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Determination of total phenolic content and antioxidant activities of five different brands of Ethiopian coffee

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ABSTRACT

High consumption of coffee has been considered to have negative health consequences, often attributed to the stimulant effects of caffeine. However, coffee is also one of the largest sources of antioxidants in the diet and contains various compounds with potential beneficial effects on glucose metabolism, inflammation and blood vessel function. The present work was carried out to determine the total phenolic content and antioxidant activity of five different Ethiopian coffee varieties, namely Sidamo, Yirgacheffe, Harare, Wollega and Jimma coffee. Total phenolic content was determined by the Folin-Ciocalteu method and expressed as Gallic acid equivalents whereas antioxidant activities were determined by 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging, reducing power and total antioxidant activity. The tested coffee beans contained appreciable amounts of total phenolic contents (54.87-80.51 g GAE /g); DPPH Scavenging capacity (73.33-84.16%), reducing power (0.634 ± 0.62 - 0.887 ± 0.14) and total antioxidant activity (0.198-0.346) at 0.2 mg/mL extract concentration. The study showed that total phenolic content and antioxidant activities of the coffee extracts were capable of protecting against free radical mediated damage and may have applications in preventing and curing various diseases.

Keywords: Branded Ethiopian Coffee, Antioxidant, DPPH radical scavenging, phenolic content

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1. Introduction

In living systems, free radicals are produced as part of normal metabolic processes and by exposure to ionizing radiations and (Andreyev *et al*., 2005; Valko *et al*., 2006) other environmental pollutants. Oxidative stress, which represents a consequence of an imbalance between the production of free radicals and the body's ability to defend against them, has been implicated in the development of many diseases (Hallowell, 2007). It has been suggested that an initial cause of dangerous diseases such as cancer, Parkinson's, diabetes, premature aging, Alzheimer's, ischemia injury and inflammation are free radical attack on biomolecules (Yashin *et al*., 2011, Mohammed *et al*., 2013).

Antioxidants are compounds that protect the harmful action of free radicals by scavenging the free radicals, primarily highly reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Mohammed *et al*., 2013). These harmful effects of free radicals and oxidative stress can be reduced by regular consumption of foods and beverages which exhibit antioxidant activity.

Coffee is one of excellent sources of bioactive compounds with antioxidant activity such as caff eic, chlorogenic, coumaric, ferrulic and sinapic acids (Farah et al., 2006; O'Keefe et al., 2013) and Melanoidins (brown pigments) which are synthesized during the roasting process of coffee. Additionally, because of its high content of chlorogenic acids, studies performed in Denmark, United States, Japan and Brazil have reported that coffee is the most important contributor to antioxidant intake in their diets (Torres and Farah, 2010). Although antioxidant activities of different geographical locations in which coffee varieties are grown have been demonstrated recently (Jayaprakasha and Jaganmohan, 2000; Złotek et al.,2016), the antioxidant properties of Ethiopian coffee are poorly known and few reports on the relationship between total phenolic content and antioxidant activity is available. Therefore, the aim of the present investigation was to determine the total phenolic contents and antioxidant activity of five brands of Ethiopian coffee extracts.

2. Materials and Methods

2.1 Chemicals and Reagents

In this study, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), potassium ferricyanide ($K_3[Fe(CN)_6]$), trichloroacetic acid ($C_2HCl_3O_2$), Gallic acid, Ethanol, Folin-Ciocalteu phenol reagent and ferric chloride ($FeCl_3$) were purchased from Sigma Aldrich. All other chemicals used were of analytical grade and obtained from standard chemical suppliers.

2.2 Sample Collection

Coffee beans (Arabica) of five brands of Ethiopian coffee namely; Wolega, Sidama, Yirgachefe Harer and Jimma coffee were roasted in the same ways collected from the respective Ethiopia commodity exchange (ECX) office and used for the analysis.

