EJSIT
European Journal of Science, Innovation and Technology
ISSN: 2786-4936
www.ejsit-journal.com
Volume 4 | Number 3 | 2024

Phytochemical Profile, Antioxidant and Antimicrobial Activity Screening of Different Extracts of the Beans of *Coffea robusta*

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ABSTRACT

Coffea robusta plays a significant role in both research and the trading market. It is utilized in the food, cosmetic, and medicinal industries because of its caffeine and elevated phytochemical content. Beans of *Coffea robusta* were successfully extracted in aqueous, acetonic and methanolic solvents. Results of quantitative estimation of total phenolic and flavonoids showed that the greatest amounts of flavonoids and polyphenols were detected in the methanolic extract of 23.9 ± 0.2 mg QEs/g dry mass and 154.4 ± 0.9 mg GAE/g dry mass, respectively. The highest antioxidant scavenging activity was found in aqueous was determined for (87.2 ± 1.2%). HPLC analysis of methanolic extract showed the presence of many important compounds among them: Ferulic acid, Chlorogenic acid, Caffeic acid, Catechin, Epicatechin and Rutin. Finally, the results of antibacterial activity revealed that methanolic extract of *Coffea robusta* bean in concentration of (200mg/ml) showed a significant against different bacterial strain like *E. coli* and *Staphylococcus aureus*.

Key words: *Coffea robusta*, phytochemicals, HPLC, antioxidant, antibacterial, *E. coli*

INTRODUCTION

For centuries, people have used the substances that produced by plants to treat illness and promote good health due to its physiological effects, as well as its enticing flavor and aroma. Coffee as a popular beverage that is consumed by many people worldwide. The Rubiaceae family encompasses a notable species of coffee referred to as *Coffea robusta*. Caffeine is the primary constituent in coffee, commonly utilized as a stimulant. Multiple studies have demonstrated that coffee possesses various health benefits, including diuretic, antibacterial, and antioxidant properties (Almeida et al., 2006; Alsunni et al., 2015).

Phytochemicals are natural bioactive compounds produced by plants as either a by-products or as defensive agents against parasites or against environmental stress (Birben et al., 2012).

Numerous studies have demonstrated the important of some phytochemicals for human health (Birben et al., 2012).

As antioxidants to reduce oxidative damage to macromolecules, defending the body against a variety of illnesses and as antimicrobial agents (Hečimović et al., 2011).

The *Coffea robusta* plant traditionally named (coffee) is a rich source of alkaloids, particularly caffeine, which gives coffee its bitter taste and acts as a diuretic and peripheral vasoconstrictor in addition to stimulating the central nervous system (International Coffee Organization, 2024).

Therefore, the current study's main objective is to assess the phytochemicals, antioxidants, and antimicrobial qualities of three distinct extracts of roasted *Coffea robusta* beans.

MATERIALS AND METHODS

Sample Collection

Roasted beans of *Coffea robusta* obtained from a local market and kept in dark dry sterilized plastic package. Powdered crude 5g were soaked in three solvents separately, 80% methanol, 80% acetone and distilled water, 50 ml of each poured in 100ml flask for overnight using a magnetic stirrer at in ambient temperature, centrifuged 3000 rpm for 15 minutes and supernatant then collected in a hot air oven at 40°C, and solvents were to evaporate the solvents. The remaining layer of plant extract was collected via scraping from the bottom of dishes, then weight was recorded and kept in a sealed dark glass container, stored at -20°C till next step (Lateef et al., 2019).

Quantitative Estimation of Total Phenolics and Flavonoids

Total polyphenols determination

Folin-Ciocalteu (F-C) assay used to estimate total polyphenols (TP) (Miliauskas et al., 2004). Initially, 0.5 mL of (1.0 mg/mL) either plant extract as unknown or (5, 10, 15, 20 and 25 mg/mL) of aqueous solutions of Gallic acid as standard were mixed separately with 2500 ul 1N (F-C) reagent. Next, 2500 ul of 20% Na_2CO_3 solution was added to the mixture and rest for 30 minutes in the shade, then the absorbance was detected at 760nm wavelength. A standard curve was designed, total phenol then measured as mg Gallic acid equivalent per gram of dry mass (mg GE/g DM).

