© 2026 Journal of Pharmacy & Pharmacognosy Research, 14 (2), 2332, 2026
ISSN 0719-4250
https://jppres.com

DOI: https://doi.org/10.56499/jppres_14.2.2332

Original Article

Comparative phytochemical profiling and evaluation of antioxidant and wound healing activities of Arabica and Robusta coffee beans and spent grounds

[Perfiles fitoquímicos comparativos y evaluación de las actividades antioxidantes y de cicatrización de heridas de los granos de café arábica y robusta y de los posos gastados]

Pimolwan Siriparu[1], Juthamat Ratha[2], Ploenthip Puthongking[2,3]*

[1]Graduate School, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, 40002, Thailand.
[2]Melatonin Research Group, Khon Kaen University, Khon Kaen, 40002, Thailand.
[3]Division of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, 40002, Thailand.
*E-mail: pploenthip@kku.ac.th

Abstract

Context: Solid waste from spent coffee grounds (SCG) has sharply increased due to a growing global coffee market. Concerning environmental impact, the value-added of solid waste from coffee drinking, especially in the pharmaceutical industry, was investigated in this research.

Aims: To compare the phytochemical content, antioxidant activity, and wound healing properties of Arabica (AB) and Robusta (RB) coffee bean extracts and SCG extracts (AG and RG, respectively).

Methods: The total phenolic content (TPC), the total flavonoid content (TFC), high-performance liquid chromatography (HPLC), and gas chromatography–mass spectrometry (GC-MS) were used to analyze the extracts. In addition, the antioxidant and wound healing activities of the extracts were evaluated.

Results: The coffee beans showed stronger antioxidant activity than the SCG extract. Robusta exhibited higher antioxidant activity than Arabica, consistent with its higher TPC. HPLC analysis identified melatonin and tryptophan in all samples, with greater concentrations in the coffee bean extracts, especially AB. GC-MS analysis indicated Robusta contained more caffeine. Interestingly, the AG extract had the lowest caffeine content (6.87%) but produced the fastest wound closure (84.8% after 48 h, compared with 77.5% for the control), achieving >98% closure after 72 h.

Conclusions: Robusta contained higher antioxidant and polyphenol levels than Arabica, while coffee beans generally possessed more beneficial phytochemicals than spent grounds. However, the AG extract showed the greatest potential for promoting wound healing, suggesting its possible use in biomedical or cosmetic applications.

Keywords: by-products; indolamines; phenolic acids; spent coffee grounds (SCG); scratch assay.

Resumen

Contexto: Los residuos sólidos de posos de café gastados (SCG) aumentan considerablemente debido al creciente mercado mundial del café. En cuanto al impacto ambiental, en esta investigación se evaluó el valor agregado de los residuos sólidos del consumo de café, especialmente en la industria farmacéutica.

Objetivos: Comparar el contenido fitoquímico, la actividad antioxidante y las propiedades curativas de los extractos de granos de café arábica (AB) y robusta (RB), y de los extractos SCG (AG y RG, respectivamente).

Métodos: Para analizar los extractos se utilizaron el contenido fenólico total (TPC), el contenido total de flavonoides (TFC), la cromatografía líquida de alta resolución (HPLC) y la cromatografía de gases con espectrometría de masas (GC-MS). Además, se evaluaron las actividades antioxidantes y cicatrizantes de los extractos.

Resultados: Los granos de café mostraron una actividad antioxidante más fuerte que la del extracto de SCG. Robusta exhibió una actividad antioxidante superior a la de la arábica, en consonancia con su mayor TPC. El análisis por HPLC identificó melatonina y triptófano en todas las muestras, con concentraciones más altas en los extractos de granos de café, especialmente en AB. El análisis GC-MS indicó que robusta contenía más cafeína. Curiosamente, el extracto AG presentó el contenido de cafeína más bajo (6,87%), pero produjo el cierre de la herida más rápido (84,8% después de 48 h, en comparación con 77,5% para el control), logrando >98% de cierre después de 72 h.

Conclusiones: El robusta contenía niveles más altos de antioxidantes y polifenoles que el arábica, mientras que los granos de café generalmente poseían fitoquímicos más beneficiosos que los posos de café. Sin embargo, el extracto AG mostró el mayor potencial para promover la cicatrización de heridas, lo que sugiere su posible uso en aplicaciones biomédicas o cosméticas.

Palabras Clave: ácidos fenólicos; ensayo de rayado; indolaminas; posos de café (SCG); subproductos.

ARTICLE INFO
Received: January 9, 2025.
Accepted: February 17, 2026.
Available Online: March 7, 2026.

AUTHOR INFO
ORCID:
0000-0002-4182-5565 (PS)
0000-0002-5069-2071 (PP)
0000-0003-0775-5329 (JR)

Abbreviations: AB: Arabica coffee bean; AG: Arabica spent coffee ground; CA: caffeic acid; ChA: chlorogenic acid; FA: ferulic acid; GA: gallic acid; MLT: melatonin; *p*-CA: *p*-coumaric acid; *p*-HO: *p*-hydroxybenzoic acid; PCCA: protocatechuic acid; QE: quercetin; RB: Robusta coffee bean; RG: Robusta spent coffee ground; RN: rutin; SA: sinapic acid; SCG: spent coffee ground; SE: serotonin; SyA: syringic acid; TFC: total flavonoid contents; TPC: total phenolic contents; Trp: tryptophan.

INTRODUCTION

The global coffee market has increased in recent years, with demand rising from 171.2% in 2018 to a preliminary estimate of 177.0% in 2023 (ICO, 2024). This escalating consumption has led to a corresponding surge in coffee waste production (Bomfim et al., 2022; Tokimoto et al., 2005). The two major green coffee bean cultivars in the coffee industry are Arabica (*Coffea arabica*) and Robusta (*Coffea canephora*); they account for 60% and 40% of coffee production, respectively (ICO, 2024). Coffee beans are rich in phytochemicals, including macromolecules (polysaccharides, polypeptides, and fatty acids) and micromolecules (alkaloids, indole amides, and polyphenols). These compounds exert a myriad of biological activities, including antioxidation, anti-inflammation, and wound healing (Ali et al., 2022; Bobková et al., 2020; Lemos et al., 2022). The compounds responsible for antioxidant activity include caffeine (Freitas et al., 2023; Vieira et al., 2020), chlorogenic acid (ChA; Freitas et al., 2023; Jeszka-Skowron et al., 2022; Opitz et al., 2017), caffeic acid (CA; Freitas et al., 2023), and ferulic acid (FA; Sugiyanta et al., 2020). In addition, melatonin (MLT) and serotonin (SE: Ramakrishna et al., 2012) show antioxidant and anti-inflammatory properties (Arnao & Hernández-Ruiz, 2019; Bonnefont-Rousselot & Collin, 2010; Galano et al., 2011; Jeszka-Skowron et al., 2022; Morvaridzadeh et al., 2020). Yuwono (2014) added coffee powder to dressings in an attempt to aid wound healing in patients with diabetes. It showed positive effects by controlling wound moisture, exerting hemostatic, antioxidant, and antimicrobial activities, and reducing scar formation. The ability of coffee beans to ameliorate inflammation could promote collagen regeneration and dermal cell epithelialization (Yuwono, 2021).

As coffee consumption has grown, so has the waste associated with its production. Spent coffee grounds (SCG) constitute approximately 45%–50% of coffee solid waste (Bevilacqua et al., 2023). Approximately 6–8 million tonnes of SCG are generated annually worldwide (Bevilacqua et al., 2023). Lee et al. (2023) reviewed the potential uses of SCG, including as a fermentation substrate for fertilizer and bio-insecticide in agriculture; a source of bioethanol and lipids for biodiesel; and as a source for foods, cosmetics, and biomedical applications. The ability of SCG to be utilized is due to its phytochemical content. SCG typically retains up to 50% of the bioactive compounds in the original coffee beans. It is rich in polysaccharides, has a higher sugar content (mannose and galactose) than whole beans, a higher protein fraction (Murthy & Madhava Naidu, 2012), and abundant phenolic compounds (Lee et al., 2023). Given this potential use, the present study investigated the phytochemical content and biological activities (i.e., antioxidant and wound healing effects) of coffee beans and their waste. This study revealed bioactive compounds responsible for these activities in both beans and SCG.

MATERIAL AND METHODS

Chemicals and reagents

All organic solvents were purchased from ACI Labscan (Bangkok, Thailand). High-performance liquid chromatography (HPLC) standards, including gallic acid (GA), protocatechuic acid (PCCA), *p*-hydroxybenzoic acid (*p*-HO), syringic acid (SyA), ChA, CA, *p*-CA, FA, sinapic acid (SA), rutin (RN), quercetin (QE), melatonin (MLT), serotonin (SE), and, tryptophan (Trp), were purchased from Sigma Aldrich (St. Louis Mo., USA). The reagents 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) disodium salt (ABTS), 2,2-diphenyl-1 picrylhydrazyl (DPPH), potassium persulfate, 2,4,6-Tris(2-pyridyl)-1,3,5-triazine, 2,2´-Azobis(2-methylpropionamidine) dihydrochloride, and aluminium chloride hexahydrate were also purchased from Sigma Aldrich (St. Louis, MO, USA). Other chemicals used in the study were purchased from the following sources: iron (III) chloride from Ajax Finechem (New South Wales, Australia), sodium carbonate from Kemaus (New South Wales, Australia), and the Folin-Ciocalteu reagent from LOBA Chemis (Mumbai, India). Dulbecco's Modified Eagle Media powder, penicillin-streptomycin, fetal bovine serum (FBS), trypsin-EDTA, and trypan blue were purchased from Gibco (New York, USA), and (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide was from Invitrogen™ (Oregon, USA).

Sample preparation

Medium-roasted Arabica (*Coffea arabica)* and Robusta (*Coffea canephora*) coffee beans were purchased from a local market in Khon Kaen, Thailand, in 2022. SCG was prepared by grinding the roasted coffee beans and pressing with an espresso machine. Then, the SCG was dried at 60°C for 24 h and stored in zipped bags in a dry place until extraction.

For extraction, the coffee sample was combined with methanol (1:2, w/v) and subjected to ultrasonic extraction three times, each for 30 min at 28 kHz and

220 V. The extracts were filtered, and methanol was removed using a rotary evaporator. All extractions were kept at –20°C until use.

Phytochemical content analysis

Total phenolic content (TPC) and total flavonoid content (TFC)

The TPC and TFC were determined according to the procedure described by Siriparu et al. (2022). To determine TPC, the coffee extract or gallic acid (GA) solutions (standard control), 7% sodium carbonate, and 10% Folin-Ciocalteu reagent (1:4:5; v/v/v) were incubated for 30 min; then the absorbance at 760 nm was measured with a microplate reader (Sunrise TM, Grödig, Austria). The gallic acid calibration curve ($y = 0.0037x + 0.0391$, $R^2 = 0.9999$) was used to calculate the TPC, presented as gallic acid equivalents per gram dry weight (mg GAE/g DW). For the TFC, the extract or a quercetin solution was incubated with 2% aluminum chloride (1:1, v/v) for 30 min, and the absorbance at 415 nm was measured. The TFC was determined from the quercetin calibration curve ($y = 0.0418x + 0.0813$, $R2 = 0.9977$), expressed as milligrams quercetin equivalents per gram DW (mg QE/g DW).

Quantifications of phenolics and indoleamine compounds by HPLC

Phenolic and indoleamine compounds in coffee beans and SCG extracts were analyzed using the fully validated method (validated parameters: LOD, LOQ, linear equation, coefficient of correlation, and concentration range) described in our previous study (Siriparu et al., 2022). Eleven standard phenolic compounds were quantified, including gallic acid (GA), protocatechuic acid (PCCA), *p*-hydroxybenzoic acid (*p*-HO), syringic acid (SyA), chlorogenic acid (ChA), caffeic acid (CA), *p*-coumaric acid (*p*-CA), ferulic acid (FA), sinapic acid (SA), rutin (RN), and quercetin (QE) with an HPLC-DAD/UV V instrument (Shimadzu Co., Kyoto, Japan). This assay used a C-18 column (4.6 × 250 mm, 5 µm diameter) and the compounds were subjected to gradient elution with 1% acetic acid (solvent A): 100% acetonitrile (solvent B) as follows; 0–5 min, 5% solvent B; 5–15 min, 9% solvent B; 15–22 min, 11% solvent B; 22–38 min, 18% solvent B; 38–43 min, 23% solvent B; 43–44 min, 90% solvent B; 44–55 min, 80% solvent B; and 55-70 min, 5% solvent B. Absorbance was measured at 280, 320, and 370 nm. The levels of 11 phenolic compounds were calculated based on their standard calibration curves, and the results are presented as µg/g DW.

