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Determination of Antioxidant and Antibacterial Activities of Yirgacheffe Arbica Coffee Leaves

Belete Tewabe Gebeyehu

Department of Chemistry, Debre Berhan University, Debre Berhan, Ethiopia

Abstract

The use of coffee plants is more focused on the coffee beans as a brewing drink or as a food additive. Parts of the coffee plant, such as leaves, are considered as waste and have not been properly utilized. The current study investigates the antioxidant activities and antibacterial activities of Arabica coffee leaves from Yirgacheffe, Gidio Zone, Ethiopia. As a reference, the crude extracts of green/raw and medium roasted beans of coffee were screened for their in vitro antioxidant properties and antibacterial activities. Antioxidant activities were measured by DPPH assay and the reducing power. The findings of this research show that the coffee leaves possessed the highest antioxidant activities in both DPPH and reducing power assays. A linear correlation between concentrations of coffee extract of the leaves and reducing power was observed with a coefficient of $y = 0.00312x + 0.0802$, ($R^2 = 0.99472$). These results indicated the extract of leaves of coffee has a strongest antioxidant activities compared with green and medium roasted coffee extracts. Moreover, coffee extract has found to be more effective against gram positive bacteria; S.aureus than negative bacteria strains; E. coil. Antioxidant activities for leaf of coffee samples were slightly higher than for the corresponding green and roasted samples while antibacterial activities was significantly lower in leaf of coffee compared to that of green and roasted samples ($p < 0.05$). Thus, coffee leaf is a very promising resource in the areas of food and pharmaceutical industry, especially, in the beverage industry.

Keywords: Coffee leaves, antioxidant activity, antibacterial, DPPH assay, reducing power

*Corresponding author email: *beletetewabe@gmail.com, beletetewabe@dbu.edu.et*

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Introduction

Coffee is one of the most extensively consumed beverages in the world due to its pleasant taste and aroma (Seninde & Chambers, 2020). Although coffee has been mainly taken to its stimulating and refreshing effects, a number of studies have been elucidated the possible beneficial effects of coffee consumption on human health and have shown that coffee exhibits potent of medicinal activities against noninfectious as well as infectious diseases (George, Ramalakshmi, & Mohan Rao,

2008). Some studies suggested that coffee has some compounds that have specific activity, such as antibacterial (Kenconojati, Ulkhaq, Budi, & Azhar, 2019), antioxidant (Cämmerer & Kroh, 2006; Tewabe Gebeyehu, 2015; Yashin, Yashin, Wang, & Nemzer, 2013), anti-diabetic (Campos Florin, Bardales Valdivia, Caruajulca Guevara, & Cueva Llanos, 2013) and anti-inflammatory (Surma, Sahebkar, & Banach, 2023).

In addition, green coffee bean phytochemicals have been included in skin care products to delay the signs of aging of the skin and show a tendency to reduce visceral fat, the risk of diabetes, obesity, and coronary heart disease. Green coffee beans are rich in polyphenolic antioxidants such n-coumarin, ferulic, caffeic and the chlorogenic acids (Mussatto, Machado, Martins, & Teixeira, 2011).

However, due to the Maillard reaction, roasting coffee dramatically changes the content of polyphenols (Cämmerer & Kroh, 2006). When coffee is roasted, the amount of chlorogenic acids falls while the amount of high molecular compounds and melanoidins—which also have antiradical properties—increases(Seninde&Chambers, 2020).

The coffee beans have been widely investigated due to their economic importance and the specific quality attributes associated with coffee beverages and human health (Seninde & Chambers, 2020). In regions where coffee is cultivated, coffee leaves have historically been used to treat or lessen a variety of ailments or disorders (X. Chen, 2019; Mees et al., 2018). For example, coffee leaves have been used in treating asthenia in Haiti (Ferrazzano, Amato, Ingenito, De Natale, & Pollio, 2009), relieving fever in Mexico and curing diarrhea and intestinal pain in Africa. Furthermore, sun-dried coffee leaves have been consumed as a tea in Indonesia, Jamaica, India, Java, Sumatra, Ethiopia and South Sudan since the 1800s. However, the majority of coffee plant leaves have been viewed as agricultural waste in the production process of coffee.