Total flavonoids determination

The quantification of total flavonoids (TF) was conducted by a colorimetric approach utilising aluminium chloride (AlCl3) and sodium hydroxide (NaOH). (Murthy et al., 2009). Briefly, 50 ul of either of each extract1.0 mg/mL as unknown or (10, 20, 30, 40, 50 and 60 mg/mL) of catechin as standards were mixed separately with 2000 ul of distilled water, 0.15 mL of solution of sodium nitrite (NaNO2) with concentration of 150 g/L, and 150 ul of AlCl3.6H2O solution with a concentration of 100 g/L. After a duration of 6 minutes, a quantity of 2000 ul of NaOH solution with a concentration of mol/L was introduced, and the total volume was adjusted to 5000 ul by adding distilled water. The entire mixture was allowed to settle in darkness for a duration of 15 minutes, after that the absorbance read at 510nm. Then standard curve was generated and the total flavonoid content was measured as milligrams of catechin equivalent per gram of dry mass (CE mg /g dry mass).

Assessment of Antioxidant Activity

The antioxidant activity of the extracts was assessed via measuring the DPPH radical scavenging activity (RSA %), as described in (Nonthakaew et al., 2015) using 96-well microplates. Initially, 100 µl of 500 µg/ml from plant extract was added to a reaction well as the sample-blank. In another well, 100 µl of a 0.2 mmol/L DPPH in a 95% methanol was combined with the extract as the sample +DPPH. Then, 100 µl of 95% methanol and 100 µl of 0.2 mmol/L DPPH were each placed in two different wells respectively. Subsequently, the sample was kept in a dark setting at the ambient temperature for an interval of 30 minutes. The microplate reader measured the absorbance of each well at a wavelength of 517 nm.

The DPPH (RSA) Radical Scavenging Activity percentage was calculated using equation which implement the absorbance values of each well. The DPPH Radical Scavenging Activity (RSA) may be calculated using the formula (Patay et al., 2016):

$$RSA = [\ ((sample + DPPH) - (sample\ blank)) / (DPPH\ blank) - (solvent\ blank)] \times 100.$$

HPLC Sample Preparation

Separation solution was prepared by dissolving 50 mg of crude *Coffea robusta* methanol extract in 10 ml of 80% ethanol. The solution was then exposed to ultra-sonication for 25 min at 25°C, then centrifuged at 7,500 rpm for 15 min. Clear supernatant then subjected to charcoal treatment to eliminate colours, and then it was evaporated under vacuum for drying. The dried crude was re-suspended in volume 1.0 ml of methanol (HPLC grade) by vortexing. It was then filtered using a 2.5 um filter and stored at 4°C for the subsequent analysis. Twenty microliters of the crude sample were injected into the HPLC system using the optimal separation conditions that were previously established using the pure standard (Ramirez et al., 1988). Liquid chromatography (LC) separation was conducted with a Shimadzu 10AV-LC system equipped with a binary delivery pump. The eluted peaks were analyzed using a UV-Vis 10 A-SPD spectrophotometer (Patay et al., 2016).

Antibacterial Activity

Four bacterial strains, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*, were cultured for 24 hours at 37°C on nutritional agar medium and subsequently preserved at 4°C. Antibacterial activity was detected in methanol extracts of *Coffea robusta* utilizing the agar well diffusion assay (Riyanti et al., 2016). The agar plates were infected with bacterial test strains. Two stock solutions of plant extract were produced at concentrations of 100 and 200 mcg/ml, respectively. Subsequently, 100 µl of the plant solution, 100 mcg/ml chloramphenicol as a positive antibiotic standard, and methanol as a standard solvent control were individually introduced into each well using a sterile syringe and permitted to diffuse at room temperature for 2 hours. The plates were incubated for 24 hours at 37°C to facilitate bacterial growth (Ramirez et al., 1988; Riyanti et al., 2016).