The quantifications of indoleamines: melatonin (MLT), serotonin (SE), and tryptophan (Trp) were quantified by HPLC with fluorescence detection (Agilent, Santa Clara, CA, USA). This process used a C-18 column (4.6 × 250 mm, 5 µm inner diameter) and gradient elution with methanol (solvent A) and 50 mM phosphate buffer at pH 4.5 (solvent B): 0–15 min, 25% solvent A; 15–45 min, 40% solvent A; and 45–60 min, 25% solvent A. The fluorescence detection involved excitation at 286 nm and emission at 346 nm. The MLT, SE, and Trp concentrations were calculated using the standard calibration curves and are presented as ng/g DW.

Determination of other compounds by gas chromatography–mass spectrometry (GC-MS)

The GC-MS protocol was adapted from a previous study (Burana-Osot et al., 2016). The samples were dissolved in methanol (10 mg/mL) and analyzed with a GC-MS-QP2020 instrument (Shimadzu Co.). Briefly, 1 µL of sample (split 1:20) was injected onto a DB-5 MS capillary column (30 m × 0.25 mm × 0.25 µm). The oven temperature was programmed to increase from 40°C (2 min hold) to 320°C at 5°C/min, with a final hold of 15 min, for a total run time of 103 min. The column temperature program included holds at 100°C (2 min), 150°C (5 min), 200°C (10 min), 250°C (5 min), and 320°C (15 min). Mass spectra were acquired in the electron ionization mode at 70 eV, with the ion source at 250°C, over a mass range of m/z 35–500. Peaks were identified by comparing the retention times and mass spectra to the NIST17 library (similarity index [SI] ≥ 80%) (Stein et al., 2017).

Antioxidant capacities

2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) disodium salt (ABTS) assay

The (ABTS) assay was adapted from the method described by Brand-Williams et al. (1995). The ABTS reagent was prepared by incubating 7 mM ABTS• with 2.45 mM potassium for 12 h in the dark. The ABTS reagent was diluted 1:15 (v/v) with distilled water before use. The extract and Trolox solution (final concentration 50 µg/mL in methanol) was mixed with the ABTS reagent in a 1:1 (v/v) ratio and incubated for 30 min. Methanol and Trolox solution were used as the blank and positive controls, respectively. The antioxidant activity of the coffee extract was calculated as a percent inhibition using Equation [1], based on its absorbance at 415 nm compared to the Trolox standard.

$$\text{Inhibition (\%)} = \frac{A_{control} - A_{treated}}{A_{control}} \times 100 \quad [1]$$

Where $A_{control}$: the absorbance of the control group; $A_{Treated}$: the absorbance of the treated group with coffee extracts or Trolox

The IC_{50}, defined as the concentration required for 50% inhibition, was determined from a linear

calibration curve (plotting percent inhibition of the coffee extracts and Trolox against concentration).

2,2-Diphenyl-1 picrylhydrazyl radical (DPPH) assay

The DPPH assay was adapted from the method described by Arabshahi-Delouee & Urooj (2007). The 0.2 mM DPPH• reagent was prepared in methanol. The extract and Trolox solutions (final concentration 50 µg/mL in methanol) were mixed with 0.2 mM DPPH• reagent in a 1:2 (v/v) ratio and then incubated in the dark for 30 min. Methanol and Trolox were used as the blank and positive controls, respectively. The absorbance at 517 nm was measured. The percent inhibition and IC_{50} were determined as described for the ABTS assay.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed according to the method reported by Iqbal et al. (2012). The FRAP reagent was prepared by mixing 20 mM ferric chloride, 10 mM 2,4,6-tris(2-pyridyl)-s-triazine, and 300 mM acetate buffer (pH 3.6) in a 1:1:10 (v/v/v) ratio. The extract was mixed with the FRAP reagent (1:4, v/v) and incubated in the dark for 5 min. The absorbance at 595 nm was then measured. The FRAP value was determined based on a standard curve of ferrous sulfate ($FeSO_4 \bullet 7H_2O$) and is presented as millimoles of Fe^{2+} equivalents per gram dry weight (mmol Fe^{2+} /g DW).

Cell culture

HaCaT cells, an immortalized human keratinocyte cell line, were obtained from American Type Culture Collection (Manassas, VA, USA). They were cultured in DMEM supplemented with 1% PenStrep and 10% FBS at 37°C with 5% CO_2. HaCaT cells were cultured in flasks until they reached approximately 80% confluence. At this point, they were trypsinized (0.25% trypsin-EDTA) and seeded onto plates for *in vitro* assays.

Cell viability assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to measure the activity of mitochondrial enzymes, an indicator of living cells(Mahakunakorn et al., 2024). HaCaT cells were seeded at a density of 1×10^5 cells per well (in 100 µL) in a 96-well plate and allowed to grow overnight. Then, the cells were treated with various coffee extracts (final concentrations of 1, 5, 10, 50, and 100 µg/mL) prepared in serum-free media containing <1% DMSO. The control group was treated with serum-free medium. After incubation for 24 h, MTT solution (0.5 mg/mL) was added to each well, and the cells were incubated for 3 h. Then, the formazan crystals formed in viable cells were dissolved in DMSO, and the absorbance at 570 nm was measured using a microplate reader. A higher absorbance corresponds to greater cell viability. The percentage of viable cells relative to the control group was calculated using Equation [2].

$$\text{Cell viability (\%)} = \frac{A_{Treated}}{A_{Control}} \times 100 \quad [2]$$

where $A_{Treated}$: the absorbance of coffee extract solutions treated groups; $A_{Control}$: the absorbance of a serum-free media group.

Wound healing assay

The influence of coffee extracts on HaCaT cell migration and wound closure was evaluated using a scratch assay adapted from Grada et al. (2017). The cells were seeded in a 24-well plate at a density of 1×10^5 cells per well (in 500 µL) and incubated overnight at 37°C and 5% CO_2. A sterile 0.2 mL micropipette tip was used to gently scratch the monolayer. The cells were washed once with 1× PBS (pH 7.4) to remove detached cells. Eace extract was prepared in serum-free DMEM at a concentration of 1 µg/mL (w/v) and added to the scratched HaCaT cells. The negative control group was serum-free media, and the positive control was medium with 10% FBS, representing normal healthy growth conditions (Nie & Deters, 2013).

The wound closure process was monitored at 24, 48, 72, 96, and 120 h by using the EVOS™ FL Auto 2 Imaging System (Thermo Fisher Scientific, Waltham, MA, USA) at 4× magnification with a scale bar of 650 µm. Images of the scratched areas were captured randomly for each treatment group at each time point. The ImageJ software was then used to quantify the wound-closure area. The percentage of wound closure was calculated using Equation [3] by comparing the initial wound area (0 h) to the remaining wound area at each time point.

$$\text{Wound closure (\%)} = \frac{W_0 - W_n}{W_0} \times 100 \quad [3]$$

where W_0: the initial wound area; W_n: the wound area at each time point.

Multivariate analysis

Multivariate analysis was conducted to examine the chemical composition and biological effects of the four coffee extracts using MetaboAnalyst 6.0 (https://www.metaboanalyst.ca/). The analysis included phytochemical profiles obtained by HPLC and GC-MS, antioxidant activity measurements, and wound closure data. All data were compiled into a comma-separated values (.csv) file and preprocessed for comparability: The values were normalized to the sample median, log-transformed to reduce skewness, and scaled using Pareto scaling. The processed data were then analyzed using principal component analysis (PCA) and hierarchical clustering analysis (HCA),

with Euclidean distances and the Ward clustering method applied to identify patterns and groupings among the coffee extracts.

Statistical analysis

All results were reported as the mean ± SD (n = 3). The data were analyzed with one-way analysis of variance with Tukey's test using SPSS Statistics 28.0 (International Business Machines Corporation, New York, USA). A p-value <0.05 was considered to indicate a statistically significant difference.

RESULTS AND DISCUSSION

Sample preparation and analysis of the phytochemical content

The coffee bean is well-known for its rich content of phytochemicals, such as polyphenols, alkaloids, and indoleamines, associated with antioxidant and anti-inflammatory responses (Ali et al., 2022). According to the coffee industry, coffee is a popular drink worldwide, and demand for coffee beans has also increased, resulting in massive SCG waste. Moreover, SCG extracts have been reported as an alternative material for developing pharmaceutical products due to their composition of biochemical compounds such as polyphenols and alkaloids (Lee et al., 2023). Thus, the value-added in the pharmaceutical field, especially the wound healing effects of coffee beans and SCG extracts derived from Arabica and Robusta, was investigated. The comparison of phytochemical compounds using HPLC and GC-MS analyses was also studied.

All samples were extracted with methanol to yield AB, AG, RB, and RG extracts at 6.06%, 3.53%, 6.49%, and 2.95%, respectively. These extracts were used for all subsequent experiments in this study. Phytochemical content revealed that the TPC ranged from 18.63 to 71.34 mg GA/g DW; the order from highest to lowest was RB, AB, RG, and AG. The TFC ranged from 1.51 to 3.75 mg QE/g DW; the order from highest to lowest was AB, AG, RB, and RG (Table 1). Quantitative analysis by HPLC showed that 5 of the 11 phenolic acids—GA, PCCA, ChA, and p-CA—were quantified, with contents ranging from 154.89 to 1,150.78 µg/g DW, as shown in Table 1. The chromatograms are presented in the supplementary material (Figs. S1-S4). ChA was the most abundant phenolic compound, consistent with the studies by Rojas-González et al. (2022) and Makiso et al. (2024), who reported that ChA was the major bioactive compound in coffee. The trend of phenolic acids contents in SCG was lower than that of coffee beans in all samples.

Inflammation is a complex biological response triggered by tissue damage, infections, and allergens. It is characterized by a cytokine storm and an imbalance between oxidants and antioxidants (Gallo et al., 2017). MLT exhibits cytoprotective effects through its antioxidant and anti-inflammatory actions. scavenges free radicals both directly and indirectly (Bonnefont-Rousselot & Collin, 2010; Morvaridzadeh et al., 2020; Galano et al., 2011) and inhibits pro-inflammatory cytokine production via the nuclear factor kappa B (NF-κB) signaling pathway (Tan & Reiter, 2022). Hence, MLT may reduce the risk of non-communicable diseases (NCDs) such as cardiovascular disease, obesity, and type 2 diabetes, conditions often associated with chronic inflammation and oxidative stress (Seyedsadjadi & Grant, 2020). A decline in endogenous MLT production may contribute to the rising prevalence of type 2 diabetes. While insulin influences glucose metabolism, exogenous MLT has been shown to affect glucose homeostasis by acting as a circadian regulator (Watanabe et al., 2023). Plant-based sources can increase serum MLT levels. For example, walnuts contain MLT (3.7 ng/g DW) and increase serum MLT levels in rats following oral consumption (Reiter et al., 2005). Similarly, dietary Trp, an essential amino acid and precursor for MLT synthesis, can significantly increase serum MLT levels compared to untreated controls (Yaga et al., 1993).

Therefore, this research is interested in evaluating melatonin (MLT), serotonin (SE), and tryptophan (Trp) in coffee beans and their solid waste product. The results showed that all samples contained measurable MLT and Trp in ng/g DW scale, as shown in Table 1, but SE was detected at very low levels, insufficient for reliable quantification by HPLC. Among the samples, the AB extract had the highest MLT and Trp contents, consistent with previous reports showing higher indoleamine content in Arabica than in Robusta coffee (Martins & Gloria, 2010; Ramakrishna et al., 2012). Trp plays a critical role in MLT biosynthesis in both plants and animals (Tan et al., 2012). Typically, plant MLT (phytomelatonin) levels exceed those in animals, likely due to greater Trp availability, suggesting that MLT content may be proportional to Trp levels in plant tissues (Tan et al., 2012).

