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Antioxidant and antimicrobial activity of green and roasted coffee beans on human oral pathogens

[1]Norazlin, A., [1]Muhammad-Adib, A., [1]Wan-Razarinah, W.A.R., [2]Roohinejad, S., [3]Koubaa, M. and [1,*]Raseetha, S.

[1]*Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia*

[2]*Burn and Wound Healing Research Center, Division of Food and Nutrition, Shiraz University of Medical Sciences, Shiraz, Iran*

[3]*Université de Technologie de Compiègne, ESCOM, Integrated Transformations of Renewable Matter, Centre de Recherche Royallieu, CS 60319, CEDEX, 60203 Compiègne, France*

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Abstract

This study aimed to determine the antioxidant content (total phenolics, chlorogenic acid (CGA), and caffeine), proximate composition, and antimicrobial activity of green and roasted coffee beans (coffee arabica and coffee canephora var Robusta) and evaluate their effect on three selected human oral pathogens (i.e., *Porphyromonas gingivalis*, *Streptococcus mutans* and *Lactobacillus casei*). Total phenolic content (TPC) was significantly higher in green *C. robusta* (5.48 mg/g) compared to green *C. arabica* (4.67 mg/g). However, there was significantly lower phenolic content in both roasted coffee types. Similarly, CGA content was higher in green *C. robusta* coffee (2.39 mg/g) compared to roasted *C. robusta* coffee (0.74 mg/g). Caffeine content was significantly higher in roasted *C. robusta* (1.36 mg/g) compared to green *C. robusta* (1.23 mg/g) and green *C. arabica* (1.04 mg/g). The extract of green *C. robusta* produced a larger mean diameter of inhibition zones at all concentrations against bacteria tested compared to *C. arabica*. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of all samples on *L. casei* were 50 mg/mL, while on *S. mutans* were 240 mg/mL. The MIC and MBC for green and roasted *C. robusta* and *C. arabica* tested on *P. gingivalis* were 100 mg/ml and 200 mg/mL, respectively. The results showed that green and roasted Robusta coffee had higher chlorogenic acid, total phenolic contents, and good antimicrobial activity compared to its counterparts. This study suggests the feasibility of using Robusta coffee in the food industry to increase the functionality of beverages.

1. Introduction

Coffee is an amazing beverage that has beneficial properties and can act as an antioxidant (Farah, 2011). The two common coffee species are Arabica (*Coffea arabica*) and Robusta (*Coffea canephora*). Both are different in various ways including their physical aspects, chemical composition, ideal growing climates, and characteristics of the brew made with ground roasted seeds (Belay, 2011). Green (unroasted) and roasted coffee beans have been studied in the literature. The main constituent of the green coffee extract is mainly chlorogenic acid (CGA). It is a phenolic compound from the hydroxycinnamic acid family, which is depleted by the roasting process (Marcason, 2013). According to Chatterjee and Patel (2015), the presence of CGA in green unroasted coffee beans effectively helps in maintaining blood sugar levels and blood pressure and reduces heart diseases. Besides, CGA potentially has an anti-diabetic effect due to its function as a novel insulin sensitizer that potentiates insulin action, similar to the therapeutic action of metformin (McCarty, 2005). Moreover, clinical trials demonstrated that CGA in coffee can modulate glucose uptake, gastrointestinal

*Corresponding author.
Email: *raseetha@uitm.edu.my*

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hormone, insulin secretion, and weight loss in the human body (Thom, 2007). Nevertheless, the desired aroma and flavour of coffee beans used for the beverage preparation are developed during the coffee roasting process, where the coffee beans undergo a series of reactions that cause the desired changes in the chemical and physical composition (Chatterjee and Patel, 2015).