2.3 Preparation of extracts

Coffee extracts were prepared according to the method of (Budryn et. al., 2009) with some modifications. The roasted beans of individual coffee samples were ground using the laboratory hammer mill (High Speed Universal Disintegrator) Grinder Model FW80, FW100) to obtain a particle size of 20 µm. This ground material was then used in the preparation of aqueous and ethanolic extracts. 2.5 gm of each ground roasted coffee was extracted with 250 ml distilled water in a temperature range of 80-90°C for 10 min. For ethanol extract, the same grams of each ground roasted coffee were macerated in 250 ml of ethanol for 24 hours. Thereafter, the sample was decanted and filtered by using filter paper and then supernatants were evaporated under vacuum at 40°C using rotary evaporator. Finally, soft crude extracts were stored in refrigerator at 4°C until further investigation.

2.4 Determination of total phenolic content

The total phenolic content of various brands of coffee samples was estimated by the Folin - Ciocalteu method (Paras and Hardeep, 2010) with little modifications, using Gallic acid as a standard phenolic compound. A 1 ml of Folin-Ciocalteu reagent (diluted ten times) was added to each diluted coffee bean sample followed by the addition of 1 ml Na_2CO_3 (75 g/lit). The absorbance of the resulting blue colour solution was measured at 765 nm using Spectrophotometer (Spectronic 20, Genesys, USA) against blank af-