GC-MS data were analyzed by comparing retention times and mass spectra with the NIST 17 GC-MS reference library. For reliable compound identification, an SI of 70%–90% is generally recommended (Matyushin et al., 2020); in this study, an SI≥ 80% was used for identification. The GC-MS results are presented in Table 2, and representative GC-MS chromatograms are provided in the supplementary data (Figs. S5-S12). Eight compounds were identified, including one alkaloid (caffeine), three fatty acids (methyl palmitate, methyl linoleate, and methyl linolelaidate), and four primary amides (palmitoleamide, erucamide, hexadecanamide,

and octadecanamide). Caffeine was the most abundant compound across all extracts, ranging from 6.87% to 81.97%. It is a major bioactive compound known for its antioxidant and anti-inflammatory properties (Hall et al., 2015). Among the samples, the RB extract contained the highest caffeine level, followed by the RG, AB, and AG extracts. The higher caffeine content in the RB extract compared with the AB extract is consistent with previous reports (Bravo et al., 2012; Jeszka-Skowron et al., 2022).

The coffee bean extracts (AB and RB) had notably higher phytochemical content than the SCG extracts (AG and RG). This finding is in line with what Bravo et al. (2012) reported: hot water and brewing-related pressure decrease the phytochemical content in Arabica and Robusta SCG extracts. This brewing effect explains the significant reduction in phytochemicals in the AG and RG extracts compared with the AB and RB extracts. Moreover, there have been numerous reports on differences in the phytochemical profiles and levels depending on the specific coffee cultivar (Bravo et al., 2012; Jeszka-Skowron et al., 2022; Martins & Gloria, 2010), the roasting level (Bevilacqua et al., 2023; Bobková et al., 2020; Freitas et al., 2023; Liao et al., 2022; Martins & Gloria, 2010; Yust et al., 2023).

Table 1. The phytochemical contents of coffee bean and spent coffee ground extracts.

Phytochemical content		Sample			
		AB	AG	RB	RG
Colorimetric assays					
TPC (mg GA/g DW)		44.14 ± 0.80^{b}	18.63 ± 0.97^{d}	71.34 ± 1.83^{a}	34.12 ± 0.65^{c}
TFC (mg QE/g DW)		3.75 ± 0.04^{a}	3.07 ± 0.02^{b}	2.49 ± 0.03^{c}	1.51 ± 0.01^{d}
HPLC analysis					
Phenolics (µg/g DW)	GA	15.77 ± 1.11^{a}	4.28 ± 0.06^{c}	10.16 ± 1.96^{b}	3.96 ± 0.26^{c}
	PCCA	27.04 ± 2.46^{a}	11.36 ± 0.11^{b}	30.04 ± 5.34^{a}	ND
	ChA	475.35 ± 18.68^{b}	154.89 ± 1.73^{d}	1,150.78 ± 42.68^{a}	364.47 ± 1.00^{c}
	CA	8.80± 0.33^{b}	4.16 ± 0.04^{c}	12.08 ± 0.47^{a}	8.85 ± 0.17^{b}
	p-CA	6.67 ± 0.25^{a}	ND	ND	ND
Indolamines (ng/g DW)	MLT	1,038.83 ± 5.56^{a}	180.99 ± 13.46^{c}	327.54 ± 11.43^{b}	152.46 ± 16.57^{c}
	Trp	1,043.275 ± 2.75^{a}	264.283 ± 9.83^{d}	938.295 ± 18.95^{b}	602.281 ± 1.81^{c}

Data are presented as mean ± SD (n = 3), means within each row, means with different superscript letters (a-d) are significantly different ($p<0.05$, Tukey's test). AB: Arabica coffee bean; AG: Arabica SCG; RB: Robusta coffee bean; RG: Robusta SCG; ND: Not detected; GA: gallic acid; QE: quercetin; DW: dry weight; PCCA: protocatechuic acid; ChA: chlorogenic acid; CA: caffeic acid; *p*-CA: *p*-coumaric acid; MLT: melatonin; Trp: tryptophan.

Table 2. Phytochemical compounds detected in the coffee bean and spent coffee ground extracts by GC-MS.

No	Name	Classification	CAS	SI (%)	Formula	Fragmentation pattern (%)	Relative peak area (%)			
							AB	AG	RB	RG
1	Caffeine	Alkaloid	58-08-2	96	$C_8H_{10}N_4O_2$	194(100), 165, 149,137, 122, 109, 94, 82, 70, 67, 55	43.93	6.87	81.97	72.00
2	Methyl palmitate	Fatty acid	112-39-0	94	$C_{17}H_{34}O_2$	270, 227, 199, 185, 171, 157, 143, 129, 115, 101, 87, 74(100), 69, 55, 43	0.75	1.07	0.67	0.51
3	Methyl linoleate	Fatty acid	112-63-0	94	$C_{19}H_{34}O_2$	294, 164, 109, 95, 81, 67(100), 55, 41	0.45	1.25	-	-
4	Methyl linolelaidate	Fatty acid	2462-85-3	90	$C_{19}H_{34}O_2$	36, 123, 109, 95, 81, 67(100), 55, 41	-	-	0.45	0.31
5	Palmitoleamide	Primary amide	106010-22-4	95	$C_{16}H_{31}NO$	154, 140, 126, 112, 72, 59(100), 55, 41	7.43	4.99	1.20	3.80
6	Erucamide	Primary amide	112-84-5	90	$C_{22}H_{43}NO$	154, 126, 97, 72, 59(100), 55, 41	6.27	-	3.71	5.27
7	Hexadecanamide	Primary amide	629-54-9	92	$C_{16}H_{33}NO$	255, 226, 212, 142, 128, 114, 86, 72, 59(100), 55, 43	1.10	1.19	-	2.15
8	Octadecanamide	Primary amide	124-26-5	88	$C_{18}H_{37}NO$	254, 240, 198, 184, 170, 156, 128, 114, 98, 86, 72, 59(100), 41	2.41	0.8	-	-

AB: Arabica coffee bean; AG: Arabica SCG; RB: Robusta coffee bean; RG: Robusta SCG; SI: similarity index.

Table 3. Antioxidant activity of the coffee bean and spent coffee ground extracts.

Sample	ABTS		DPPH		FRAP value (mmole Fe^{2+}/100 g DW)
	IC_{50} value (µg/mL)	Antioxidation classification	IC_{50} value (µg/mL)	Antioxidation classification	
AB	35.68 ± 0.07^{c}	Very strong	50.75 ± 0.12^{c}	Strong	3.03 ± 0.20^{b}
AG	66.05 ± 0.64^{d}	Strong	94.52 ± 0.16^{d}	Strong	2.12 ± 0.01^{c}
RB	25.17 ± 0.16^{a}	Very strong	32.38 ± 0.17^{a}	Very strong	3.83 ± 0.32^{a}
RG	32.34 ± 0.30^{b}	Very strong	34.71 ± 0.16^{b}	Very strong	4.00 ± 0.06^{a}
Trolox	4.00 ± 0.03	Very strong	3.53 ± 0.03	Very strong	3495.51 ± 70.37

Data are presented as mean ± SD (n = 3), means within each column, means with different superscript letters (a-d) are significantly different (p<0.05, Tukey's test), AB: Arabica coffee bean; AG: Arabica SCG; RB: Robusta coffee bean; RG: Robusta SCG.

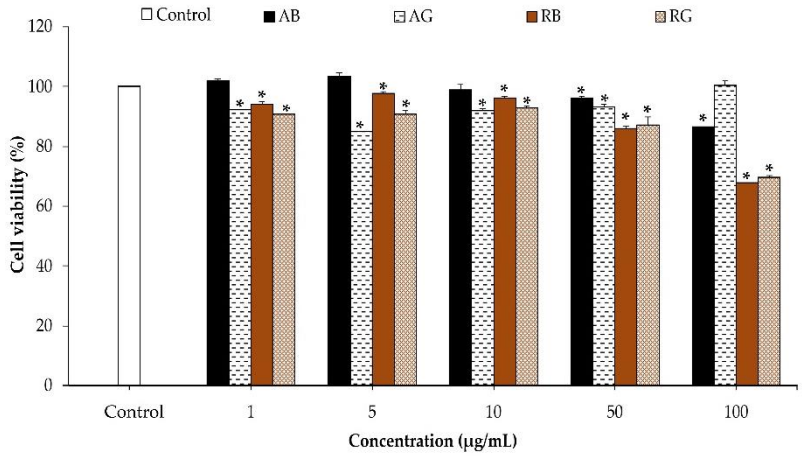

Figure 1. Viability of the HaCaT cells when incubated with various concentrations of the extracts.

Data are presented as mean ± SD (n = 3); * indicates a significant difference from the control group (p<0.05, Tukey's test). AB: Arabica coffee bean; AG: Arabica SCG; RB: Robusta coffee bean; RG: Robusta SCG.

Antioxidant activity

The antioxidant activity of AB, AG, RB, and RG was evaluated using ABTS, DPPH, and FRAP assays, and the results are presented in Table 3. Antioxidant activity was classified based on the IC_{50}: very strong for IC_{50} < 50 µg/mL, strong for 50 µg/mL < IC_{50} < 100 µg/mL, medium for 101 µg/mL < IC_{50} < 250 µg/mL, weak for 250 µg/mL < IC_{50} < 500 µg/mL, and no activity for when IC_{50} > 500 µg/mL (Kusumawati et al., 2021). The ABTS IC_{50} ranged from 25 to 66 µg/mL (very strong to strong), and the DPPH IC_{50} ranged from 32 to 95 µg/mL (very strong to strong). The coffee bean extracts (AB and RB) showed higher antioxidant activity in both assays, with the RB extract exhibiting the highest. The RB extract had the highest FRAP value, followed by the AB, RG, and AG extracts. Among all samples, the RB extract showed the highest levels of almost all phytochemical compounds.

Many studies have reported that stronger antioxidant activity correlated with higher amounts of their phenolic compounds (Bobková et al., 2020). This trend aligns with previous studies by Choi & Koh (2017) and Bravo et al. (2012), who reported lower antioxidant activity in Arabica and Robusta SCG extracts compared with the respective coffee bean extracts. This reduction is likely due to the coffee brewing process, where hot water and pressure extract bioactive compounds into the beverage, leaving fewer active compounds in the SCG. Several studies have demonstrated that stronger antioxidant activity in coffee correlates with higher levels of phenolic compounds (Bobková et al., 2020), particularly the major bioactive constituents ChA (Freitas et al., 2023; Hussein et al., 2022; Jeszka-Skowron et al., 2022; Opitz et al., 2017) and caffeine (Hussein et al., 2022; Jeszka-Skowron et al., 2022; Klingel et al., 2020; Rodrigues et al., 2023).

Wound healing

As mentioned, coffee bean powder has been reported to promote wound healing (Yuwono, 2014; 2021). Therefore, the wound healing ability of the AB, RB, AG, and RG extracts was evaluated in an *in vitro* model with HaCaT cells. First, the effect of the extracts on cell viability was evaluated using the MTT assay according to ISO-10993-5 (2009). The tested concentrations (1, 10, 50, and 100 µg/mL) of each extract were not toxic to the cells (Fig. 1). However, 100 µg/mL of the RB and RG extracts reduced cell viability to <70%. Based on these results, a concentration of 1 µg/mL was chosen for the wound healing assay.

Table 4. Percentage wound closure in HaCaT cells after treatment with the Arabica and Robusta bean and spent ground extracts.

Sample	Wound closure (%)				
	24 h	48 h	72 h	96 h	120 h
10% FBS	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}
Control	68.48 ± 1.70^{b}	77.49 ± 4.82^{b}	94.85 ± 7.28^{a}	96.32 ± 5.20^{a}	98.16 ± 2.61^{a}
AB	36.31 ± 1.02^{d}	42.85 ± 2.00^{d}	49.77 ± 1.78^{c}	55.07 ± 0.19^{c}	62.24 ± 2.70^{c}
AG	62.41 ± 3.01^{bc}	84.80 ± 5.90^{b}	98.27 ± 2.59^{a}	99.06 ± 1.33^{a}	100.00 ± 0.00^{a}
RB	56.53 ± 0.80^{c}	63.80 ± 2.95^{c}	74.72 ± 5.21^{b}	77.45 ± 4.51^{b}	82.73 ± 1.36^{b}
RG	63.18 ± 2.08^{b}	72.65 ± 2.11a^{c}	73.55 ± 1.65^{b}	78.75 ± 4.97^{b}	81.20 ± 4.62^{b}

Data are presented as mean ± SD (n = 3). In each row, means with different superscript letters (a–d) are significantly different ($p<0.05$, Tukey's test).