Phenolic compounds occur in various parts of the plant including bark, root, and leaves, as they belong to the secondary plant metabolites. These molecules also act as antioxidants with an important role in protecting the organisms from oxidative damage as well as in delaying the formation of free radicals. The antioxidant properties of phenolic compounds are mainly due to their redox potential, which allows them to act as reducing agents, hydrogen donators and metal chelators (Lattazio, 2013). Besides, coffee has been classified as one of the major dietary sources of antioxidants in which the antioxidant activity is attributed to the phenolics such as CGA, caffeic acid, ferulic acid, *p*-coumaric acid, as well as the Maillard reaction products that are formed during the roasting process (Tamilmani and Pandey, 2015). The total content of CGA on the dry weight basis in *C. arabica* is 5 to 8% and in *C. canephora* is 7 to 10%, thus indicating that Robusta coffee has a substantially higher content of CGA (Clifford, 1985).

The amount of caffeine (1,3,7-tri methylxanthine) in food items varies and coffee has been considered a major dietary source of caffeine depending on the serving size (71-220 mg caffeine/serving) (Ludwig *et al*., 2014). Caffeine content per cup also varies greatly depending on different factors such as serving size (25-190 mL), preparation method (either boiled, filtered, espresso or instant), and type of coffee bean used (Arabica or Robusta) (Neha *et al*., 2009). Coffee made from Robusta contained 4-5% higher caffeine compared to Arabica (Kreicbergs and Dimins, 2011). It is also characterized by its bitterness and less aromatic flavour and odour. Caffeine has heat-stable properties and it would stimulate the central nervous system as an adenosine receptor antagonist. The stimulatory effects of caffeine include enhanced perception, increased capacity to remain awake for a longer period, and reduced fatigue (Schaik *et al*., 2021; Xie *et al.*, 2021).

Meanwhile, the human oral microbiome refers to microorganisms that reside in the human oral cavity, which represents a very diverse community of oral microbes with approximately 700 species identified (Duran-Pinedo and Frias-Lopez, 2015). The number of oral infectious diseases has risen quite steeply due to oral microorganisms, and include dental caries, periodontal disease, endodontic infections, alveolar osteitis, and tonsillitis (Dewhirst *et al.*, 2010). Furthermore, a few oral microbiomes are always associated with systemic diseases such as bacterial endocarditis and aspiration pneumonia (Aas *et al.*, 2005). *Streptococcus mutans* and *Lactobacillus* spp. are the main Gram-positive oral bacteria that are associated with dental caries, a disease that occurs due to high sugar intake. Other bacteria that cause periodontal diseases are the *Fusobacterium* and *Porphyromonas gingivalis* (Wade, 2013).

Due to the abovementioned properties of CGA and caffeine, this study aimed to determine the antioxidant contents (i.e., CGA and caffeine), proximate composition, and antimicrobial activity of green and roasted coffee beans (*Coffea arabica* and *Coffea canephora* var *Robusta*), as well as to evaluate their effects on three selected human oral pathogens namely *Porphyromonas gingivalis*, *Streptococcus mutans* and *Lactobacillus casei*.

2. Materials and methods

2.1 Preparation of green and roasted coffee beans

Both fresh green and roasted coffee beans (Robusta and Arabica) were purchased from a coffee plantation (Kilang Kopi FAMA, Banting, Selangor). The coffee beans were rinsed with deionized water and then oven-dried at 50°C for 24 h (the final moisture content was approximately ≈12% of dry weight). The samples were then ground into a fine powder using a hammer mills grinder to pass 0.5 -1.0 mm sieve screen for less than 1 min. The ground green and roasted coffee beans powder were stored at room temperature in an air-tight container before analysis. The chemicals used in this study are all purchased from Merck Sdn Bhd (Malaysia) and are of analytical grade.

2.2 Extraction of chlorogenic acid (CGA)

Solvent extraction of CGA was performed according to Raseetha *et al.* (2016). Approximately 10 g of the ground coffee bean (green and roasted) was weighed and loaded into glass column extraction. Isopropanol was used to extract CGA. The samples were extracted with isopropanol:water (60:40) at the ratio of 1:10. About 100 mL of solvent and 2 g of activated carbon were added to extract CGA from the ground coffee powder. Then, the mixture was magnetically stirred (70 rpm) using an incubator shaker for 30 min at 80°C in dark conditions to eliminate the possibility of oxidation due to external factors. The mixture was cooled and filtered using filter paper (0.45 µm) under vacuum conditions. The filtrate was centrifuged for 15 min at 3500 rpm. Lastly, the extract was drained and distilled on a rotary evaporator at 50°C under reduced pressure (40 mbars). The extract was stored at 4°C before analysis.