RESULTS AND DISSCUSSION

Phytochemical Components

The phytochemical components, antioxidant properties, and antibacterial activity of various plant extracts are of significant interest in both the pharmaceutical and nutrient supplement industries. Thus, natural additives have tended to substitute the synthetic antioxidants and antibacterial agents with natural ones. The amount of total phenolic compounds in *Coffea robusta* extraction samples was assessed using the Standard Gallic acid curve. The outcomes derived from each extraction solvent are shown in the Table. 1. The greatest amount of total phenolics was found in methanolic extract (154.4 ± 0.9 mgGAE/ g dry mass), while the acetone extract has only 141.9 ± 0.3 mgGAE/ g dry mass. The overall phenolic content in plant extracts is influenced by the solvent's polarity. The use of a highly polar extraction solvent resulted in a high yield due to the strong solubility of phenols in polar solvents (Singleton et al., 1999).

The results of the current investigation indicated that no significant differences in flavonoid concentrations were observed across various solvent extracts. The crude methanolic extract contained the highest concentration of flavonoids (23.9 ± 0.2 mg QEs/g) compared to the acetone and aqueous extracts, which had 13.3 ± 0.4 mg QEs/g and 12.1 ± 0.5 mg QEs/g, respectively. Numerous investigations have identified a substantial association between the total contents of flavonoids and phenolics. Their results indicated that roasting conditions could influence the polyphenolic components in coffee. Their demonstration indicated that light and medium roasting techniques are more effective in maintaining important components during coffee roasting (Orthofer et al., 1999).

Antioxidant Activity

As a quick and simple method for estimation the antioxidant activity of vary plant extracts, DPPH assay implanted in this study. Among three extracts of *Coffea robusta*, high DPPH RSA% activity was found in aqueous extract (87.2 ± 1.2%) followed by acetone extract (68.9 ± 1.0%), while methanol extract showed the lower antioxidant activity (50.6 ± 0.6%). A previous study observed a high antioxidant activity in green robusta coffee beans comparing to roasted ones (Stanković et al., 2010). Anyway, this difference might due to the partial degradation of chlorogenic acid during roasting process (Takahashi et al., 2017). Current study also showed that the aqueous extract had greater antioxidant activity than methanol extract. This agrees with Złotek et al. (Vasanthi et al., 2012).

Table 1: Phytochemical content and antioxidant activity of three coffee extraction solutions

	Extraction solvent	Polyphenols (mg GE/g DM)	Flavonoids (mg CE/g DM)	DPPH RSA (%)
1	Aqueous	148.8 ± 0.8	12.1 ± 0.5	87.2 ± 1.2
2	Methanol	154.4 ± 0.9	23.9 ± 0.2	50.6 ± 0.6
3	Acetone	141.9 ± 0.3	13.3 ± 0.4	68.9 ± 1.0

HPLC Analysis

Phytochemical profile analysis for methanol extract of *Coffea robusta* showed the presence of many phenolic and flavonoid compounds. Among them, chlorogenic acid was found dominantly in concentration of 500.61 ug/ml. Rutin was the lowest concentration of 159.39 ug/ml, while caffeic acid, isochlorogenic acid, epicatechin, catechin and ferulic acid were found in concentrations of (413.92, 402.45, 323.67, 315.2 and 251.93, respectively). This comes in agreement with (Ramirez-Martinez et al., 1988) (Figure 1).