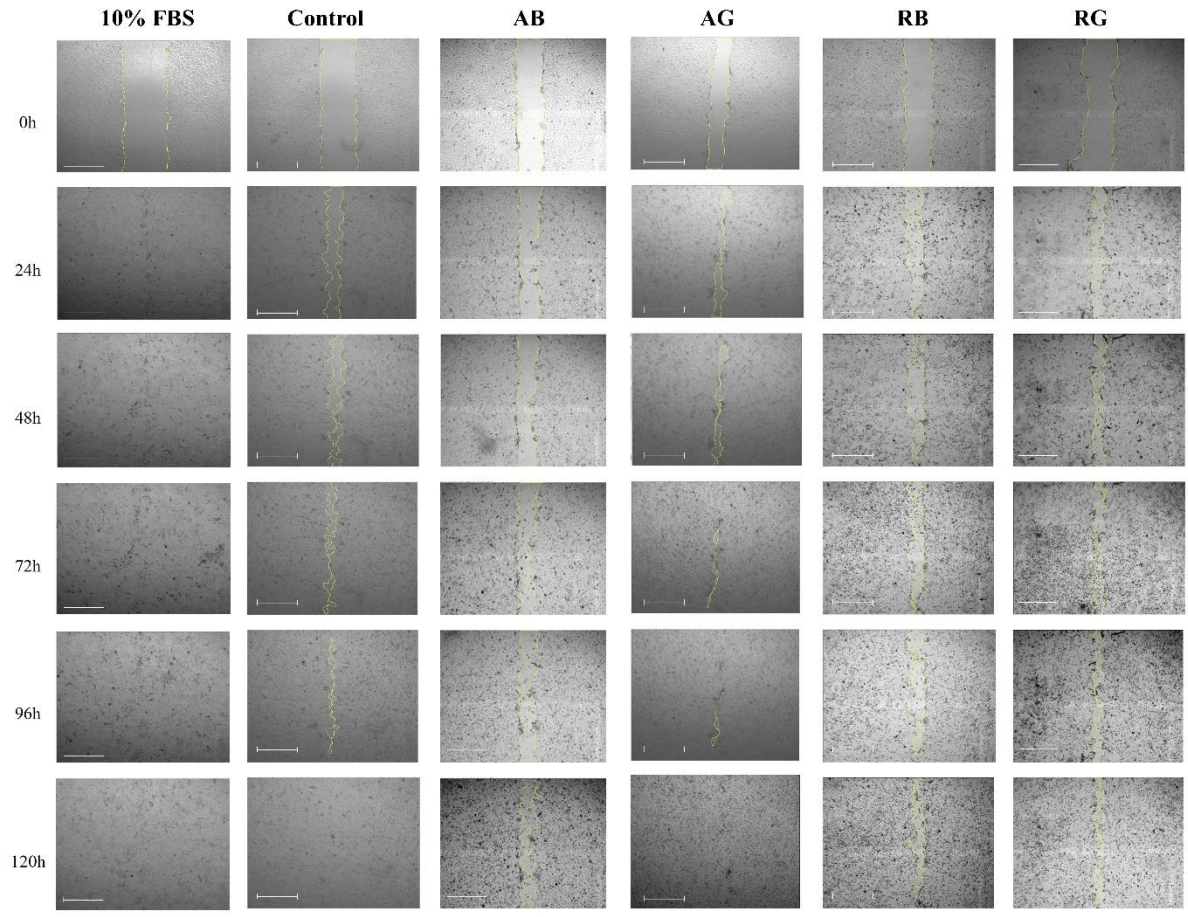

Figure 2. Wound closure in HaCaT cells after treatment with 10% FBS and coffee extracts.

AB: Arabica coffee bean; AG: Arabica SCG; RB: Robusta coffee bean; RG: Robusta SCG extracts. The scale bars are 650 µm. Magnification 4×.

The wound healing assay results are presented in Table 4 and Fig. 2. The wound healing area was measured by observing the rate of wound closure after treatment with coffee extracts for 24, 48, 72, 96, and 120 h. 10% FBS was used as a positive control group, representing normal, healthy growth conditions (Nie & Deters, 2013). The positive control (10% FBS) led to complete wound closure within 24 h. In contrast, wound closure in the cells treated with the AB, AG, RB, or RG extract gradually increased over time. After 48 h, wound closure was 84.80% in the cells treated with the AG extract, higher than that in the control group. Remarkably, the cells treated with the AG extract achieved 98.27% closure by 72 h, the highest wound

closure among the extracts at this time point, despite having the lowest antioxidant, polyphenol, and caffeine contents. Specifically, the AG extract contained 6.4–11.93 times less caffeine than the other extracts. Caffeine been reported to inhibit keratinocyte migration through the integrin/FAK/Akt signaling pathway (Meisaprow et al., 2021), which may explain why the AG extract with the lowest caffeine content promoted the fastest wound closure. Previous studies also suggest that caffeine can suppress epithelialization and keratinocyte migration, and may even induce cell death (Ojeh et al., 2016). Epithelialization of epidermal keratinocytes is a key process in wound healing (Enoch & Leaper, 2007). Therefore, coffee by-products, particularly Arabica spent grounds, represent a promising natural source of wound healing agents that promote keratinocyte migration while maintaining strong antioxidant activity.

Multivariate analysis

Principal component analysis (PCA) was used to evaluate the relationship between the phytochemical compound content, antioxidant activity, and wound healing effect of each extract. The biplot in Fig. 3 shows that the first two principal components (PCs) explained 81.2% of the total variation (55.5% for PC1 and 27.5% for PC2). Notably, the loading vectors aligned along the same axes (PC1 and PC2), indicating strong correlations between specific phytochemical compounds and biological activities. PC1 was primarily associated with high contributions from antioxidant capacity (FRAP, DPPH, and ABTS) and several phytochemical compounds, including TPC, Trp, ChA, CA, caffeine, methyl linoleate, methyl linolelaidate, palmitoleamide, erucamide, and octadecanamide. In contrast, PC2 was associated with high contributions from wound healing activity and from phytochemicals such as TFC, MLT, GA, PCCA, *p*-CA, and hexadecanamide. Interestingly, methyl palmitate contributed positively to both PCs. Antioxidant capacity showed strong positive correlations with TPC, Trp, ChA, CA, caffeine, methyl linolelaidate, palmitoleamide, erucamide, and octadecanamide. Wound healing activity, on the other hand, correlated positively with FRAP, DPPH, methyl palmitate, and palmitoleamide. The PCA biplot further revealed that the RB extract exhibited dominant antioxidant activity, whereas AG demonstrated superior wound healing potential among all coffee samples.

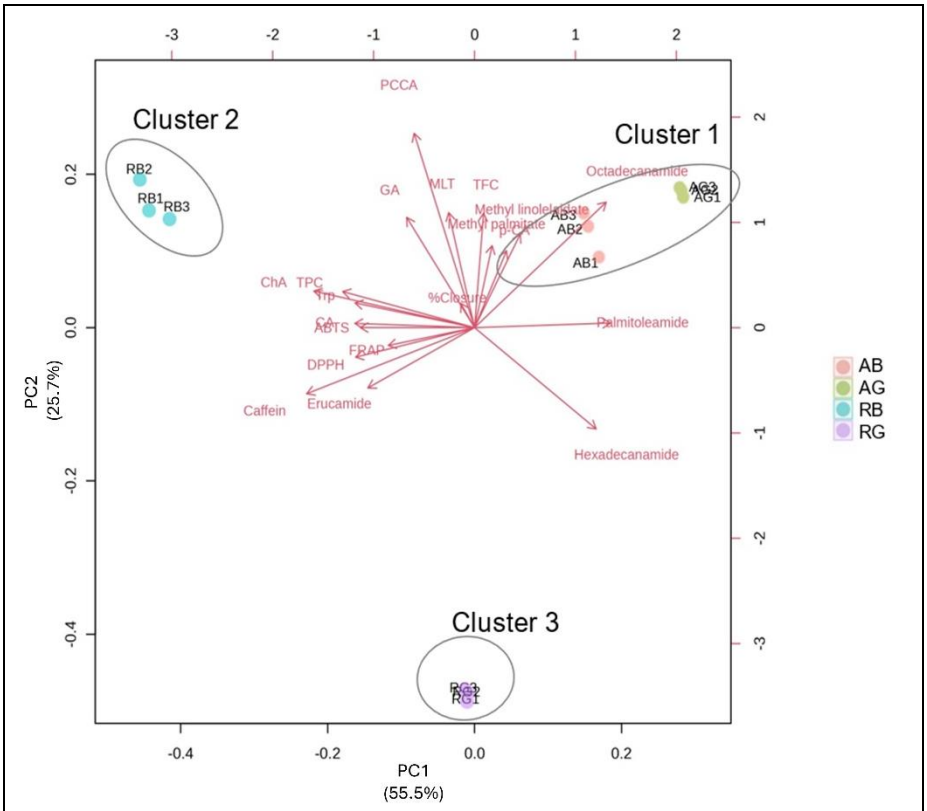

Figure 3. Biplot from principal component analysis (PCA) and Hierarchical cluster analysis (HCA) of the whole data set of coffee extracts by their phytochemicals, antioxidant activities, and %wound closure in HaCaT cells.

AB: Arabica coffee bean; AG: Arabica SCG; RB: Robusta coffee bean; RG: Robusta SCG extracts; GA: gallic acid; TFC: total flavonoid compounds; TPC: total phenolic compounds; PCCA: protocatechuic acid; ChA: chlorogenic acid; CA: caffeic acid; MLT: melatonin; Trp: tryptophan; ABTS: 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) disodium salt; FRAP: Ferric reducing antioxidant power; DPPH: 2,2-diphenyl-1 picrylhydrazyl.

HCA grouped the samples based on their phytochemical profiles and antioxidant and wound healing effects (Fig. 3). The extracts were divided into three clusters: cluster 1, comprising AG and AB; cluster 2, RB; and cluster 3, RG. Cluster 1 was positively associated with wound healing and correlated with octadecanamide, methyl linoleate, hexadecanamide, palmitoleamide, methyl palmitate, *p*-CA, MLT, PCCA, GA, and TFC. In contrast, Cluster 2 exhibited strong antioxidant activity (ABTS, DPPH, and FRAP) and showed positive correlations with PCCA, GA, ChA, CA, TPC, Trp, erucamide, caffeine, and methyl linolelaidate. Cluster 3, in comparison, displayed weak wound healing and antioxidant activities, which corresponded to its lower phytochemical content.

CONCLUSION

Coffee beans and solid waste products are good alternative sources of phytochemical compounds with antioxidant and wound healing activities. This finding suggests that Robusta coffee beans are a potential natural antioxidant source with high polyphenol and caffeine contents, and that Arabica SCG is a potential natural source that can promote wound healing by modulating HaCaT cell migration. The use of SCG to promote health can help to reduce massive waste from coffee drinks, promote environmental sustainability, and add value to coffee waste products.

CONFLICT OF INTEREST

The research was conducted entirely free from any involvement in commercial, financial, or personal matters that might be perceived as a possible conflict of interest or that could have influenced the outcomes or interpretation of the study.

ACKNOWLEDGMENTS

This research was supported by the National Science, Research, and Innovation Fund (NSRF), Thailand, through the Fundamental Fund (FF), Khon Kaen University, Thailand. The first author, Pimolwan Siriparu, gratefully acknowledges the Research Fund for Supporting Lecturer to Admit High Potential Student to Study and Research on His Expert Program Year, 2022 (652H226), from the Graduate School, Khon Kaen University.

GENERATIVE ARTIFICIAL INTELLIGENCE (AI)

The authors declare that no generative artificial intelligence (AI) or AI-assisted technologies were used in the preparation, writing, or editing of this manuscript. All content was developed solely by the authors, who take full responsibility for its accuracy and integrity.