Depending on the stage of leaf growth, coffee leaves contain a variety of phytochemicals (X. M. Chen, Ma, & Kitts, 2018). According to some reports, coffee leaves may contain all of the key metabolites found in coffee beans. In addition, they contain mangiferin, a xanthonoid with great antioxidant potential as well as therapeutic and pharmacological properties. Mangiferin (Talamond et al., 2008) was identified in coffee leaves during 2008 and it is unique in that it has anti-inflammatory, anti-diabetic, anti-hyperlipidemic, antioxidant, anti-microbial, and neuroprotective

activities(Jyotshna, Khare, & Shanker, 2016).

Although, very few studies have targeted the leaves of coffee plants, coffee leaves have a long history for use as ethno-medicine and tea beverage by locals from countries where coffee plants grow. Many previous studies on health benefits from coffee focus on the use of the coffee beans. There are relatively few studies concerning the antioxidant activities coffee leaves. Moreover, to the best of my knowledge, reports on the evaluation of the antioxidant activities in leaves of coffee plant taken from the same garden of Yirgacgheffe coffee in comparison with the content in green and roasted coffee beans are still lacking. This study therefore aims at assessing antioxidant and sntibacterial activities of coffee plant organs such as coffee leaves, green and roasted coffee beans for the first time.

Material and Methods

Samples

Fresh leaves and beans of coffee, , growing in the garden of Yirgacheffe district, Gidio zone , Ethiopia, were collected and used as plant material for evaluation of the antioxidant activities of Yirgacheffe coffee segments. Both the seeds and leaves were taken from the same garden two years old coffee. All the samples were thoroughly washed rinsed with tap water and were dried without direct sun light.

Chemicals

Trichloroacetic acid, 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical, iron (III) chloride was obtained from Sigma Aldrich, India. Potassium ferricyanide, sodium dihydrogen phosphate and disodium hydrogen phosphate were purchased from Lach-Ner (Brno, Czech Republic). All the remaining reagents were analytical grade from several suppliers and included: ascorbic acid, methanol. All other chemicals and solvents were of the highest analytical grade obtained from several suppliers. Phosphate buffer (pH 6.6) was prepared from sodium dihydrogen phosphate and disodium hydrogen phosphate.

Preparation samples

Green coffee beans (50 g) were initially dried without sun to a constant weight (7 % weight loss). Roasting of green coffee beans was performed using a traditional way of roasting coffee in Ethiopia. All, leaves, green and roasted coffee beans were ground into a fine powder and sieved through a 55-mesh screen.

Sample Extraction

The leaves, green and roasted coffee samples were ground and extracted using methanol solvent. Similarly, the medium-roasted powder was extracted with methanol after sieving. Twenty-five-gram powder of each sample was dissolved in 250 mL methanol and allowed to shake for 72 hours in a shaker (GFL, Model 3020, Germany) at 25° C and filtered with Whatman No. 1 filter paper. The methanol solvent was evaporated using a rotary evaporator (BUCHI, Germany) at 50 ° C followed by oven drying to remove the remaining solvent and kept in a refrigerator until used for antioxidant test.

Analysis of antioxidant activities

Assays of 1, 1-diphenyl-1-picrylhydrazyl (DPPH) radical

The antioxidant capacity of coffee extracts (leaf, green and roasted beans) to scavenge DPPH free radicals was assessed as described by(Mishra, Ojha, & Chaudhury, 2012) Chen, Ma, and Kitts (2017), with minor modifications. Briefly, 1mL of 0.1 mM solution of DPPH (Sigma Aldrich, India) in methanol was mixed with an equal volume of the methanol extracts at different concentrations. The mixture was then incubated for 30 min at room temperature in the dark. The absorbance of solutions in a 1-cm cuvette at 517 nm was measured at 2-min intervals against a blank (MeOH) using UV-7504 spectrophotometer (Shanghai, China) until the absorbance reached a plateau. Ascorbic acid was used as the standard. Samples were prepared and measured in triplicates

The percentage inhibition of the DPPH free radical was calculated as:

$$\% \text{ Inhibition} = \frac{Ac - As}{Ac - Ab} * 100$$

Where; As = Absorbance of sample = 0.1 mmol DPPH with sample in methanol, Ac = Absorbance of control = 0.1 mmol/L DPPH alone in methanol, Ab = Absorbance of blank in methanol solvent in absence of DPPH and sample.