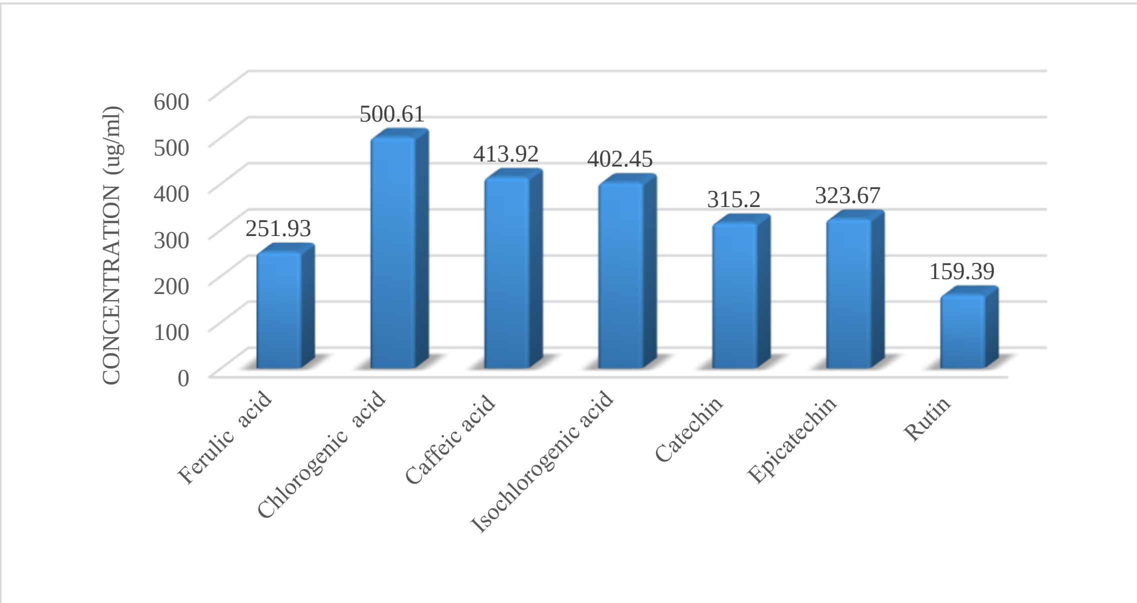

Figure 1: HPLC analysis show the phytochemical contents of methanol extract of *Coffea robusta*

Antibacterial Assay

The results of antibacterial activity of methanol extract of *Coffea robusta* beans were obtained by implementing agar diffusion method against four used strains of bacteria were shown in Table 2.

Generally, comparing to 100 mcg/ml Chloramphenicol as standard antimicrobial agent, methanol extract in concentration of 200 mcg/ml exhibited moderate antibacterial activity, while in concentration of 200 mcg/ml showed less or no antibacterial activity. The highest zone of inhibition was gained against *E. coli* which was 22.5cm. While the lowest inhibition zone was shown against *Klebsiella pneumonia* within 10.4cm. Notably, concentration of 100mcg/ml of methanol extract exhibited no antibacterial activity against *Klebsiella pneumonia*. Numerous studies have verified the existence of the alkaloid caffeine in roasted Robusta coffee beans, while additional research has shown the antibacterial efficacy of Robusta coffee against E. coli and S. aureus, employing the disc diffusion method, attributed to the presence of caffeine as a bacterial growth inhibitor (Vaz et al., 2011; Złotek et al., 2016).

Almeida et al. noted that the elevated levels of phenolics in coffee exhibit antibacterial activity via altering the structure of the cytoplasmic membrane, hence interrupting the proton motive force and electron flow (Almeida et al., 2006). Additionally, Almeida et al. noted that the elevated phenolic content in coffee extract may demonstrate antibacterial properties by modifying the cytoplasmic membrane structure and affecting proton and electron flow (Almeida et al., 2006).

Table 2: Antimicrobial activity of methanolic extraction of *Coffea robusta* beans against *B. subtilis, E. coli, S. aureus* and *K. pneumonia*

	Bacterial strains	**Methanol extract**		**Chloramphenicol (Standard)**
		100 mcg/ml	***200 mcg/ml***	***100 mcg/ml***
1	*B. subtilis*	0.9± 0.12	1.6 ± 1.56	3.0 ± 0.11
2	*E. coli*	1.0± 0.17	2.1 ± 0.08	2.9 ± 0.13
3	*S. aureus*	0.9± 0.09	1.9 ± 1.44	3.1 ± 1.12
4	*K. pneumoniae*	0.00	1.4 ± 0.78	2.7 ± 0.09

CONCLUSIONS AND FUTURE PROSPECTS

The elevated concentration of phytochemical components, such as phenolics and flavonoids, in *Coffea robusta* beans indicates their potential as natural antioxidants. Moreover, additional research is required to examine the existence of established bioactive compounds and ascertain their roles as antioxidant or antibacterial agents, given that the current study concentrated on the crude extract of *Coffea robusta*.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

The author declare his own funding of this article

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