REFERENCES

Ali A, Zahid HF, Cottrell JJ, Dunshea FR (2022) A comparative study for nutritional and phytochemical profiling of Coffea arabica (*C. arabica*) from different origins and their antioxidant potential and molecular docking. Molecules 27(16): 5126. https://doi.org/10.3390/molecules27165126

Arabshahi-Delouee S, Urooj A (2007) Antioxidant properties of various solvent extracts of mulberry (*Morus indica* L.) leaves. Food Chem 102(4): 1233–1240. https://doi.org/10.1016/j.foodchem.2006.07.013

Arnao MB, Hernández-Ruiz J (2019) Melatonin: a new plant hormone and/or a plant master regulator? Trends Plant Sci 24(1): 38–48. https://doi.org/10.1016/j.tplants.2018.10.010

Bevilacqua E, Cruzat V, Singh I, Rose'Meyer RB, Panchal SK, Brown L (2023) The potential of spent coffee grounds in functional food development. Nutrients 15(4): 994. https://doi.org/10.3390/nu15040994

Bobková A, Hudáček M, Jakabová S, Belej Ľ, Capcarová M, Čurlej J, Bobko M, Árvay J, Jakab I, Čapla J, Demianová A (2020) The effect of roasting on the total polyphenols and antioxidant activity of coffee. J Environ Sci Health B 55(5): 495–500. https://doi.org/10.1080/03601234.2020.1724660

Bomfim ASC, de Oliveira DM, Walling E, Babin A, Hersant G, Vaneeckhaute C, Dumont MJ, Rodrigue D (2022) Spent coffee grounds characterization and reuse in composting and soil amendment. Waste 1(1): 2–20. https://doi.org/10.3390/waste1010002

Bonnefont-Rousselot D, Collin F (2010) Melatonin: action as antioxidant and potential applications in human disease and aging. Toxicology 278(1): 55–67. https://doi.org/10.1016/j.tox.2010.04.008

Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Technol 28(1): 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Bravo J, Juániz I, Monente C, Caemmerer B, Kroh LW, de Peña MP, Cid C (2012) Evaluation of spent coffee obtained from the most common coffeemakers as a source of hydrophilic bioactive compounds. J Agric Food Chem 60(51): 12565–12573. https://doi.org/10.1021/jf3040594

Burana-Osot J, Phattanawasin P, Luangthuwapanit P, Khamwut P, Sotanaphun U (2016) Determination of volatile constituents from crude drugs of Phikud Navakot by gas chromatography-mass spectrometry. Thai Bull Pharm Sci 11(2): 45–60.

Choi B, Koh E (2017) Spent coffee as a rich source of antioxidative compounds. Food Sci Biotechnol 26(4): 921–927. https://doi.org/10.1007/s10068-017-0144-9

Enoch S, Leaper DJ (2007) Basic science of wound healing. https://doi.org/10.1016/j.mpsur.2007.11.005

Freitas VV, Rodrigues Borges LL, Dias Castro GA, Henrique Dos Santos M, Teixeira Ribeiro Vidigal MC, Fernandes SA, Stringheta PC (2023) Impact of different roasting conditions on the chemical composition, antioxidant activities, and color of *Coffea canephora* and *Coffea arabica* L. samples. Heliyon 9(9): e19580. https://doi.org/10.1016/j.heliyon.2023.e19580

Galano A, Tan DX, Reiter RJ (2011) Melatonin as a natural ally against oxidative stress: a physicochemical examination: melatonin as an antioxidant. J Pineal Res 51(1): 1–16. https://doi.org/10.1111/j.1600-079X.2011.00916.x

Gallo J, Raska M, Kriegova E, Goodman SB (2017) Inflammation and its resolution and the musculoskeletal system. J Orthop Translat 10: 52–67. https://doi.org/10.1016/j.jot.2017.05.007

Grada A, Otero-Vinas M, Prieto-Castrillo F, Obagi Z, Falanga V (2017) Research techniques made simple: analysis of collective

cell migration using the wound healing assay. J Invest Dermatol 137(2): e11–e16. https://doi.org/10.1016/j.jid.2016.11.020

Hall S, Desbrow B, Anoopkumar-Dukie S, Davey AK, Arora D, McDermott C, Schubert MM, Perkins AV, Kiefel MJ, Grant GD (2015) A review of the bioactivity of coffee, caffeine and key coffee constituents on inflammatory responses linked to depression. Food Res Int 76(Part 3): 626–636. https://doi.org/10.1016/j.foodres.2015.07.027

Hussein H, Abouamer W, Ali H, Elkhadragy M, Yehia H, Farouk A (2022) The valorization of spent coffee ground extract as a prospective insecticidal agent against some main key pests of *Phaseolus vulgaris* in the laboratory and field. Plants 11(9): 1124. https://doi.org/10.3390/plants11091124

ICO (2024) Coffee Market Report July 2024. https://ico.org/documents/cy2023-24/cmr-0724-e.pdf [Consulted 22 May 2024].

Iqbal S, Younas U, Chan KW, Zia-Ul-Haq M, Ismail M (2012) Chemical composition of *Artemisia annua* L. leaves and antioxidant potential of extracts as a function of extraction solvents. Molecules 17(5): 6020–6032. https://doi.org/10.3390/molecules17056020

ISO (2009) 10993–5: 2009 biological evaluation of medical devices—part 5: tests for *in vitro* cytotoxicity. International Organization for Standardization, Geneva: 34.

Jeszka-Skowron M, Frankowski R, Zgoła-Grześkowiak A, Płatkiewicz J (2022) Comprehensive analysis of metabolites in brews prepared from naturally and technologically treated coffee beans. Antioxidants 12(1): 95. https://doi.org/10.3390/antiox12010095

Klingel T, Kremer JI, Gottstein V, Rajcic De Rezende T, Schwarz S, Lachenmeier DW (2020) A review of coffee by-products including leaf, flower, cherry, husk, silverskin, and spent grounds as novel foods within the European Union. Foods 9(5): 665. https://doi.org/10.3390/foods9050665

Kusumawati AH, Farhamzah F, Alkandahri MY, Sadino A, Agustina LS, Apriana SD (2021) Antioxidant activity and sun protection factor of black glutinous rice (*Oryza sativa* var. *Glutinosa*). Trop J Nat Prod Res 5(11): 1958–1961. https://doi.org/10.26538/tjnpr/v5i11.11

Lee YG, Cho EJ, Maskey S, Nguyen DT, Bae HJ (2023) Value-added products from coffee waste: a review. Molecules 28(8): 3562. https://doi.org/10.3390/molecules28083562

Lemos MF, de Andrade Salustriano N, de Souza Costa MM, Lirio K, Da Fonseca AFA, Pacheco HP, Endringer DC, Fronza M, Scherer R (2022) Chlorogenic acid and caffeine contents and anti-inflammatory and antioxidant activities of green beans of Conilon and Arabica coffees harvested with different degrees of maturation. J Saudi Chem Soc 26(3): 101467. https://doi.org/10.1016/j.jscs.2022.101467

Liao YC, Kim T, Silva JL, Hu WY, Chen BY (2022) Effects of roasting degrees on phenolic compounds and antioxidant activity in coffee beans from different geographic origins. LWT-Food Sci Technol 168: 113965. https://doi.org/10.1016/j.lwt.2022.113965

Mahakunakorn P, Sangchart P, Panyatip P, Ratha J, Damrongrungruang T, Priprem A, Puthongking P (2024) *In vitro* cytoprotective and *in vivo* anti-oral mucositis effects of melatonin and its derivatives. PeerJ 12: e17608. https://doi.org/10.7717/peerj.17608

Makiso MU, Tola YB, Ogah O, Endale FL (2024) Bioactive compounds in coffee and their role in lowering the risk of major public health consequences: a review. Food Sci Nutr 12(2): 734–764. https://doi.org/10.1002/fsn3.3848

Martins ACCL, Gloria MBA (2010) Changes on the levels of serotonin precursors—tryptophan and 5-hydroxytryptophan—during roasting of Arabica and Robusta coffee. Food Chem 118(3): 529–533. https://doi.org/10.1016/j.foodchem.2009.05.016

Matyushin DD, Sholokhova AY, Karnaeva AE, Buryak AK (2020) Various aspects of retention index usage for GC-MS library search: a statistical investigation using a diverse data set. Chemom Intell Lab Syst 202: 104042. https://doi.org/10.1016/j.chemolab.2020.104042

Meisaprow P, Aksorn N, Vinayanuwattikun C, Chanvorachote P, Sukprasansap M (2021) Caffeine induces G0/G1 cell cycle arrest and inhibits migration through integrin αv, β3, and FAK/Akt/c-Myc signaling pathway. Molecules 26(24): 7659. https://doi.org/10.3390/molecules26247659

Morvaridzadeh M, Sadeghi E, Agah S, Nachvak SM, Fazelian S, Moradi F, Persad E, Heshmati J (2020) Effect of melatonin supplementation on oxidative stress parameters: a systematic review and meta-analysis. Pharmacol Res 161: 105210. https://doi.org/10.1016/j.phrs.2020.105210

Murthy PS, Madhava Naidu M (2012) Sustainable management of coffee industry by-products and value addition—A review. Resources, Resour Conserv Recycl 66: 45–58. https://doi.org/10.1016/j.resconrec.2012.06.005

Nie W, Deters AM (***2013)*** Tamarind seed xyloglucans promote proliferation and migration of human skin cells through internalization via stimulation of proproliferative signal transduction pathways. Dermatol Res Pract 2013: 359756. https://doi.org/10.1155/2013/359756

Ojeh N, Stojadinovic O, Pastar I, Sawaya A, Yin N, Tomic-Canic M (2016) The effects of caffeine on wound healing. Int Wound J 13(5): 605–613. https://doi.org/10.1111/iwj.12327

Opitz SEW, Goodman BA, Keller M, Smrke S, Wellinger M, Schenker S, Yeretzian C (2017) Understanding the effects of roasting on antioxidant components of coffee brews by coupling on-line ABTS assay to high performance size exclusion chromatography. Phytochem Anal 28(2): 106–114. https://doi.org/10.1002/pca.2661

Ramakrishna A, Giridhar P, Sankar KU, Ravishankar GA (2012) Melatonin and serotonin profiles in beans of *Coffea* species. J Pineal Res 52(4): 470–476. https://doi.org/10.1111/j.1600-079X.2011.00964.x

Reiter RJ, Manchester LC, Tan D (2005) Melatonin in walnuts: influence on levels of melatonin and total antioxidant capacity of blood. Nutrition 21: 920–924. https://doi.org/10.1016/j.nut.2005.02.005

Rodrigues R, Oliveira MBPP, Alves RC (2023) Chlorogenic acids and caffeine from coffee by-products: a review on skincare applications. Cosmetics 10(1): 12. https://doi.org/10.3390/cosmetics10010012

Rojas-González A, Figueroa-Hernández CY, González-Rios O, Suárez-Quiroz ML, González-Amaro RM, Hernández-Estrada ZJ, Rayas-Duarte P (2022) Coffee chlorogenic acids incorporation for bioactivity enhancement of foods: a review. Molecules 27(11): 3400. https://doi.org/10.3390/molecules27113400

Seyedsadjadi N, Grant R (2020) The potential benefit of monitoring oxidative stress and inflammation in the prevention of non-communicable diseases (NCDs). Antioxidants 10(1): 15. https://doi.org/10.3390/antiox10010015

Siriparu P, Panyatip P, Pota T, Ratha J, Yongram C, Srisongkram T, Sungthong B, Puthongking P (2022) Effect of germination and illumination on melatonin and its metabolites, phenolic content, and antioxidant activity in mung bean sprouts. Plants 11(21): 2990. https://doi.org/10.3390/plants11212990

Stein SE, Wallace W, Ji W, Little J, Markey S, Mallard WG, Mikaia A, Neta P, Sparkman OD, Linstrom P, Mirokhin Y, Tchekhovskoi D, Yang X, Andriamaharavo R, de Leoz L, Liang Y, Pu L, Todua N, Tretyakov K, Zhang Y (2017) NIST standard reference

database 1A. Standard Reference Data, NIST, Gaithersburg, MD, USA. Available at: https://chemdata.nist.gov/dokuwiki/lib/exe/fetch.php?media=chemdata:nist17:nistms_ver23man.pdf [Consulted 22 May 2024].