2.3 Purification of chlorogenic acid (CGA)

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Purification of CGA was carried out according to Suarez-Quiroz *et al*. (2014). The aqueous coffee extract obtained from each of the green and roasted extraction methods of CGA was adjusted to pH 3 with phosphoric acid. Next, approximately 40 g/L of activated carbon (AC) was added and magnetically stirred for 30 min at 60°C. After cooling to room temperature, the mixture was vacuum filtered through diatomaous earth (celite) with 1 cm thickness. CGA was desorbed from AC using ethanol 96% (v/v). A small amount of drying agent, anhydrous sodium sulfate was added into the mixture and finally, dried in a rotary evaporator under vacuum conditions at 60°C with 120 rpm. The residue was centrifuged for 6 min at 14000 rpm, and then filtered using a syringe filter (0.45 µm) and analyzed by HPLC.

2.4 Quantification of chlorogenic acid (CGA)

CGA was analysed according to Ayelign and Sabally (2013). A standard solution of CGA (Sigma Aldrich, USA) (100 µg/L) was prepared in distilled water and used for the quantification. Standard calibration curves were established for the range 0.1–3 ppm. 100 µl of the filtrate was diluted 10 times in deionized water, and 20 µl was injected in HPLC-DAD (Model 1200, Agilent Technologies, USA). The samples and standards were run in the HPLC system with the following settings: Column: (Agilent ZORBAX Eclipse Plus C18; 250 × 4.6 mm i.d., 5 µm). The mobile phase consisted of 2% (v/v) acetic acid as solvent A and 98% acetonitrile as solvent B at a flow rate of 1 $mL.min^{-1}$. The ratio of the mobile phase acetic acid to acetonitrile was 70:30. The chromatograms were recorded from a diode array and data was collected at 330 nm.

2.5 Extraction of caffeine from coffee beans

Caffeine extraction was performed by mixing 100 mL of distilled water with 20 g of crushed coffee beans (0.5 -1.0 mm) in a beaker for 10 min. The resulting mixture was then filtered. Then, 2 g of Na_2CO_3 were added to the filtrate. The extraction of caffeine was carried out with 3×100 mL of ethyl acetate. The combined organic layer was dried over anhydrous Na_2SO_4. The solvent was distilled using a rotary evaporator (40°C) and the crystals of caffeine formed were dissolved in 5 mL of deionised water. The caffeine content was analysed using a UV-Vis spectrophotometer (Bota *et al*., 2015). A series of standard calibration curves were built between 1-5 mg/g. Caffeine content was measured at 272 nm.

2.6 Determination of total phenolic content (TPC)

About 1 mL of coffee extract was placed in test tubes, followed by 0.1 mL of Folin-Ciocalteu (F-C) reagent and 0.9 mL of distilled water as described by Jeszka-Skowron *et al*. (2016). The tubes were allowed to stand for 5 minutes. 1 mL of sodium carbonate (Na_2CO_3) solution (70 g L^{-1}) and 0.4 mL of water were added and allowed to stand again for 10 minutes for stabilization of the blue colour. The absorbance was measured at 765 nm and the data were expressed as gallic acid equivalent (GAE) in mg kg^{-1} of dry mass extract.

2.7 Chemical analysis

The moisture content was determined using the air oven method (AOAC, 2000). The fat content was determined using the soxhlet extraction method (AOAC 1995). The protein content was measured using the Kjeldahl method (AOAC, 2000). The crude fibre was determined using the acid-alkali digestion method (AOAC, 2000) and the ash content was measured according to AOAC (2000).

