit pathogenic bacteria while supporting beneficial gut microbiota. Variability in antibacterial efficacy among studies may arise from differences in extraction methods, solvent polarity, coffee variety, roasting degree, and bacterial strain specificity. Therefore, further purification and characterization of active constituents are necessary to enhance the antibacterial potential of SCG-derived products and clarify their mechanisms of action.

■ CONCLUSION

This study provides a comprehensive assessment of the nutritional composition, phytochemical constituents, physicochemical characteristics, and biological activities of SCGs collected from Sakon Nakhon Province, Thailand. The results revealed that SCGs are rich in macronutrients such as carbohydrates, proteins, and fibers, and contain essential fatty acids, including linoleic, oleic, and α-linolenic acids. Phytochemical analysis confirmed the presence of flavonoids, phenolics, and terpenoids. High OM and OC contents indicate the suitability of SCGs as an organic soil amendment; however, their slightly acidic pH and moderate phytotoxicity (GI < 80%) require pretreatment or co-composting before agricultural use. Nutrient profiling also reflected variations related to coffee origin and processing methods. The SCG extract showed selective antibacterial activity against *E. coli* but not *B. subtilis*, suggesting potential use as a targeted antimicrobial agent against Gram-negative bacteria. It should be noted that this study focused on single-source sampling from a single café, which is a limitation. Therefore, future studies are recommended to include SCGs from multiple sources and regions to validate and generalize the findings. Such extended research will further support the development of comprehensive SCG valorization strategies for agriculture, soil improvement, cosmetics, and community-based enterprises. These findings demonstrate the multifaceted value of SCGs as a sustainable, value-added resource for agriculture, cosmetics, and antimicrobial applications, contributing to sustainable waste utilization and the development of a circular bioeconomy.

■ ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support from the Basic Research Support Fund provided by the Research and Development Institute, Sakon Nakhon Rajabhat University, Thailand, for the fiscal year 2025 (Fund code: P1-1.1/2569). We also appreciate the valuable support and collaboration from the Community Chemistry Innovation Research Unit, the Chemistry Program, and the Environmental Science Program within the Faculty of Science and Technology at Sakon Nakhon Rajabhat University. Special thanks are extended to the Biomass Energy Research Laboratory, affiliated with the Center of Excellence on Alternative Energy, for their technical assistance and partnership throughout this study.

■ CONFLICT OF INTEREST

The authors declare no financial conflicts of interest or personal relationships that could have influenced the research reported in this paper.

■ AUTHOR CONTRIBUTIONS

Aekkaphon Thammayod: Conceptualization; Formal analysis; Data curation; Investigation. Wuttichai Roschat: Conceptualization; Formal analysis; Data curation; Investigation; Writing - Original draft preparation; Writing – Reviewing and Editing. Nathaporn Jirawattanasomkul: Methodology; Investigation; Data curation; Formal analysis. Sunti Phewphong: Resources; Methodology; Investigation; Formal analysis. Tappagorn Leelatam: Methodology; Investigation; Formal analysis. Janeeya Khunchalee: Formal analysis; Data curation; Methodology. Preecha Moonsin: Formal analysis; Data curation; Methodology. Prawit Nuengmatcha: Supervision; Formal analysis; Data curation; Writing – Reviewing and Editing. All Authors read and agreed to the final version of this manuscript for publication.

■ REFERENCES

[1] Choe, U., 2025, Valorization of spent coffee grounds and their applications in food science, *Curr. Res. Food Sci.*, 10, 101010.

[2] Freitas, V.V., Borges, L.L.R., Vidigal, M.C.T.R., dos Santos, M.H., and Stringheta, P.C., 2024, Coffee: A comprehensive overview of origin, market, and the quality process, *Trends Food Sci. Technol.*, 146, 104411.

[3] Grand View Research, 2024, *Coffee Market (2025 - 2030)*, https://www.grandviewresearch.com/industry -analysis/coffee-market, accessed on July 3, 2025.