Sugiyanta S, Notopuro H, Nugraha J, Soetjipto S, Handajani R (2020) The effect of roasting temperature on ferulic acid levels of robusta coffee bean with thin layer chromatography (TLC)-densitometry. Indian J Forensic Med Toxicol 14(4): 3497–3503. https://doi.org/10.37506/ijfmt.v14i4.12168

Tan DX, Hardeland R, Manchester LC, Korkmaz A, Ma S, Rosales-Corral S, Reiter RJ (2012) Functional roles of melatonin in plants, and perspectives in nutritional and agricultural science. J Exp Bot 63(2): 577–597. https://doi.org/10.1093/jxb/err256

Tan DX, Reiter RJ (2022) Mechanisms and clinical evidence to support melatonin's use in severe COVID-19 patients to lower mortality. Life Sci 294: 120368. https://doi.org/10.1016/j.lfs.2022.120368

Tokimoto T, Kawasaki N, Nakamura T, Akutagawa J, Tanada S (2005) Removal of lead ions in drinking water by coffee grounds as vegetable biomass. J Colloid Interface Sci 281(1): 56–61. https://doi.org/10.1016/j.jcis.2004.08.083

Vieira AJSC, Gaspar EM, Santos PMP (2020) Mechanisms of potential antioxidant activity of caffeine. Radiat Phys Chem 174: 108968. https://doi.org/10.1016/j.radphyschem.2020.108968

Watanabe K, Nakano M, Maruyama Y, Hirayama J, Suzuki N, Hattori A (2023) Nocturnal melatonin increases glucose uptake via insulin-independent action in the goldfish brain. Front Endocrinol 14: 1173113. https://doi.org/10.3389/fendo.2023.1173113

Yaga K, Reiter RJ, Richardson BA (1993) Tryptophan loading increases daytime serum melatonin levels in intact and pinealectomized rats. Life Sci 52(14): 1231–1238. https://doi.org/10.1016/0024-3205(93)90106-D

Yust BG, Wilkinson F, Rao NZ (2023) Variables affecting the extraction of antioxidants in cold and hot brew coffee: a review. Antioxidants 13(1): 29. https://doi.org/10.3390/antiox13010029

Yuwono HS (2014) The new paradigm of wound management using coffee powder. J Surg 2(2): 25–29. https://doi.org/10.12691/js-2-2-2

Yuwono HS (2021) Why the coffee powder is the best topical wound dressing? Eur J Health Sci 3(6): 4–7. https://doi.org/10.24018/ejmed.2021.3.6.1077

AUTHOR CONTRIBUTION:

Contribution	Siriparu P	Ratha J	Puthongking P
Concepts or ideas			x
Design	x	x	x
Definition of intellectual content	x		x
Literature search	x		x
Experimental studies	x	x	x
Data acquisition	x	x	x
Data analysis	x	x	x
Statistical analysis	x	x	x
Manuscript preparation	x		x
Manuscript editing	x		x
Manuscript review	x	x	x

Citation Format: Siriparu P, Ratha J, Puthongking P (2026) Comparative phytochemical profiling and evaluation of antioxidant and wound healing activities of Arabica and Robusta coffee beans and spent grounds. J Pharm Pharmacogn Res 14(2): 2332. https://doi.org/10.56499/jppres_14.2.2332

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Open Access: This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/ licenses/by/4.0/), which permits use, duplication, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license and indicate if changes were made.

Supplementary data

Supplementary data 1: Chromatogram of HPLC from coffee extract

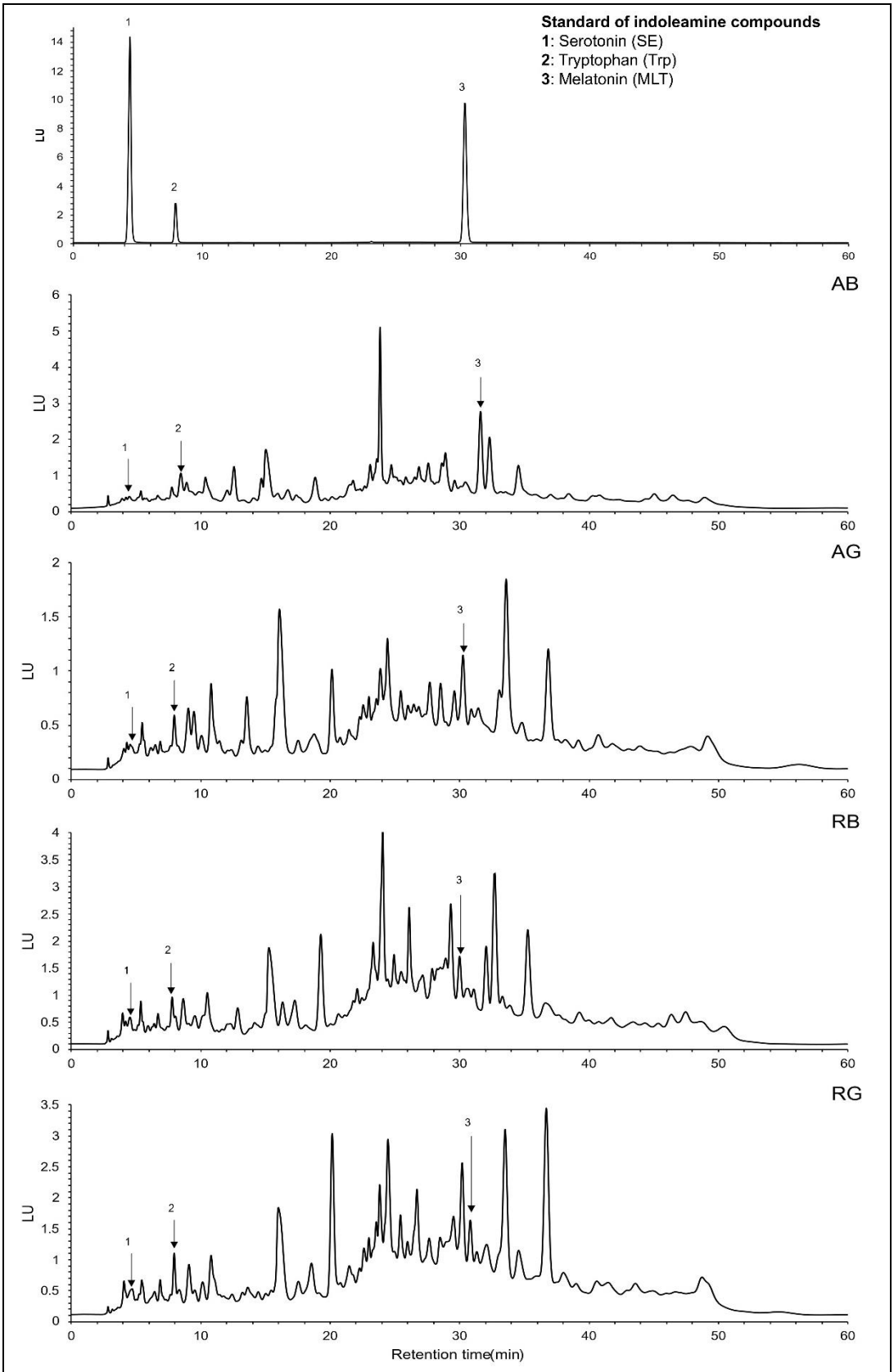

Figure S1. Chromatogram of indoleamine compounds in coffee extract from HPLC-FD.
AB: Arabica coffee bean; AG: Arabica SCG; RB: Robusta coffee bean; RG: Robusta SCG.

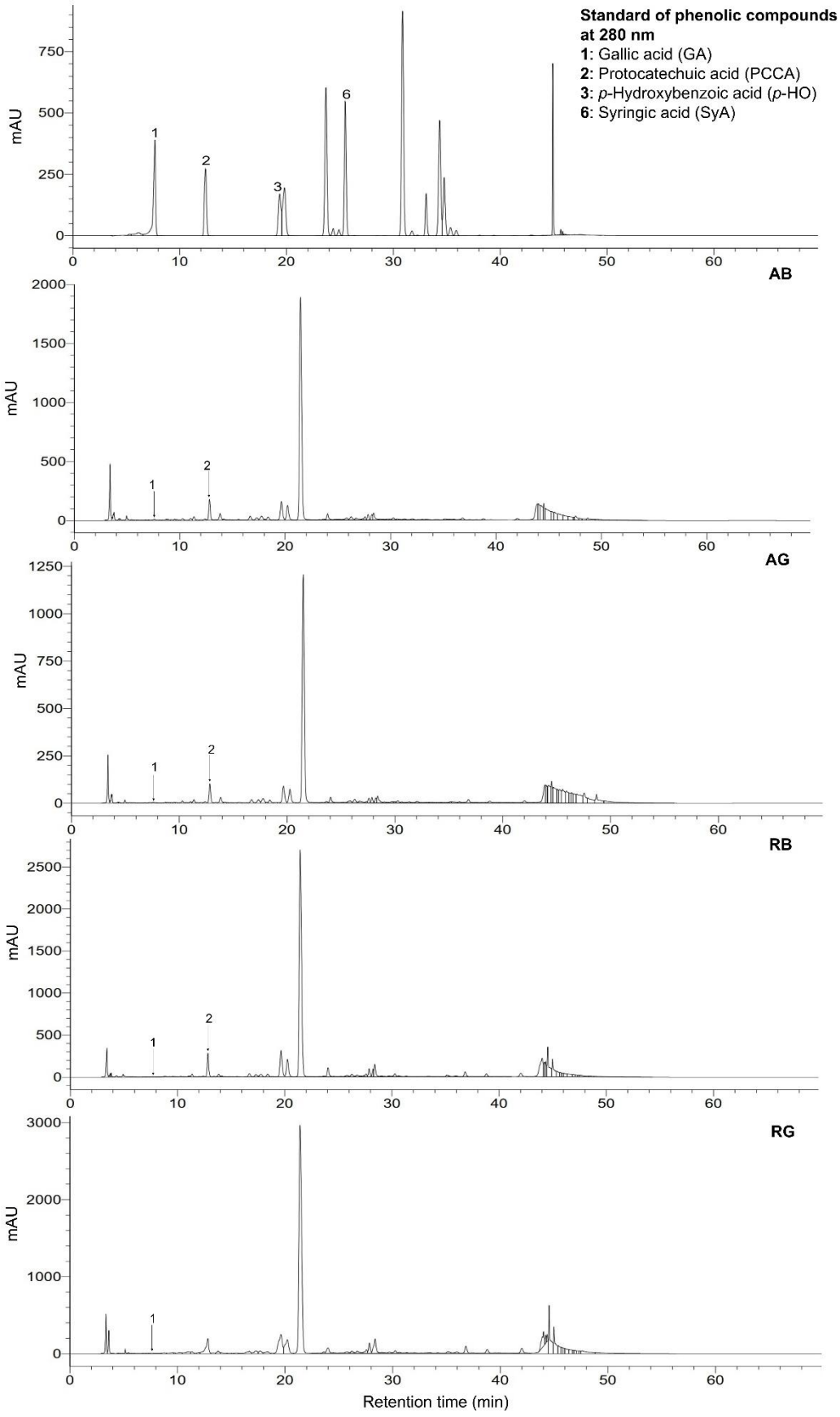

Figure S2. Chromatogram of phenolic compounds in coffee extract from HPLC-UV/DAD at 280 nm.
AB: Arabica coffee bean; AG: Arabica SCG; RB: Robusta coffee bean; RG: Robusta SCG.