2.8 Antimicrobial activity

2.8.1 Preparation of pre-reduced media for anaerobic culture

An amount of 1 mL of 0.1% Resazurin was added to the prepared TSA, TSB, MRS agar and MRS broth. The media then was added with 0.5 g of L-Cysteine HCl, 5 g of yeast extract, along with 1 mL of hemin and 0.2 mL of vitamin K_1. The mixture was boiled until the colour of the media changed from purple to the native colour of the media. All of the media were autoclaved at 121˚C for 15 minutes. The TSA and MRS agar plates were prepared by cooling the pre-reduced liquid agar to 45˚C. An amount of 60 mL of liquid agar was poured into a sterile 150 mm petri dish. The agar then was allowed to solidify at room temperature. TSB and MRS broth were prepared by pouring the pre-reduced broth into sterile culture bottles (Uchino and Ken-Ichiro, 2011).

2.8.2 Preparation of stock cultures and sub-cultures

S. mutans and *P. gingivalis* were cultured on the prepared TSA agar slant and allowed to grow anaerobically in the anaerobic gas jar. *L. casei* was cultured on the prepared MRS agar slant and allowed to grow anaerobically in the anaerobic gas jar supplied with an anaerobic gas pack. All of the bacteria were maintained in the agar slant at -4˚C in the refrigerator until further use.

For sub-cultures, a small amount of the bacterial stock culture was transferred to a prepared agar media by the streak plate method. The bacterial culture was incubated anaerobically for 24 hours at 37˚C to allow for the growth of the bacteria. The colony produced was inoculated into the culture bottles containing the prepared broth. The cultures were incubated anaerobically at 37˚C for 48 hours and allowed bacteria growth.

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2.8.3 Screening on antimicrobial activity of extracts

The screening of the antimicrobial activity was carried out by using the Kirby-Bauer disk susceptibility test. An amount of 200 µl of the inoculum was pipetted onto the surface of the prepared agar plate. The inoculum was spread evenly by using a hockey stick spreader. Each sterile disk was impregnated with 20 µl of each coffee extract. The impregnated disks were carefully arranged and placed on the side of the agar by using flame-sterilized forceps. The disks were pressed gently onto the surface of the agar plate. A disk impregnated with amoxicillin was used as positive control while a disk impregnated with 95% ethanol was used as a negative control. Both disks for the positive and negative controls were placed at the centre of the agar plates. The plates were incubated anaerobically in the anaerobic gas jar accompanied by an anaerobic gas pack for 48 hours at 37˚C. The diameter of the zone of inhibition produced around the disks was measured and recorded by using a ruler (mm). This test was repeated 3 times to obtain the mean value.

2.8.4 Minimum inhibitory concentration (MIC)

The MIC was evaluated by using a sterile microtitre plate. The coffee extracts were diluted to a final concentration of 200 mg/mL. All wells were filled with 200 µl of TSB for *S. mutans* and *P. gingivalis* while for *L. casei*, 200 µl of MRS broth was used. An amount of 100 µL of the extract was pipetted to the first well. Two-fold serial dilutions were performed by pipetting 100 µL of the first well into the next consecutive well until the end of the row. In the last well, 100 µL of the mixture was discharged to make the total mixture in each well 200 µL. An amount of 10 µL of the tested inoculum was pipetted in each well. The microtitre plate was incubated anaerobically at 37˚C for 24 hours. The experiment was repeated 3 times and the well containing no extract was used as a control. The lowest concentration of the sample that exhibited no visible growth of microorganisms after incubation was considered MIC (Vipra *et al.*, 2013).

2.8.5Minimum bactericidal concentration (MBC)

All wells that showed no visible growth were subcultured onto the prepared agar plate. The agar plate was incubated anaerobically at 37˚C for 24 hours. The lowest concentration of the extract that produced no growth on the agar plates was considered the MBC (Fasolato *et al.,* 2015). This experiment was repeated 3 times to obtain the mean value.