[4] Visual Capitalist, 2024, *Visualizing Global Coffee Production in 2024*, https://www.visualcapitalist.com/visualizing-global-coffee-production-in-2024/, accessed on July 3, 2025).

[5] Ribeiro, E., Rocha, T.S., and Prudencio, S.H., 2021, Potential of green and roasted coffee beans and spent coffee grounds to provide bioactive peptides, *Food Chem.*, 348, 129061.

[6] Bevilacqua, E., Cruzat, V., Singh, I., Rose'Meyer, R.B., Panchal, S.K., and Brown, L., 2023, The potential of spent coffee grounds in functional food development, *Nutrients*, 15 (4), 994.

[7] ReportLinker, 2025, *Forecast: Coffee Consumption in Thailand*, https://www.reportlinker.com/dataset/4af189f678491371a49f404163187986dad95b5d, accessed on July 3, 2025.

[8] ReportLinker, 2024, *Thailand Coffee Industry Outlook 2024–2028*, https://www.reportlinker.com/clp/country/464/726412, accessed on July 3, 2025.

[9] May, G., and Folkerts, J., 2021, Breaking new grounds for coffee, *Food Sci. Technol.*, 35 (2), 28–31.

[10] Hu, Y., Li, J., Wu, Y., Zhang, D., Qi, Z., and Yang, R., 2025, Spent coffee ground and its derivatives as soil amendments—Impact on soil health and plant production, *Agronomy*, 15 (1), 26.

[11] de Bomfim, A.S.C., de Oliveira, D.M., Walling, E., Babin, A., Hersant, G., Vaneeckhaute, C., Dumont, M.J., and Rodrigue, D., 2022, Spent coffee grounds characterization and reuse in composting and soil amendment, *Waste*, 1 (1), 2–20.

[12] Dordevic, D., Gablo, N., Zelenkova, L., Dordevic, S., and Tremlova, B., 2024, Utilization of spent coffee grounds as a food by-product to produce edible

films based on κ-carrageenan with biodegradable and active properties, *Foods*, 13 (12), 1833.

[13] Cervera-Mata, A., Delgado, G., Fernández-Arteaga, A., Fornasier, F., and Mondini, C., 2022, Spent coffee grounds by-products and their influence on soil C–N dynamics, *J. Environ. Manage.*, 302, 114075.

[14] Ciesielczuk, T., Rosik-Dulewska, C., Poluszyńska, J., Miłek, D., Szewczyk, A., and Sławińska, I., 2018, Acute toxicity of experimental fertilizers made of spent coffee grounds, *Waste Biomass Valorization*, 9 (11), 2157–2164.

[15] Cervera-Mata, A., Navarro-Alarcón, M., Delgado, G., Pastoriza, S., Montilla-Gómez, J., Llopis, J., Sánchez-González, C., and Rufián-Henares, J.A., 2019, Spent coffee grounds improve the nutritional value in elements of lettuce (*Lactuca sativa* L.) and are an ecological alternative to inorganic fertilizers, *Food Chem.*, 282, 1–8.

[16] Hirooka, Y., Kurashige, S., Yamane, K., Watanabe, Y., Kakiuchi, M., Ishikawa, D., Miyagawa, T., Iwai, K., and Iijima, M., 2022, Effectiveness of direct application of top dressing with spent coffee grounds for soil improvement and weed control in wheat-soybean double cropping system, *Plant Prod. Sci.*, 25 (2), 148–156.

[17] Massaya, J., Mills-Lamptey, B., and Chuck, C.J., 2022, Soil amendments and biostimulants from the hydrothermal processing of spent coffee grounds, *Waste Biomass Valorization*, 13 (6), 2889–2904.

[18] Sinclair, C.L., Irga, P.J., Duani, G., and Torpy, F.R., 2024, Spent coffee grounds (SCGs) as a soil amendment: The effects of composting time on early sunflower development, *Environments*, 11 (12), 272.

[19] Cervera-Mata, A., Molinero-García, A., Martín-García, J.M., and Delgado, G., 2023, Sequential effects of spent coffee grounds on soil physical properties, *Soil Use Manage.*, 39 (1), 286–297.