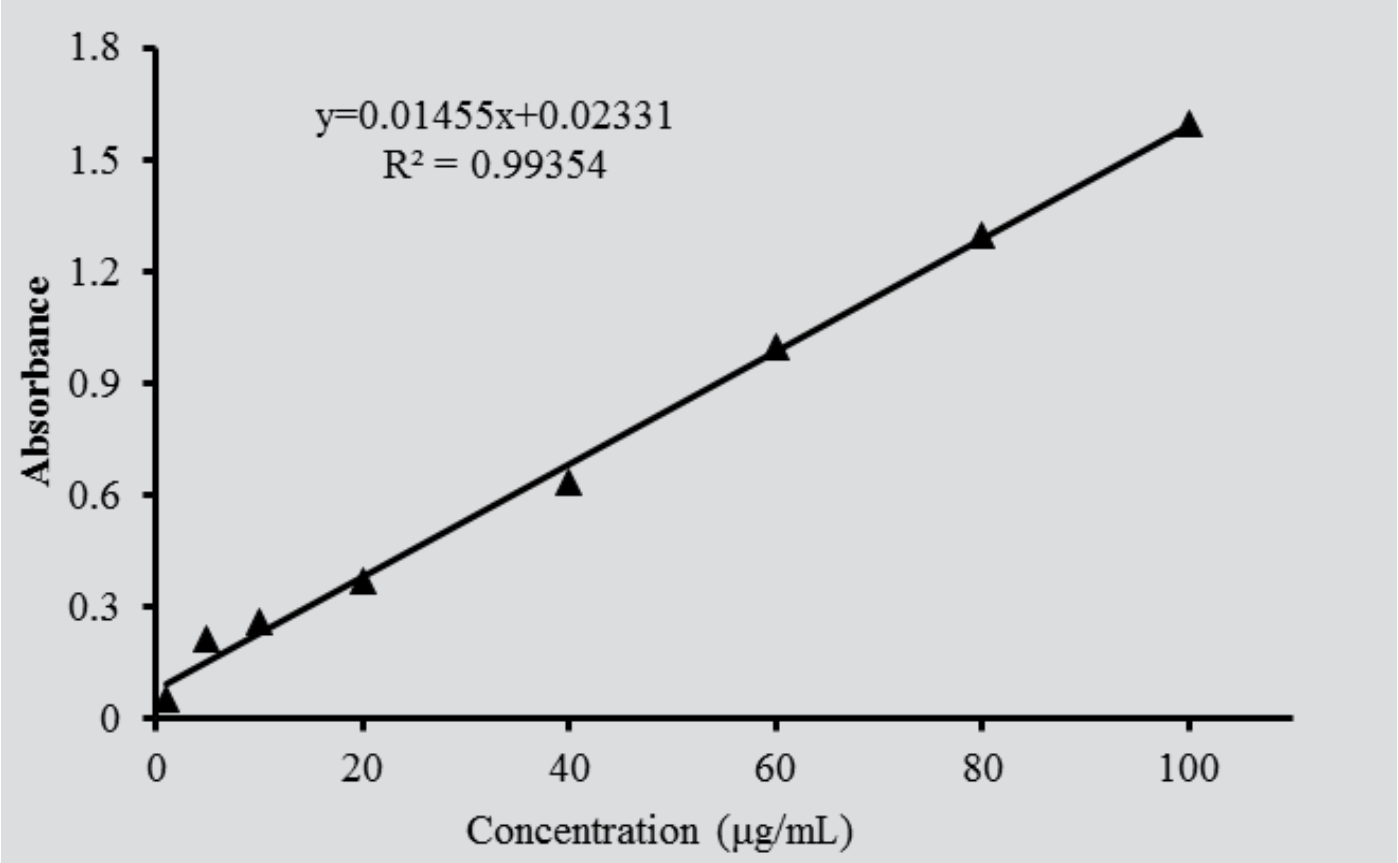

Figure 1. Calibration curve of standard Gallic acid

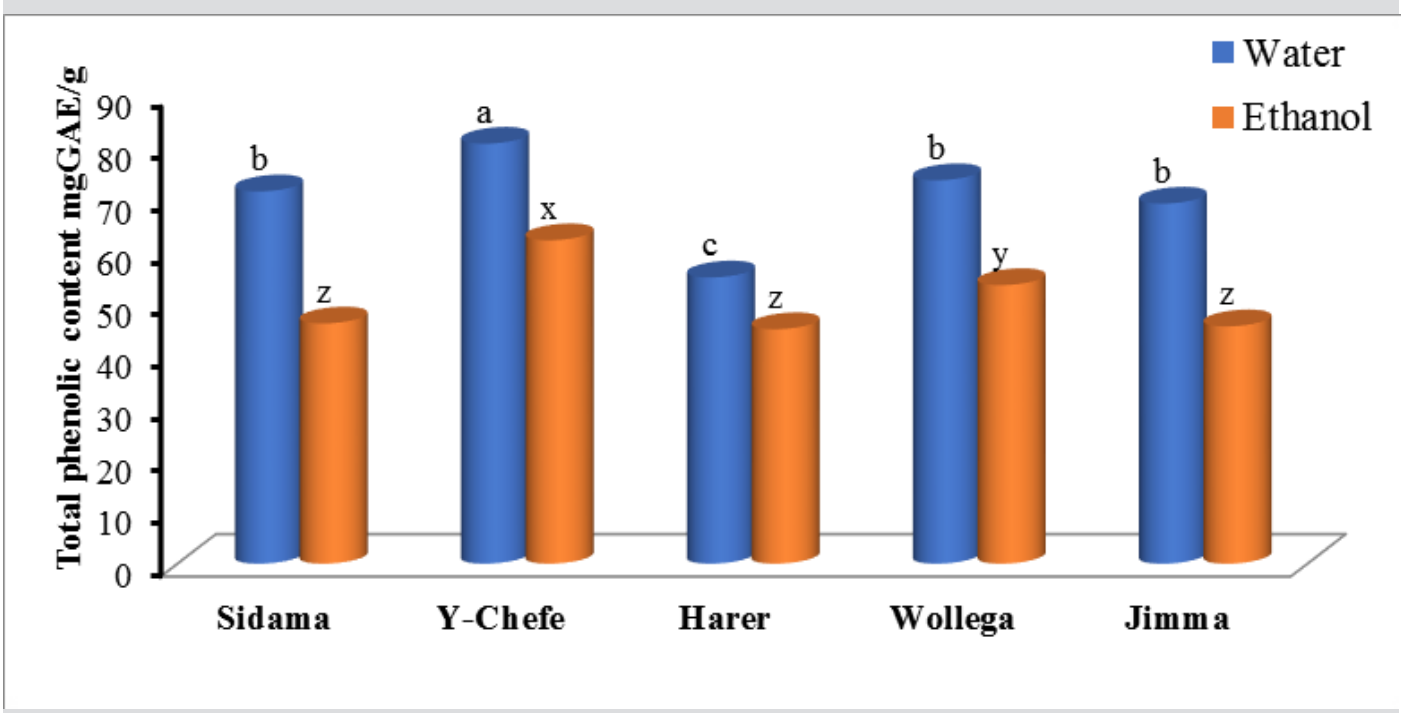

Figure:2. Total phenolic contents of aqueous and ethanol coffee extracts. Concentration sample was 200 mg/mL. Values expressed as means (n =3). Means with different letters (a,b,c for water and x,y,z for ethanol)were significantly different at the level of $p < 0.05$.

ter incubating for 90 min at room temperature. Quantification was done based on a calibration curve of Gallic acid (1-100 µg/mL) **figure 1**. All the experiment was conducted in three replicates and the results was also expressed as gram of gallic acid equivalent (mgGAE) per gm of dried extract using following formula:

C= (CxV)/m

Where C = total phenolic content of compounds, mg/g plant extract, in GAE, c = the concentration of gallic acid established from the calibration curve (mg/mL), V = the volume of extract in mL, and m = the dry weight of plant extract Gallic acid equivalence (GAE) per gram dry coffee beans.

2.5 Determination of Antioxidant Activities

2.5.1 Free Radical Scavenging Activity (DPPH)

The stable DPPH free-radical scavenging activity of the roasted coffee bean extracts was determined as described by (Chan *et al*.,2007) with slight modifications. 2 mL (0.006% in methanol) DPPH solution was mixed with 1 mL of different concentrations of coffee extracts and stirred vigorously for 30 sec. Then the solutions were allowed to stand in a dark place at room temperature for 30 min occurring chemical reactions. After 30 min, absorbance was measured against a blank at 520 nm with UV-Visible spectrophotometer (Spectronic 20, Genesys, USA). Ascorbic acid was used as standard and the solution without any extract, but with DPPH and ethanol was used as a control. DPPH free radical-scavenging activity of plant extract was calculated as:

DPPH free-radical scavenging activity (%) = (Ac-As)/Ac x100