Reducing Power assay

The reducing power of the prepared extracts was determined by the procedure described by Oyaizu (1986), with slight modifications. Briefly, different concentrations (5-200 µg/ mL) of the extracts of the samples were mixed with an equal volume of 0.2 M phosphate buffer (2.5 mL; 0.2 M; pH 6.6) and potassium ferricyanide solution (2.5 mL; 1% w/v) and incubated at 50 °C on a water bath for 20 min. After cooling, trichloroacetic acid solution (2.5 mL; 10% w/v) was added to the mixture, which was centrifuged at 3000 rpm for 10 min. Finally 2.5 mL of the upper layer was mixed with an equal volume of distilled water and then added with 0.5 mL

of 0.1 % (w/v) ferric chloride solution. The reaction mixture was left for 10 min at room temperature, and the absorbance at 700 nm was measured spectrophotometric ally against an appropriate blank. Increased absorbance of the reaction mixture indicates greater reducing power.

Antibacterial activity test

The antibacterial activity was carried out on the extract of the leaves of coffee with concentrations range of 25- 200 µg/mL against the (Staphylococcus aureus, ATCC 25923) and Gram-negative (Escherichia coli ATCC 25922). The cultures of bacteria were maintained in their appropriate agar slants at 4 ^{0}C throughout the study and used as stock cultures. The disk diffusion method was employed for the determination of antimicrobial activities of the coffee leaves extracts. Briefly, a suspension of the tested microorganism (0.1 mL of 10^8 cells/mL) was spread on the solid media plates. Filter paper disks (6 mm in diameter) were soaked with 15 µL of the coffee extracts and placed on the inoculated plates. After being kept at 4 °C for 2 h, they were incubated at 37 °C for 24 h for bacteria.

The diameters of the inhibition zones were measured in millimeters. Negative control was prepared using respective DMSO solvent. Gentamicin (10 µg/disc) was used as a positive control with the tested bacteria.

Statistical Analysis

Results were expressed as the means ± SD (n = 3). A one-way analysis of variance was used for the data analysis by using origin 8.5software. Differences with a P value of < 0.05 were considered significant. All tests were performed in triplicate.

Results and Discussion

By employing DPPH and reducing power assays, the antioxidant properties of methanol extracts of leaves, green, and medium-roasted coffee were assessed. While disc diffusion techniques were used to evaluate the antibacterial activity. The samples for this study were taken from the same Yirgacehffe garden coffee tree (Figure 1a). The raw/green coffee beans in Figure 1b were taken from sample Figure 1a. Finally, the green coffee beans were traditionally roasted at a medium degree, as indicated in Figure 1c.

Figure 1. (a)Yirgacehffe Coffee tree (b) Green coffee beans and (c) Roasted (medium) coffee beans.

DPPH radical scavenging activity

The DPPH assay is a rapid and affordable approach that is widely used to assess the anti-oxidative properties of different natural compounds (Arteaga *et al*., 2012). DPPH radical possesses a purple color (Figure 2a), with a maximum absorption at 517 nm in methanol hence, scavenging the DPPH radical by coffee antioxidants will result change in color to yellow (Figure 2a) and also a decrease in absorbance readings over time; the extent of decrease in absorbance of DPPH is proportional to the concentration of radicals that are being scavenged.

In addition, the level of discolouration reveals the antioxidant extract's capacity for scavenging free radicals. The absorbance change produced by this reaction is assessed to evaluate the antioxidant potential of the test sample.

The results in Figure 2a demonstrated that after 30 min incubation, the color of reaction mixture changed from purple to yellow. Each of the coffee extracts reduced DPPH to the yellow coloured product, di-phenylpicrylhydrazine, and the absorbance at 517 nm declined (Przybylski, Konopko, Letowski, Jodko-Piorecka, & Litwinienko, 2022).

The DPPH assay is based on the measurement of the scavenging capacity of antioxidants towards it. As shown in Figure 2b, the odd electron of nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidants to the corresponding hydrazine.