[20] Choi, Y., Rim, J.S., Na, Y., and Lee, S.R., 2018, Effects of dietary fermented spent coffee ground on nutrient digestibility and nitrogen utilization in sheep, *Asian-Australas. J. Anim. Sci.*, 31 (3), 363–368.

[21] Batbekh, B., Ahmed, E., Hanada, M., Fukuma, N., and Nishida, T., 2023, Assessment of the impact of coffee waste as an alternative feed supplementation on rumen fermentation and methane emissions in an *in vitro* study, *Fermentation*, 9 (9), 858.

[22] Carta, S., Tsiplakou, E., Nicolussi, P., Pulina, G., and Nudda, A., 2022, Effects of spent coffee grounds on production traits, haematological parameters, and antioxidant activity of blood and milk in dairy goats, *Animals*, 16 (4), 100501.

[23] de Otálora, X.D., Ruiz, R., Goiria, I., Rey, J., Atxaerandio, R., San Martin, D., Orive, M., Iñarra, B., Zufia, J., Urkiza, J., and García-Rodríguez, A., 2020, Valorisation of spent coffee grounds as functional feed ingredient improves productive performance of Latxa dairy ewe, *Anim. Feed Sci. Technol.*, 264, 114461.

[24] Liu, Y., Yuan, W., Lu, Y., and Liu, S.Q., 2021, Biotransformation of spent coffee grounds by fermentation with monocultures of *Saccharomyces cerevisiae* and *Lachancea thermotolerans* aided by yeast extracts, *LWT*, 138, 110751.

[25] Medjadbi, M., Garcia-Rodrguez, A., Atxaerandio, R., Charef, S.E., Picault, C., Ibarruri, J., Iñarra, B., San Martin, D., Serrano-Pérez, B., Martin-Alonso, M.J., and Goiri, I., 2024, Dose-dependent effect of spent coffee grounds on intake, apparent digestibility, fermentation pattern, methane emissions, microbial protein supply, and antioxidant status in Latxa sheep, *J. Anim. Sci.*, 102, skae351.

[26] AOAC, 2000, *Official methods of analysis, 17th Edition*, The Association of Official Analytical Chemists, Gaithersburg, MD, US.

[27] Preecharram, S., Posoongnoen, S., Thummawongsa, T., Sripakdee, T., Tawil, S., Poomsuk, N., and Jandaruang, J., 2023, Proximate composition and antioxidant activity of young flattened rice (Khao-Mao), *Trends Sci.*, 20 (4), 6575.

[28] Preecharram, S., Posoongnoen, S., Thummavongsa, T., Pasom, W., Panyakom, S., Sripakdee, T., and Jandaruang, J., 2024, Values, bioactive compounds, antioxidant activities of brown rice and germinated brown rice, and the pellet products, *Creative Sci.*, 17 (1), 257996.

[29] Preecharram, S., Phosri, S., Theansungnoen, T., Roschat, W., Arthan, S., Lasopha, S., and Jandaruang, J., 2024, Nutritional values and their potential applications in food products of Krabok seed (*Irvingia malayana*), *Trends Sci.*, 21 (1), 7316.

[30] Phewphong, S., Roschat, W., Namwongsa, K., Wonam, A., Kaisri, T., Duangpakdee, P., Leelatam, T., Moonsin, P., and Promarak, V., 2023, Evaluation of the nutritional, minerals, and antioxidant potential of roselle (*Hibiscus sabdariffa* Linn.) seeds from Roi Et Province in the northeastern region of Thailand, *Trends Sci.*, 20 (6), 6664.

[31] Suwannatrai, K., Namwongsa, K., Phanomkhet, N., Tawil, S., and Roschat, W., 2023, Analysis of the Nile tilapia fish's (*Oreochromis niloticus* L.) proximate composition in Sakon Nakhon province, Thailand, *Creative Sci.*, 15 (2), 251073.