2.9 Statistical analysis

All data were expressed as Mean ± Standard Deviation. All statistical analysis was conducted by using SPSS 16.0 (Statistical Programme for Scientific Students) for windows. Duncan's multiple range test was used to access the differences between means. A significant difference was considered at the level of $p<0.05$.

3. Results and discussion

3.1 Total phenolic content (TPC), caffeine, and chlorogenic acid (CGA) content of green and roasted coffee

Based on Table 1, the results showed that the total phenolic content was highest in green coffee than in roasted coffee and was significantly higher in green coffee Robusta than in green coffee Arabica. Dziki *et al.* (2015) reported that Robusta coffee extracts have significantly higher antioxidant activity in the TPC assay than Arabica coffee extract. The experimental results were consistent with previously reported data ranging between 4.5-11.1 mg/g (Kreicbergs and Dimins, 2011). Additionally, Jeszka-Skowron *et al*. (2016) reported that green coffee beans had twice the content compared to roasted coffee beans. TPC has a vital role in human health in protecting against diseases related to oxidation and free-radical damage (Vladimir-Knezevic *et al*., 2012).

Table 1. The concentration of total phenolic content (TPC), caffeine and chlorogenic acid (CGA) in coffee samples (mg/g).

	Total phenolic content mg/g	Caffeine content mg/g	Chlorogenic acid mg/g
Green Robusta	5.48 ± 0.005^{aA}	1.23 ± 0.001^{bB}	2.39 ± 0.20^{aA}
Green Arabica	4.67 ± 0.005^{bA}	1.04 ± 0.002^{cA}	2.37 ± 0.08^{aA}
Roasted Robusta	2.96 ± 0.025^{cB}	1.26 ± 0.001^{aA}	0.70 ± 0.03^{bB}
Roasted Arabica	1.79 ± 0.002^{dB}	1.00 ± 0.000^{dB}	0.29 ± 0.021^{cB}

Notes: Values with different small superscripts letters (a,b,c) were significantly different at $p<0.05$, ANOVA between four different samples. While values with different capital superscripts letters (A, B) were significantly different at $p<0.05$, T-test between two different samples (Green and Roasted Robusta, Green and Roasted Arabica).

The caffeine content was significantly higher in Robusta coffee either roasted (1.26 mg/g) or green coffee (1.23 mg/g) compared to Arabica coffee. Phan *et al.* (2012) reported that the caffeine content in Robusta coffee was two times higher than Arabica coffee, even though the caffeine content of coffee beans did not significantly change during the roasting process.

Similarly, a significantly higher concentration of chlorogenic acid (CGA) was found in green coffee compared to roasted coffee for both species. For example, CGA present in green coffee Robusta (2.395

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mg/g) was significantly higher compared to roasted coffee Robusta (0.704 mg/g). It has been reported that the concentration of CGA in Robusta coffee is 1.5-2 times higher than the concentration in Arabica coffee (Farah, 2012). High roasting temperature reduced CGA content in coffee beans, whereby the process promotes breakage of the carbon-carbon bonds of CGA, leading to isomerization and degradation (Esquivel and Jimenez, 2012). Other variables that influenced CGA content are coffee origin and variety. Arabica coffee is sensitive to rough handling thus needs a more skilled workforce to pick the coffee beans before processing. The thermal instability of CGA during the roasting process could also be one of the factors that lead to a significant difference in CGA content between green and roasted coffee. The severe the treatment of roasting process (longer roasting time and high roasting temperature) leads to higher losses and depletion of CGA (Preedy, 2014).

3.2 Proximate composition of green and roasted coffee

Based on Table 2, the moisture content in green Robusta and Arabica was significantly higher (9 g/100 g) compared to roasted coffee beans (2.2-3 g/100 g). Usually, green coffee had moisture content within the range of 8.5-13 g/100 g (Nogaim *et al*., 2013). Meanwhile, Iaccheri *et al*. (2015) reported that the moisture content of roasted coffee beans should be between 0.9-1.5 g/100 g because high moisture content would cause loss of roasted coffee beans characteristics such as brittleness and fragility, and also promotes hardening effect (anti plasticization). The experimental moisture results of roasted coffee beans are slightly higher, probably due to the coffee beans are hygroscopic matrices that can readily uptake moisture when exposed to the environment during storage.