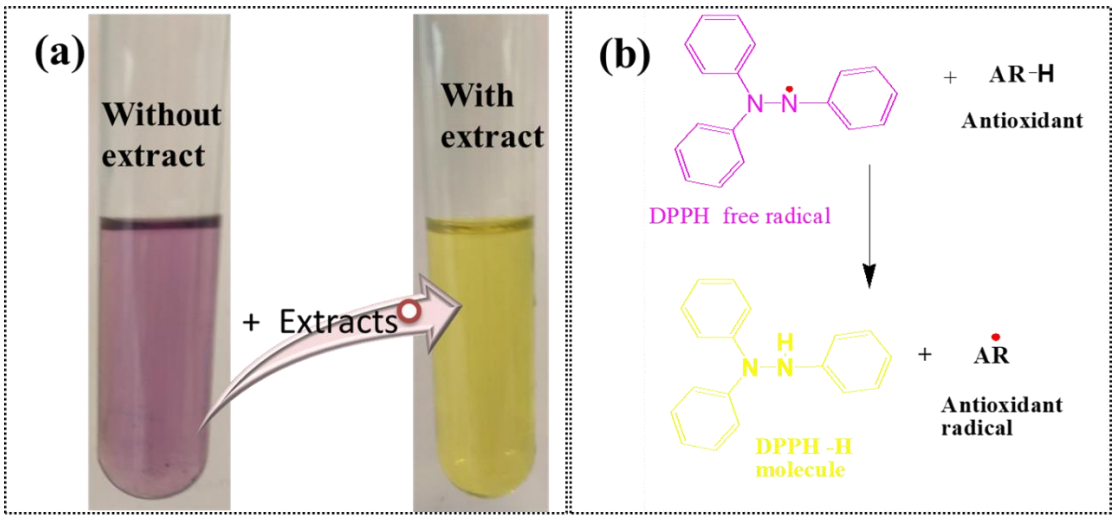

Figure 2. (a) Effect of coffee extracts on the color of the DPPH free radical and (b) the way in which coffee extract, an antioxidant, interacts with the DPPH free radical.

The extent of DPPH radical-scavenging abilities of the three coffee methanol extracts (leaves, green and roasted) along with the reference antioxidant ascorbic acid are shown in Figure 4a**.** A dose-dependent relationship was found in DPPH scavenging activity among all coffee samples in scavenging DPPH radical that was statistically significant ($p < 0.05$) compared with control. As clearly seen in Figure 4a, the scavenging activity of all coffee extracts rapidly increased as the concentrations raised from 6.25 to 200 µg/mL.

As indicated in Figure 4a, it clearly shows that among the three coffee segments, leaves had the highest % scavenging activity for all the concentrations (6.25- 200 µg/mL) of the methanol extract followed by green and roasted beans respectively. For instance, coffee leaves had the highest rate of scavenging activity at 200 g/mL, with a value of 92.17%, followed by green (90.04%) and roasted coffee beans (82.95 %). Additionally, DPPH scavenging values of methanol extracts of coffee leaves, green beans, and roasted beans were reported to be 55.22%, 52.48%, and 32.15%, respectively, at the lowest concentration (6.25 g/mL). The standard ascorbic acid was most effective at scavenging DPPH radicals, followed by leaf, green coffee, and roasted coffee, as shown in Figure 4a.

Reducing power

The measurement of reducing power can reflect several aspects of antioxidant activity in the sample. In this method ferric ions are reduced to ferrous ions resulting in a change of color from yellow to bluish green **(**Figure 3a**).** The intensity of color depends on reducing potential of the extracts present in the medium. Greater the intensity of the color results in greater absorption, hence, an increase in antioxidant activity. As shown in Figure 3b, the presence of reductants in the solution causes the reduction of the $Fe^{3+/}$Ferricyanide complex to the Fe^{2+} / ferrous form(Gulcin, 2020). Therefore, the Fe^{2+} can be monitored by measurement of the formation of Perl's Prussian blue at 700 nm.