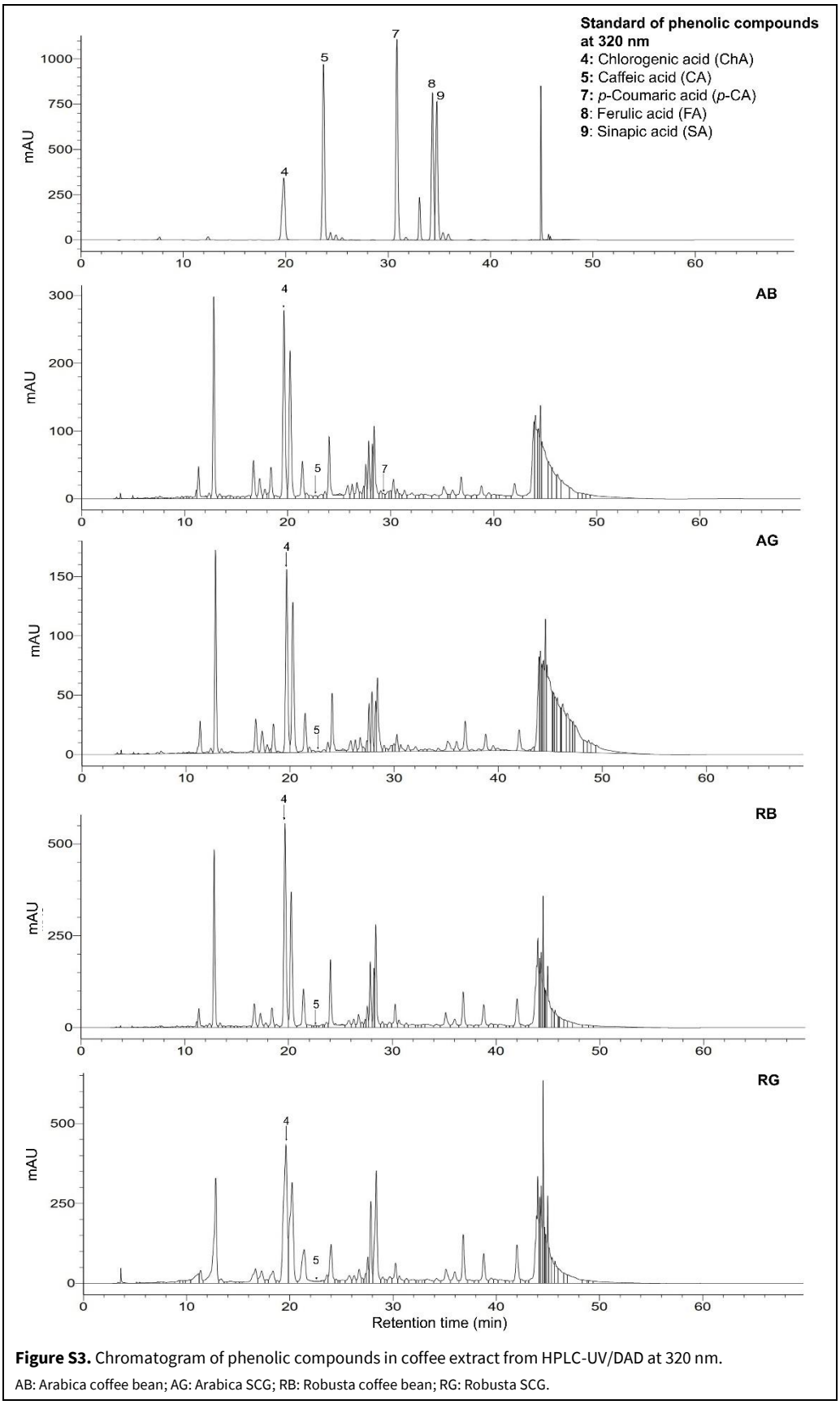

Figure S3. Chromatogram of phenolic compounds in coffee extract from HPLC-UV/DAD at 320 nm.

AB: Arabica coffee bean; AG: Arabica SCG; RB: Robusta coffee bean; RG: Robusta SCG.

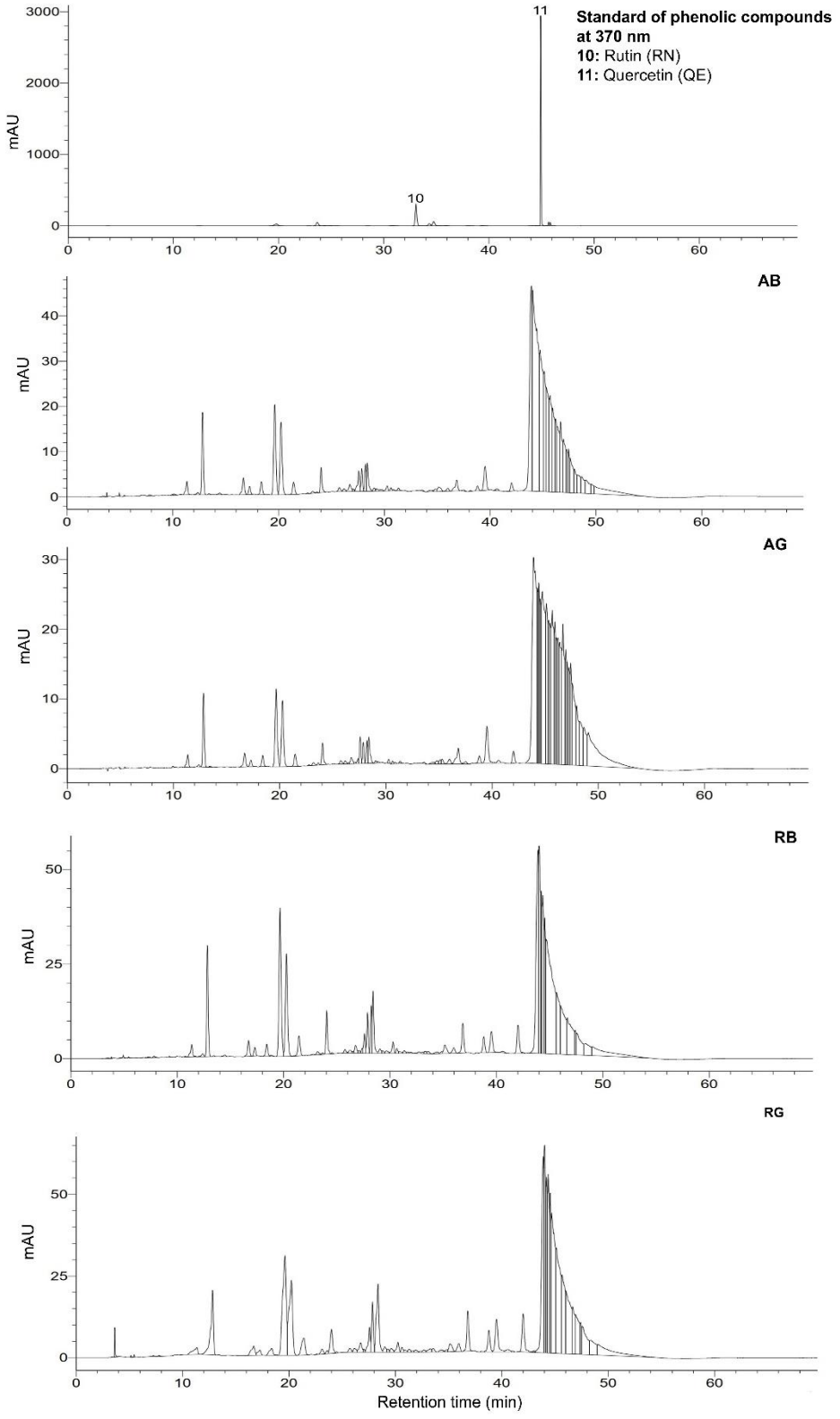

Figure S4. Chromatogram of phenolic compounds in coffee extract from HPLC-UV/DAD at 370 nm. AB: Arabica coffee bean; AG: Arabica SCG; RB: Robusta coffee bean; RG: Robusta SCG

Supplementary data 2: Mass spectrum of GC-MS from the experimental sample and the reference standard.

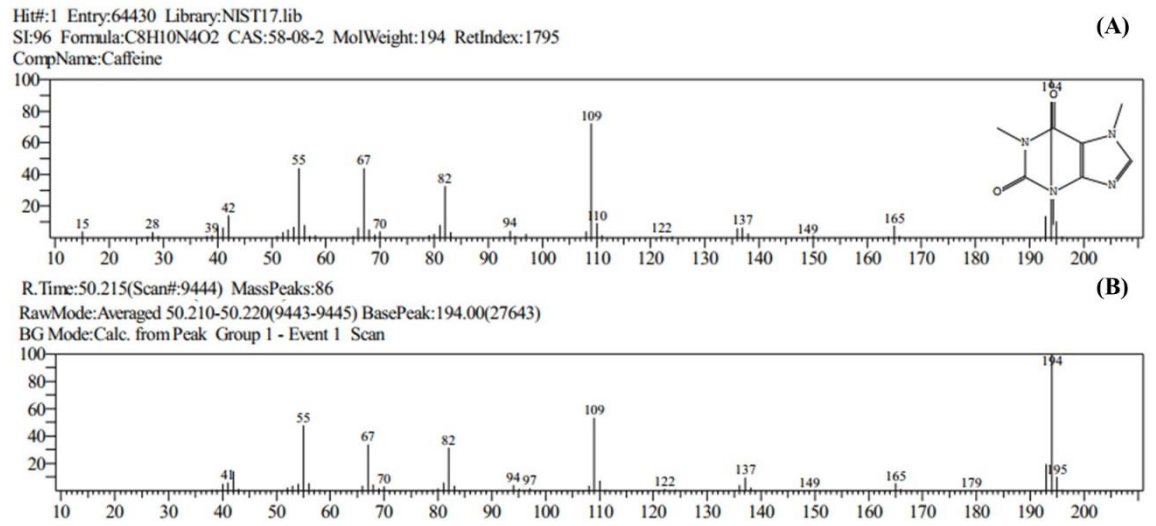

Figure S5. Mass spectrum of caffeine from the reference standard **(A)** and the experimental sample **(B)**.

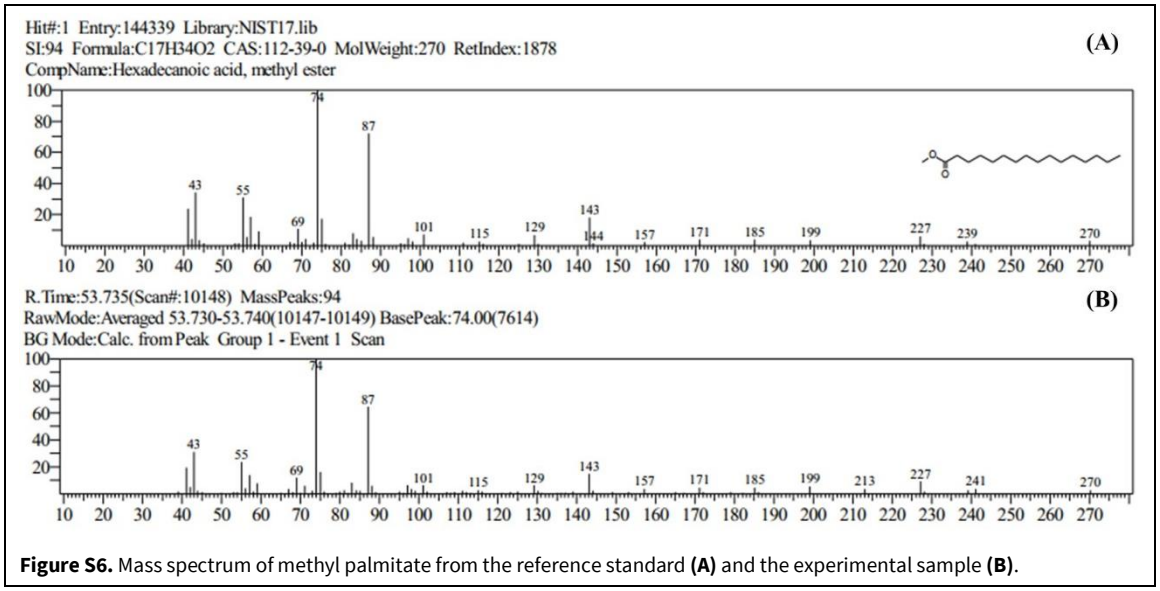

Figure S6. Mass spectrum of methyl palmitate from the reference standard **(A)** and the experimental sample **(B)**.