[32] Suwannatrai, K., Namwongsa, K., Phanomkhet, N., Nuntapanich, H., and Roschat, W., 2022, The analysis of nutritional value, total phenolic and flavonoid contents, and antioxidant activities from the ethanolic extracts of the roasted broken brown rice powder, *Creative Sci.*, 14 (2), 246426.

[33] Tawil, S., Roschat, W., Phewphong, S., Wonam, A., Kaisri, T., Namwongsa, K., Thammayod, A., Leelatam, T., Maneerat, B., Moonsin, P., Yoosuk, B., Janetaisong, P., and Promarak, V., 2025, Sustainable biodiesel production and properties enhancement from locally sourced Roselle (*Hibiscus sabdariffa* L.) as an alternative biofuel feedstock in Thailand, *Biomass Bioenergy*, 193, 107596.

[34] Moonsin, P., Roschat, W., Phewphong, S., Watthanalao, S., Maneerat, B., Thammayod, A., Leelatam, T., Suwannarong, P., Yoosuk, B., Janetaisong, P., and Promarak, V., 2025, Development of liquid biofuel properties through the blending of biodiesel from used cooking oil and pyrolysis oil from low-quality rubber waste, *Case Stud. Chem. Environ. Eng.*, 11, 101230.

[35] Singh, P.K., Singh, J., Medhi, T., and Kumar, A., 2022, Phytochemical screening, quantification, FT-IR analysis, and *in silico* characterization of potential bio-active compounds identified in HR-LC/MS analysis of the polyherbal formulation from Northeast India, *ACS Omega*, 7 (37), 33067–33078.

[36] Angeloni, S., Navarini, L., Khamitova, G., Maggi, F., Sagratini, G., Vittori, S., and Caprioli, G., 2020, A new analytical method for the simultaneous quantification of isoflavones and lignans in 25 green coffee samples by HPLC-MS/MS, *Food Chem.*, 325, 126924.

[37] Wong-Paz, J.E., Guyot, S., Aguilar-Zárate, P., Muñiz-Márquez, D.B., Contreras-Esquivel, J.C., and Aguilar, C.N., 2021, Structural characterization of native and oxidized procyanidins (condensed tannins) from coffee pulp (*Coffea arabica*) using phloroglucinolysis and thioglycolysis-HPLC-ESI-MS, *Food Chem.*, 340, 127830.

[38] Thammayod, A., Roschat, W., Leelatam, T., Phewphong, S., Maneerat, B., Leelatam, N., Thangthong, A., Moonsin, P., and Promarak, V., 2025, Development of high-quality organic fertilizer from biomass waste and the application of biochar in local areas of Sakon Nakhon province, Northeastern Thailand, *Trends Sci.*, 22 (2), 9105.

[39] Jeon, Y.J., Kang, Y.G., Eun, J.A., and Oh, T.K., 2024, Yield, functional properties and nutritional compositions of leafy vegetables with dehydrated food waste and spent coffee grounds, *Appl. Biol. Chem.*, 67, 22.

[40] Phewphong, S., Roschat, W., Jandaruang, J., Leelatam, T., Arthan, S., Poomsuk, N., Thammayod, A., Maneerat, B., Namthaisong, A., Siriwong, K., and Promarak, V., 2025, Analysis of volatile organic compounds, antioxidant, tyrosinase inhibitory, and antimicrobial activities of essential oils from citronella grass and kaffir lime, *Trends Sci.*, 22 (4), 9434.

[41] Raspo, M.A., Vignola, M.B., Andreatta, A.E., and Juliani, H.R., 2020, Antioxidant and antimicrobial activities of citrus essential oils from Argentina and the United States, *Food Biosci.*, 36, 100651.

[42] Choopan, W., and Kittiamornkul, N., 2025, Comparative effects of graphene oxide and sodium alginate on filter paper against *S. aureus* and *E. coli*, *Creative Sci.*, 17 (2), 285702.

[43] Suwonnachot, W., Chaisen, K., and Audtarat, S., 2025, *In vitro* anti-pathogenic activity of composite materials made of activated carbon from bamboo containing silver nanoparticles, *Creative Sci.*, 17 (1), 258193.