Where, A_c is the absorbance of the control solution (containing all reagents except plant extract); A_s is the absorbance of the DPPH solution containing plant extract. Ascorbic acid was used as positive control standard.

2.5.2 Ferric Ion Reducing Power

Reducing power of the extracts was determined as described by (Ardestani and Yazdanparast , 2007) with slight modification. Different concentrations (50-200 µg/mL) of coffee extracts were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The mixtures were incubated at 50˚C for 20 min. After incubation, 2.5 mL of 10% trichloroacetic acid was added and the mixture then centrifuges (INSIF, India) at 3000 rpm for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and 0.1% ferric chloride (0.5 mL). Then the absorbance of the solution was measured at 700 nm using UV-Visible Spectrophotometer (Spectronic 20, Genesys, USA). The assays were carried out in triplicate and the results were expressed as mean ± standard deviation and ascorbic acid was used as standard.

2.5.3 Total Antioxidant Activity (Phosphomolybdenum Assay)

The total antioxidant capacities of extracts were evaluated using slightly modified phosphomolybdenum method (Matthias *et al*., 2015). The assay was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and subsequent formation of a green phosphate (Mo (V) complex at acid pH. Different concentrations of crude coffee extracts (0.5-1 mg/mL) were prepared in methanol from test stock solution. 3 mL of reagent solution (prepared from 10 mL of 0.6 M sulphuric acid, 10 mL of 28 mM sodium phosphate, and 10 mL of 4 mM ammonium molybdate) was added to all the tubes and incubate at 95^{0}C for 90 min. After cooling the sample to room temperature, the absorbance of the solutions was measured at 695 nm against blank using UV spectrophotometer (Spectronic 20, Genesys, USA). Ascorbic acid was used as reference.