The ash content in roasted Robusta coffee was significantly higher (4.6 g/100 g) compared to green coffee (3.9 g g/100 g). Farah (2012) indicated that the ash content in green Robusta coffee was within the range of 4.2-4.8 g while the ash content in green Arabica coffee was within the range of 3.0-4.2 g/100 g. Ash content in green coffee varies between 4.8-6.0 % for good quality green coffee as the higher ash content indicates a higher amount of minerals (Nogaim *et al*., 2013). Ash content for roasted Robusta coffee ranges between 4.6-4.7 g/100 g while for roasted Arabica coffee is between 4.0-4.5 g/100 g sample (Clarke, 1985).

As presented in Table 2, protein content was significantly higher in green Robusta coffee (18.027 g/100 g) and significantly lower in roasted Arabica coffee (12.946 g/100 g) compared to its respective counterparts. The protein content in green coffee varies from 11-16.5 g/100 g (Farah, 2012). The experimental results of protein content in green coffee were slightly higher than the expected range. This is because protein, peptides, and free amino acids are important for the Maillard reaction. Theoretically, the total nitrogenous compounds or protein content in green Robusta coffee was slightly higher than green Arabica coffee, but coffee is not included as one of the good nutritional sources of protein as coffee lacks essential amino acids needed by the body (Farah, 2012). This is because, during the roasting process some of the coffee protein is degraded and free amino acids would be consumed in Strecker reactions, while some other amino acids join with reducing sugars in the Maillard reaction to produce low molecular weight compounds and melanoidins. Therefore, the roasting process promotes the colour changes from green to brown colour due to the presence of melanoidin polymers (Bekedam *et al*., 2008).

Fat content was significantly higher in green (12.945 g/100 g) and roasted (12.290 g/100 g) Arabica coffee compared to its respective counterparts. However, Vasconcelos *et al*. (2007) reported that the lipid content in roasted Arabica coffee beans was significantly higher

Table 2. Data on proximate composition for different coffee samples.

	Green Robusta	Green Arabica	Roasted Robusta	Roasted Arabica
Moisture (g/100 g sample)	9.003 ± 0.309^{aA}	9.063 ± 0.104^{aA}	2.216 ± 0.310^{bB}	3.085 ± 0.171^{bB}
Ash (g/100 g sample)	3.951 ± 0.116^{bB}	3.764 ± 0.181^{bB}	4.645 ± 0.606^{aA}	$4.399\pm0.145^{a,bA}$
Protein (g/100 g sample)	18.027 ± 0.299^{aA}	15.067 ± 0.162^{bA}	14.494 ± 0.844^{bB}	12.946 ± 0.102^{cB}
Fat/Lipids (g/100 g sample)	9.550 ± 0.399^{bA}	12.945 ± 1.152^{aA}	9.115 ± 0.714^{bA}	12.290 ± 0.678^{aA}
Fiber (g/100 g sample)	2.397 ± 0.035^{aA}	2.125 ± 0.086^{bA}	2.328 ± 0.145^{aA}	2.093 ± 0.011^{bA}
Carbohydrate (g/100 g sample)	57.036 ± 0.150^{bB}	57.072 ± 0.457^{bB}	65.202 ± 0.289^{aA}	65.187 ± 0.262^{aA}

Notes: Values with different small superscripts letters (a,b,c,d) were significantly different at $p<0.05$, ANOVA between four different samples. While values with different capital superscripts letters (A,B) were significantly different at $p<0.05$, T-test between two different samples (Green and Roasted Robusta, Green and Roasted Arabica).

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compared to green Arabica coffee beans. Theoretically, Arabica coffee contained higher sugar and fat content, which is double the amount of the fat content in Robusta coffee, thus making it for favourable coffee flavour (Stephanucci *et al.,* 1979).