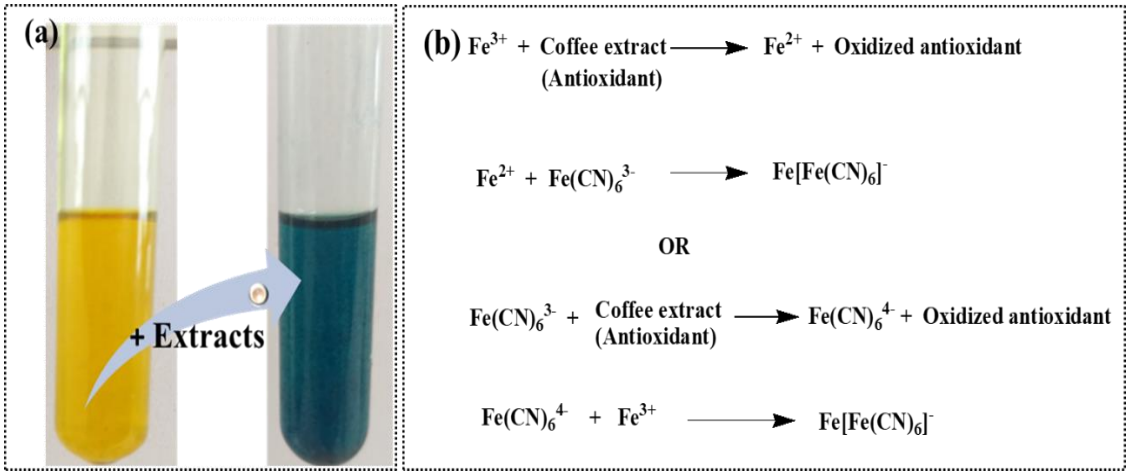

Figure 3. (a) Effects of coffee extracts on color changes of Fe3+ and (b) Reaction of coffee extract (antioxidant) with Fe3+.

As shown in Figure 4b and Table1, the methanol extract of leaves of coffee exhibited the highest antioxidant potential among the other extracts, based on the reducing power assay. The absorbance of the reducing power of leaves the coffee extracts was found to be in the range of 0.429 to 2.89 at various concentrations (6.25 to 200 µg/mL), whereas at the same concentration green/raw and roasted bean extracts of the respective coffee showed 0.429 to 2.89 and 0.122 to 1.59 respectively (Table 1). The higher the value of absorbance of the reaction mixture indicated the greater reducing power. Similar with DPPH assay, green and roasted coffee beans had lower antioxidant potential compared to leaves of coffee and the standard ascorbic acid.

Table 1. Mean ± standard deviation (n =3) of absorbance of coffee leaf, green/ raw and roasted beans at wave length 700 nm.

Concentration of Samples (µg/mL)	Average absorbance ± SD			
	Ascorbic acid (AA), standard	Leaf	Roasted coffee	Green/Raw coffee
6.25	0.98 ± 0.001	0.645 ± 0.003	0.122 ± 0.004	0.429 ± 0.003
12.5	1.184 ±0.001	1.076 ± 0.002	0.234 ± 0.004	0.654 ± 0.002
25	1.401 ± 0.001	1.384 ± 0.003	0.568 ± 0.003	1.243 ± 0.004
50	1.956 ± 0.002	1.892 ± 0.001	0.892 ± 0.004	1.625 ± 0.003
100	2.597 ± 0.001	2.493 ± 0.001	1.224 ± 0.004	2.438 ± 0.001
200	2.982 ± 0.01	2.976 ± 0.01	1.587 ± 0.003	2.897 ± 0.01

All samples showed some degree of reducing power; however, as anticipated, their reducing power was inferior to ascorbic acid, which is known to be a strong reducing agent. Like the DPPH assay the reducing power of extracts increased with increasing amount of extracts; the equation of reducing power (y) and amount of leaf (x) was y = 0.00312x + 0.080272, (r^2 = 0.99472), raw coffee y = 0.00273x + 0.01725, (r^2 = 0.99272); and roasted coffee, y = 0.0022x + 0.0022, (r^2 = 0.95586); indicating that reducing ability correlated well with amount of coffee extracts. The reducing power of coffee extracts and ascorbic acid followed the following order: Ascorbic acid > leaf > green/raw > roasted (Figure 4b). All extracts exhibited significant difference ($p < 0.05$) in antioxidant potential.

Analysis of variance showed that methanolic extracts of green (raw) beans had significant stronger ($p < 0.05$) reducing power than roasted coffee extracts. This could be due to the high amount of total phenolics(Masek et al., 2020) present in green extracts, compared to roasted extracts. The amount of phenolic compounds in green/raw coffee extracts was higher than in roasted coffee extracts(Masek et al., 2020).