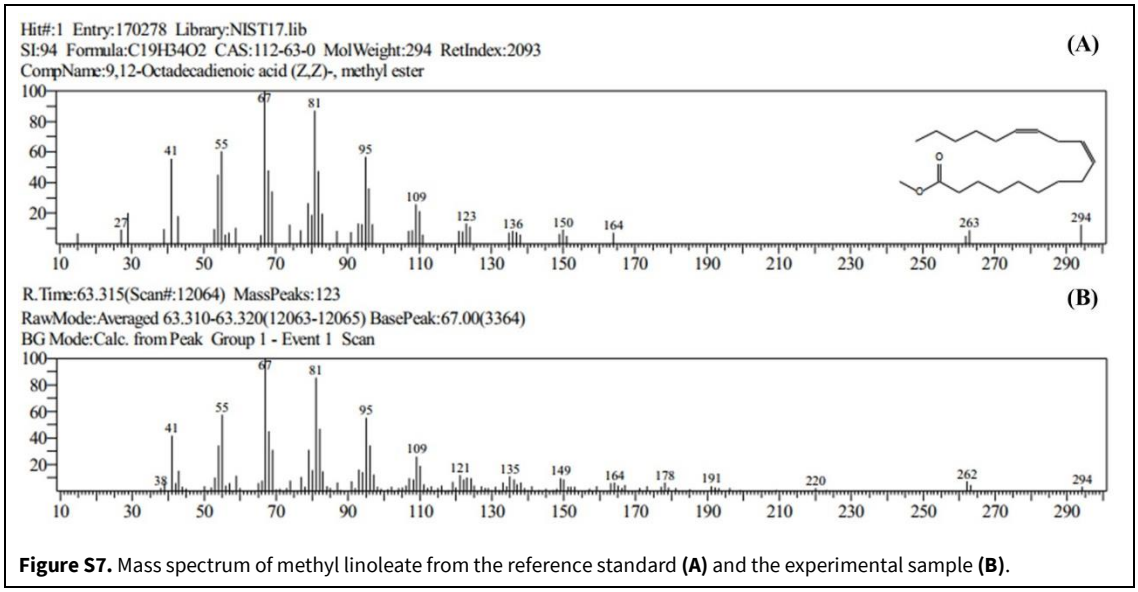

Figure S7. Mass spectrum of methyl linoleate from the reference standard **(A)** and the experimental sample **(B)**.

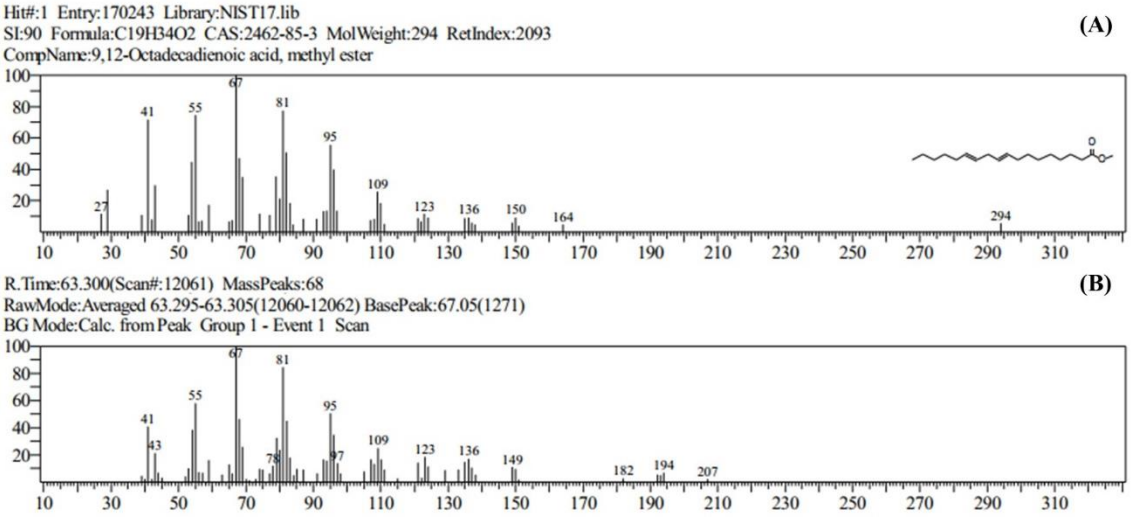

Figure S8. Mass spectrum of methyl linolelaidate from the reference standard **(A)** and the experimental sample **(B)**.

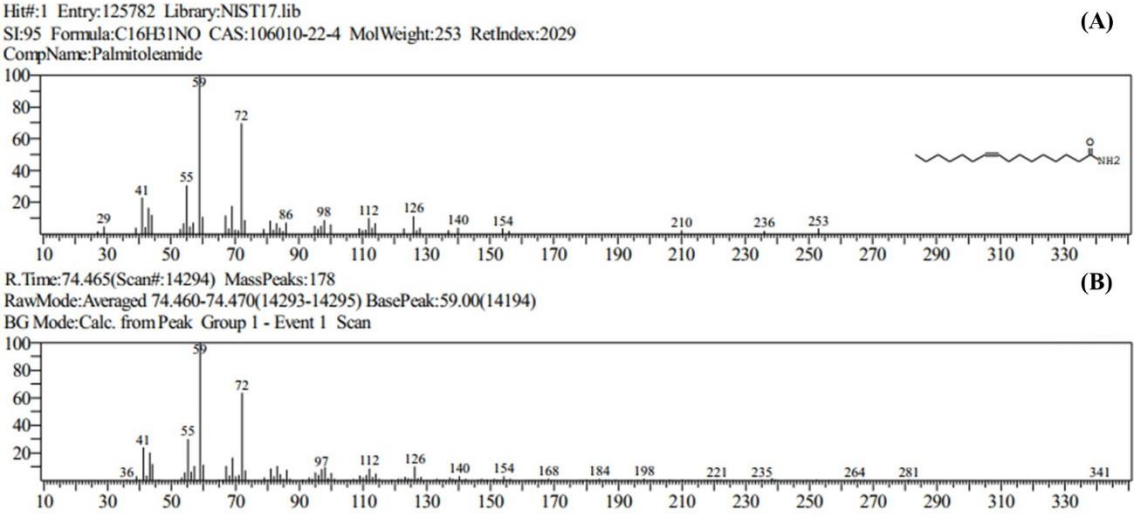

Figure S9. Mass spectrum of palmitoleamide from the reference standard **(A)** and the experimental sample **(B)**.

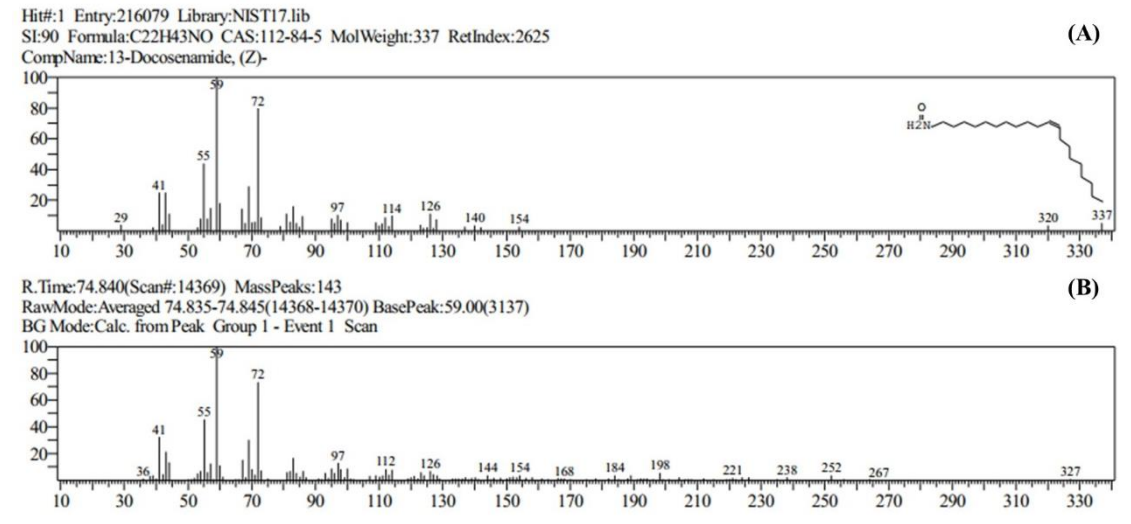

Figure S10. Mass spectrum of erucamide from the reference standard **(A)** and the experimental sample **(B)**.

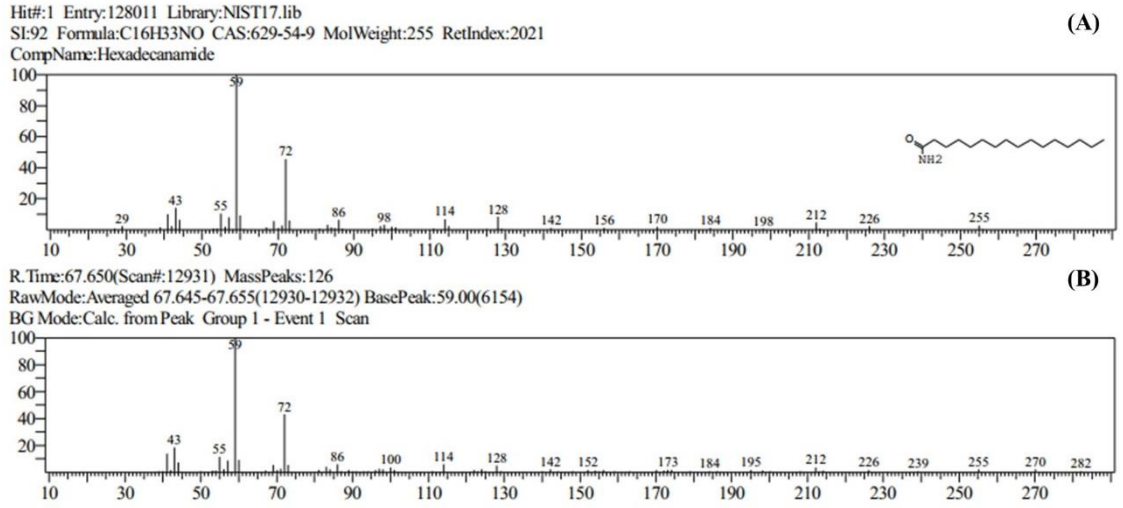

Figure S11. Mass spectrum of hexadecanamide from the reference standard **(A)** and the experimental sample **(B)**.

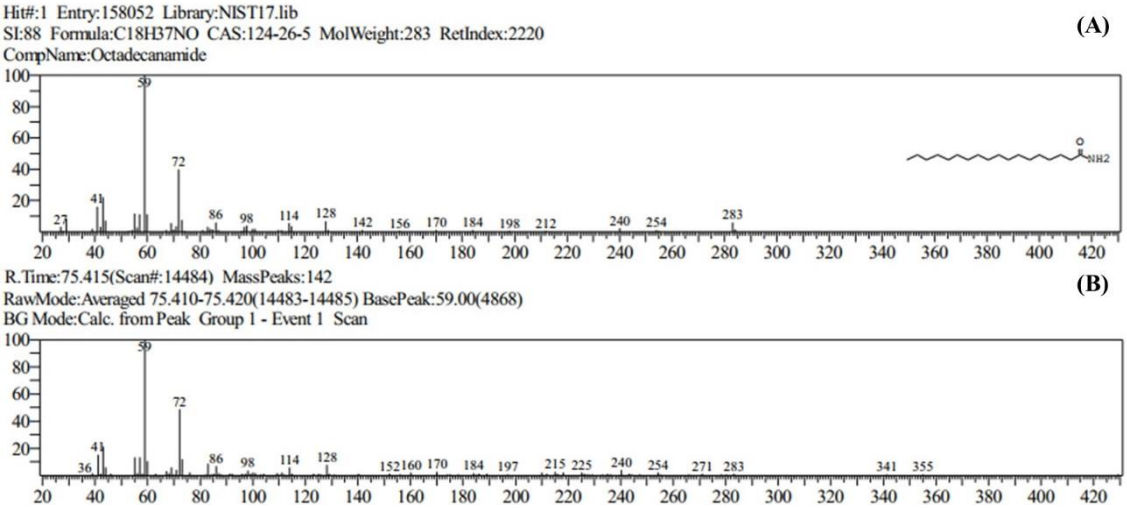

Figure S12. Mass spectrum of octadecanamide from the reference standard **(A)** and the experimental sample **(B)**.