3.3 Antimicrobial activity of green and roasted coffee

The antimicrobial activity of green and roasted Arabica and Robusta coffee extracts on the selected oral pathogens of *L. casei*, *P. gingivalis,* and *S. mutans* were determined by measuring the diameter of the inhibition zones produced at four concentrations: 200 mg/mL, 220 mg/mL, 240 mg/mL and 260 mg/mL (Table 3).

Table 3 showed the mean ± SEM diameter of inhibition zones produced by each extract against *L. casei*. The extract of green Robusta produced the largest mean diameter of the inhibition zone at all concentrations (200 mg/mL, 220 mg/mL, 240 mg/mL and 260 mg/mL) compared to the other extracts. The mean diameter of the inhibition zone increased as the concentration of the extract increased. At the concentration of 260 mg/mL, the diameter of inhibition zones was largest with green Robusta (13.33 mm) compared to green Arabica (8.66 mm). The results indicated that *L. casei* was susceptible to the extracts. According to Denyer and Maillard (2002), Gram-positive bacteria are the most sensitive to antimicrobial agents, followed by Gram-negative bacteria and mycobacteria. The high sensitivity of Gram-positive bacteria to antibiotics and antimicrobial agents is due to the absence of the outer membrane. The outer membrane gives primary protection against toxic molecules from the external environment and also provides an additional stabilizing layer around the cell (Silhavy *et al*., 2010).

Table 3 showed the extract of green Robusta produced the largest mean diameter of the inhibition zone of *P. gingivalis* at all concentrations compared to the other extracts. The table also showed that the mean diameter of the inhibition zone increased as the concentration of the extract increased. At the concentration of 260 mg/mL, the mean diameter of the inhibition zone was significantly higher in green Robusta (11.10 mm) compared to green Arabica (8.24 mm) and roasted Arabica (7.03 mm). *P. gingivalis* showed intermediate sensitivity when tested against all of the extracts. The mean diameter of inhibition zones produced on *P. gingivalis* was smaller than the mean diameter of inhibition zones produced on *L. casei.* The lower sensitivity of *P. gingivalis* towards the extract might be due to the reason that *P. gingivalis* is a Gram-negative bacterium. Unlike Gram-positive bacteria, Gram-negative bacteria are surrounded by an outer membrane. According to Denyer and Maillard (2002), the existence of an outer membrane is responsible for the impermeability of antimicrobial agents to the Gram-negative bacteria since the target sites for antimicrobial agents are located within the cell and specifically, within the cytoplasm. The presence of porin channels is also the main reason why Gram-negative bacteria are resistant to most antibiotics and antimicrobial agents. The porin channels selectively allow the uptake of beneficial nutritional substances and expel out substances that are harmful to the survival of Gram-negative bacteria such as antibiotics (Miller, 2016).

Table 3. Mean ± SEM diameter of inhibition zones of coffee extracts against *L. casei, P. gingivalis* and *S. mutans*.

	Mean diameter of inhibition zones (mm)				
Oral pathogens	Concentration of extracts (mg/mL)	Green Robusta	Green Arabica	Roasted Robusta	Roasted Arabica
L. casei	200	9.67±0.33^{a}	7.03±0.03^{c}	8.50±0.06^{a,b}	6.36±0.01^{c}
	220	10.67±0.33^{a}	7.55±0.01^{c}	8.77±0.03^{b}	6.54±0.03^{d}
	240	11.67±0.33^{a}	8.17±0.09^{c}	9.00±0.00^{b}	7.03±0.03^{d}
	260	13.33±0.33^{a}	8.66±0.03^{c}	10.67±0.33^{a,b}	8.03±0.09^{c}
P. gingivalis	200	9.07±0.12^{a}	7.11±0.07^{c}	8.53±0.03^{b}	6.25±0.01^{d}
	220	9.55±0.01^{a}	7.40±0.01^{c}	8.77±0.04^{b}	6.38±0.01^{d}
	240	10.03±0.03^{a}	7.74±0.03^{c}	8.97±0.03^{b}	6.61±0.06^{d}
	260	11.10±0.10^{a}	8.24±0.13^{c}	10.07±0.07^{b}	7.03±0.07^{d}
S. mutans	200	NIZ	NIZ	NIZ	NIZ
	220	NIZ	NIZ	NIZ	NIZ
	240	6.52±0.01^{a}	6.21±0.09^{c}	6.29±0.01^{c}	6.08±0.04^{d}
	260	7.00±0.00^{a}	6.32±0.02^{c}	6.47±0.02^{c}	6.24±0.01^{d}