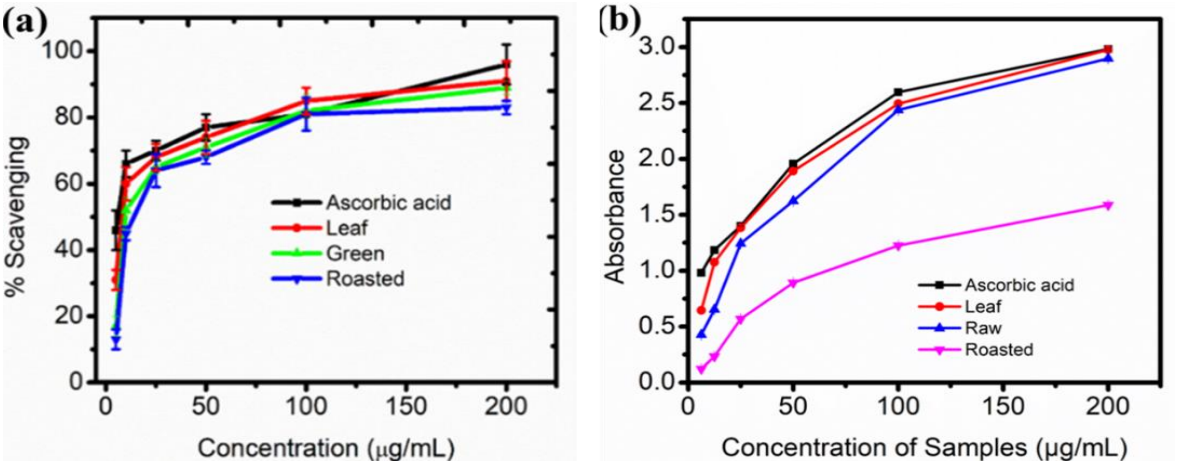

Figure 4. Antioxidant activities of coffee extracts (leaf, green/raw and roasted beans) by (a) DPPH radical scavenging assay and (b) reducing power assay.

On the hand methanolic extracts of leaves of coffee showed the highest antioxidant capacity when determined by the DPPH and reducing assays, while roasted coffee extracts showed the lowest antioxidant activity. Based on the antioxidant assays, it is thus suggested that phenolic compounds present in leaves of coffee extracts have strong scavenging ability and ferric reducing power rather than products of melanoid reactions during roasting process. This could be due to the antioxidant mechanisms of phenolic compounds towards free radicals. Beside phenolic compounds, the presence of methyl xanthine (theobromine and caffeine in green/raw coffee beans might influence the antioxidant capacity(Masek et al., 2020).

For the lowest antioxidant activities of roasted coffee beans, during coffee roasting process, majority of phenolic compounds are destroyed or they may react with free radicals from the Maillard reaction and be incorporated in browning products. But, the significantly higher scavenging properties of roasted coffee brews can be attributed to the Maillard reaction products (MRPs), especially melanoidins formed during the thermal treatment, although the remaining caffeic and chlorogenic acids and/or their degradation products may exhibit antioxidant capacity as well(Liu & Kitts, 2011).The highest values of the antioxidants may be due to the existence of abundant bioactive phytochemicals in coffee leaves.

Factors that may contribute to the antioxidant activities of different coffee samples and thus to the apparent variations between findings include country of origin, degree of roasting, processing method,

altitude at which the coffee plant was grown, soil type, average temperature, and number of sunny days per year, among others (Tasew et al., 2020). Roasting adds some antioxidant components like melanoidins due to Maillard reaction (Liu & Kitts, 2011). Similarly with this study, other highly antioxidant activities are reported to be found in larger amount in

Antibacterial activities of leaves extracts of coffee

The present study was carried out to screen and evaluate antimicrobial activity of methanol extracts of different segments of coffee. Methanol extract of parts coffee were tested against negative Gram bacteria; Escherichia coli and positive Gram; Staphylococcus aureus, which are known to be resistant to various antibiotics. The inhibitory activity of leaf, green/raw and roasted coffee was evaluated using disc diffusion method. As shown in in Table 2, the segments of coffee samples exhibited antibacterial activity against both gram positive and gram-negative bacteria. The formation of clear zone around the paper disc has shown the presence of antibacterial activity of all coffee extracts. All the coffee extracts were more effective against the Gram-positive strains (S. aureus) than the Gram-negative bacteria (E. coli). This is consistent with previous studies reporting that due to their outer lipopolysaccharide membrane (Liu & Kitts, 2011; Yashin et al., 2013).