[44] Bijla, L., Ibourki, M., Bouzid, H.A., Sakar, E.H., Aissa, R., Laknifli, A., and Gharby, S., 2022, Proximate composition, antioxidant activity, mineral and lipid profiling of spent coffee grounds collected in Morocco reveal a great potential of valorization, *Waste Biomass Valorization*, 13 (11), 4495–4510.

[45] Panpraneecharoen, S., and Rodkate, N., 2020, Proximate analysis and structural properties of Arabica spent coffee grounds, *YRU J. Sci. Technol.*, 5 (1), 10–16.

[46] Vu, D.C., Vu, Q.T., Huynh, L., Lin, C.H., Alvarez, S., Vo, X.T., and Nguyen, T.H.D., 2021, Evaluation of fatty acids, phenolics and bioactivities of spent coffee grounds prepared from Vietnamese coffee, *Int. J. Food Prop.*, 24 (1), 1548–1558.

[47] El-Desouky, N., Shoueir, K., El-Mehasseb, I., and El-Kemary, M., 2023, Synthesis of silver nanoparticles using bio-valorization coffee waste extract: photocatalytic flow-rate performance, antibacterial activity, and electrochemical investigation, *Biomass Convers. Biorefin.*, 13 (17), 15871–15885.

[48] Tinrat, S., 2022, Phytochemical screenings, antibacterial and anti-biofilm activities of *Garcinia cowa* Roxb. leaves extracts and its synergistic effect with antibiotic, *Pharm. Sci. Asia*, 49 (6), 556–567.

[49] Zainol, M.K., Mohd Subri, I., Zamri, A.I., Mohd Zin, Z., Fisal, A., and Mamat, H., 2020, Antioxidative properties and proximate analysis of spent coffee ground (SCG) extracted using ultrasonic-methanol assisted technique as a potential functional food ingredient, *Food Res.*, 4 (3), 636–644.

[50] Arauzo, P.J., Lucian, M., Du, L., Olszewski, M.P., Fiori, L., and Kruse, A., 2020, Improving the recovery of phenolic compounds from spent coffee grounds by using hydrothermal delignification coupled with ultrasound assisted extraction, *Biomass Bioenergy*, 139, 105616.

[51] Maiyah, N., Kerdpiboon, S., Supapvanich, S., Kerr, W.L., Sriprom, P., Chotigavin, N., Klaypradit, W., and Puttongsiri, T., 2025, Recovering bioactive compounds and antioxidant capacity of medium-roasted spent coffee grounds through varied hydrothermal brewing cycles, *J. Agric. Food Res.*, 20, 101789.

[52] Alghamdi, A.G., Alomran, A., Ibrahim, H.M., Alkhasha, A., and Majrashi, M.A., 2024, Spent coffee waste-derived biochar improves physical properties, water retention, and maize (*Zea mays* L.) growth in sandy soil, *Sci. Rep.*, 14 (1), 19753.

[53] Afriliana, A., Hidayat, E., Mitoma, Y., Masuda, T., and Harada, H., 2021, Studies on composting spent coffee grounds by *Aspergillus* sp. and *Penicillium* sp. in aerobic static batch temperature control, *J. Agric. Chem. Environ.*, 10 (1), 91–112.

[54] Ballesteros, L.F., Teixeira, J.A., and Mussatto, S.I., 2014, Chemical, functional, and structural properties of spent coffee grounds and coffee silverskin, *Food Bioprocess Technol.*, 7 (12), 3493–3503.

[55] Canci, L.A., de Toledo Benassi, M., Canan, C., Kalschne, D.L., and Colla, E., 2022, Antimicrobial potential of aqueous coffee extracts against pathogens and *Lactobacillus* species: A food matrix application, *Food Biosci.*, 47, 101756.

[56] Castro-Díaz, R., Silva-Beltrán, N.P., Gámez-Meza, N., and Calderón, K., 2025, The antimicrobial effects of coffee and by-products and their potential applications in healthcare and agricultural sectors: A state-of-art review, *Microorganisms*, 13 (2), 215.