Notes: Values with different small superscript letters (a,b,c,d) were significantly different at $p<0.05$, ANOVA between four different samples

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Table 4. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of coffee extracts against *L. casei*, *P. gingivalis* and *S. mutans*.

Coffee	MIC (mg/mL)			MBC (mg/mL)		
	L. casei	*P. gingivalis*	*S. mutans*	*L. casei*	*P. gingivalis*	*S. mutans*
Green Robusta	50	200	240	50	200	240
Green Arabica	50	100	240	50	100	240
Roasted Robusta	50	200	240	50	200	240
Roasted Arabica	50	100	240	50	100	240

Table 3 showed that all of the extracts did not produce any inhibition zone against *S. mutans* at the concentrations of 200 mg/mL and 220 mg/mL but the inhibition zones were present at the concentrations of 240 mg/mL and 260 mg/mL. At the concentration of 260 mg/mL, the diameter of inhibition zones was significantly higher in green Robusta (7.00 mm), compared to green Arabica (6.32 mm) and roasted Arabica (6.24 mm). A previous study by Almeida *et al*. (2012) on roasted Arabica against *S. mutans* stated that the inhibition zones produced by the extract at the concentration of 260 mg/mL were within the range of 6 mm to 8 mm which supported these findings. The least susceptible towards all of the extracts was *S. mutans*. Table 3 showed that the mean diameter of inhibition zones produced by all extracts was the smallest when tested on *S. mutans*. The results showed that *S. mutans* were very resistant to the extracts. Despite being a Gram-positive bacterium, the presence of a capsule may be caused *S. mutans* to be less receptive towards antimicrobial agents (Flayyih and Al-Ammar, 2016).

3.4 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of green and roasted coffee extracts against L. casei, P. gingivalis and S. mutans

Table 4 showed the lowest concentration of extracts (MIC) that inhibited the growth of *L. casei*, *P. gingivalis,* and *S. mutans*. The lowest concentration needed to inhibit the growth of *L. casei* for all extracts was 50 mg/mL and the lowest concentration needed to inhibit the growth of *S. mutans* for all extracts was 240 mg/mL. The MIC values of green Robusta and roasted Arabica on *P. gingivalis* were 100 mg/mL and 200 mg/mL, respectively.

MBC was tested on the extracts to determine the lowest concentration required to kill the bacteria (Table 4). The concentration that does not show any growth of bacteria on the plate after incubation was considered as the MBC. The MBC values of all extracts when tested on *L. casei* were 50 mg/mL while the MBC values of all extracts when tested on *S. mutans* were 240 mg/mL. The MBC values of green Robusta and roasted Arabica when tested on *P. gingivalis* were 100 mg/mL and 200 mg/mL, respectively.

4. Conclusion

The results of this study showed that green and roasted Robusta coffee had higher chlorogenic acid, total phenolic contents, and good antimicrobial activity compared to its counterparts. However, Arabica coffee was found to have a more pleasant and favorable coffee aroma compared to Robusta coffee due to its high content of fat and carbohydrates. The results indicated that *L. casei* was more susceptible to all the extracts, followed by *P. gingivalis* with intermediate sensitivity and *S. mutans* being the least susceptible to all the extracts. Robusta coffee could be used by food industries depending on the type of beverage application.

Conflict of interest

The authors have no competing conflicts of interest.

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