The result revealed that roasted coffee extract showed the best activity for inhibiting the growth of both bacteria next to the positive control (gentamycin). However, the leaf of coffee extract appeared to possess weak antibacterial activity (inhibition zone ranged from 3 $\pm$ 0.87 to 11 $\pm$ 1.12 against S.aurus and 3 $\pm$ 0.87 to 4 $\pm$ 0.89 mm against E.coil). While the roasted coffee possessed the highest inhibition zone against both positive S.aurus (6 $\pm$ 1.23 to 21$\pm$1.42) and negative bacteria E.coil (4 $\pm$ 0.98 to 8 $\pm$ 0.97) for the same concentrations (25 to 200 µg/mL) On the other hand, green coffee showed moderate antibacterial activity against both gram-positive bacteria and gram-negative bacteria. There were, however, significant differences among the different coffee samples in their antibacterial activity. As shown in Table 2, the size of inhibition zone grew with the increase of the extract concentration.

The differences in antibacterial and antioxidant activities with the reported one may be attributed to different the chemical composition present in leaves, green and roasted coffee segments of the plant(Mesfin, Admassu, Gobena, & Belayneh, 2022). The findings of this work

shows that antibacterial activity may not likely to be associated with antioxidant activity. For the highest antibacterial activities of roasted coffee, miliard reactions might attributes with the formation of different compounds. It has been reported that roasted coffee have potent antibacterial effects on various bacteria including B. subtilis and S. aureus (Taguri et al., 2006; Rodríguez et al., 2009). Hou and colleagues have reported the antibacterial activity of green and roasted coffee, against S. aureus, indicating that the observed activity of our extract could be due to its richness in melanoids resulted from Milliard reactions(Boulekbache-Makhlouf et al., 2010).

Finally, it can be concluded that the active chemical compounds present in leaves, green and roasted coffee should certainly find a place in the treatment of various bacterial infections (Tasew et al., 2020). The results from the present study are very encouraging and indicate that various segments from the same species coffee should be studied more extensively to explore its potential in the treatment of infectious diseases as well.

Table 2. Diameter of the Inhibition Zone obtained with segments Yirgacheffe coffee methanol extracts against S.aureus and E. coli.

Bacteria	Concentration(µg/mL)	Inhibition zone (mm)		
		Leaf	Green /raw	Roasted
S. aureus	200	11 + 1.12	13 + 0.98	21 + 1.42
	100	12 + 1.12	14 + 0.98	20 + 1.42
	50	5 + 0.97	6 + 1.23	8 + 1.12
	25	3 + 0.87	3 + 0.59	6 + 1.23
	Gentamycin(10 µg)	24.34		
E. Coli	200	4 + 0.89	7 + 1.11	8 + 0.97
	100	4 + 0.87	6 + 1.21	7 + 0.89
	50	3 + 0.75	4 + 0.45	6 + 0.54
	25	2 + 0.65	3 + 1.01	4 + 0.98
	Gentamycin (10 µg)	22 + 1.21		
DMSO	-	-	-	-

Conclusion

The antioxidant activity of Yirgacheffe coffee components was assessed in leaf, green, and roasted coffee bean extracts using 1, 1-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and reducing powers assays. In a series of in vitro tests, segments of coffee (leaf, green and roasted bean) exhibited strong antioxidant activity. Nevertheless, the leaves of coffee samples showed higher antioxidant content compared to green and roasted coffee samples taken from the same plant. The differences in the levels of antioxidant activity among the leaves, green and roasted bean extracts extracted from the same variety were noted. The observed differences in the antioxidant activity between the different segments coffee may be attributed to their varying polyphenolic content, milliard reaction products and composition of the coffee segments. The segments of coffee with the various antioxidant contents were evaluated in vitro for antibacterial activities using disc diffusion method. The coffee leaf samples demonstrated a significantly lower antibacterial activity than green and roasted coffee against both the test bacteria. Nevertheless, the leaf of coffee samples showed the higher activity against S.aurus than E. coli. The underlying antimicrobial and antioxidant mechanisms of the leaves of coffee as well as their active components need to be further studied and clarified. Additional in vivo studies and clinical trials would be needed to justify and further evaluate the potential of this leaves of as an antibacterial agent.

Conflict of interest

The authors have no conflicts of interest to declare.

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