2.6 Statistical Analysis

The sample will be analyzed for the determination of antioxidant activity of different brands of Ethiopian coffee and correlation between antioxidant levels and coffee test/aroma by using UV/Vis Spectrophotometer. All samples were treated in duplicated at least triplicates. Means, standard deviation, or relative standard deviations of all values were calculated. In all cases, standard and blank were treated in the same ways as the real samples to minimize matrix interferences during analysis.

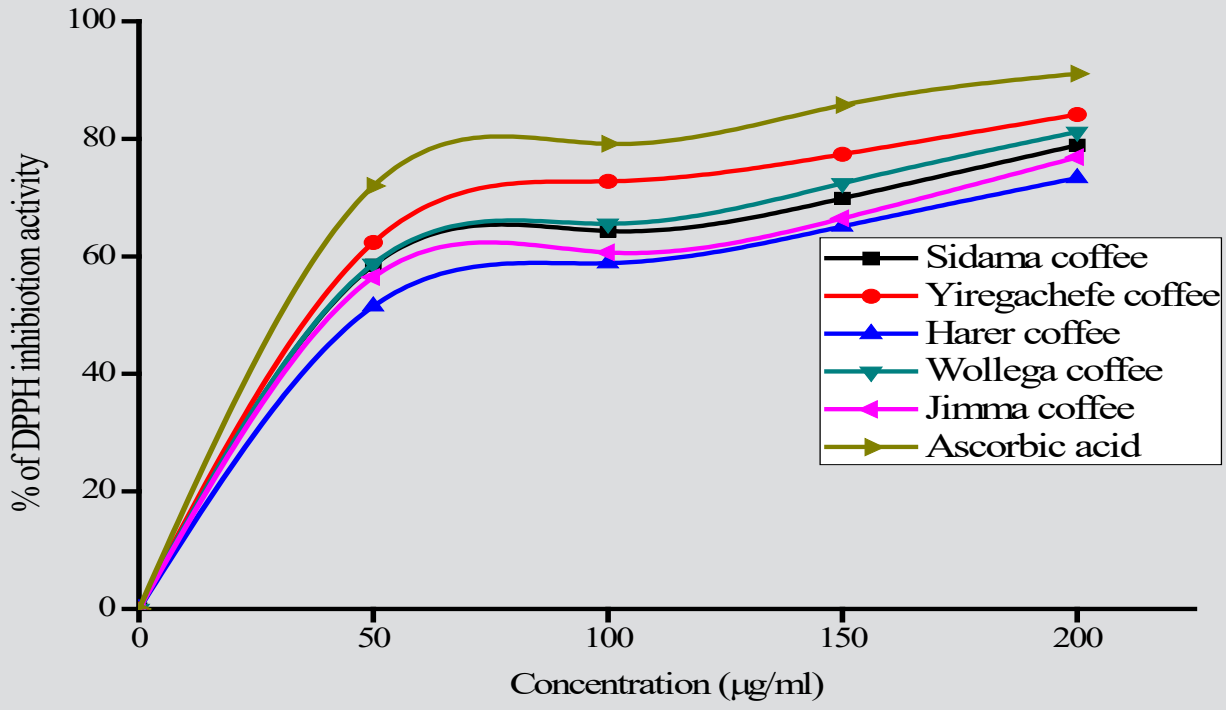

Figure. 3. Scavenging effect of water extract on DPPH radicals. Values are expressed as mean ± standard deviation (n = 3). Ascorbic acid was used as the standard.

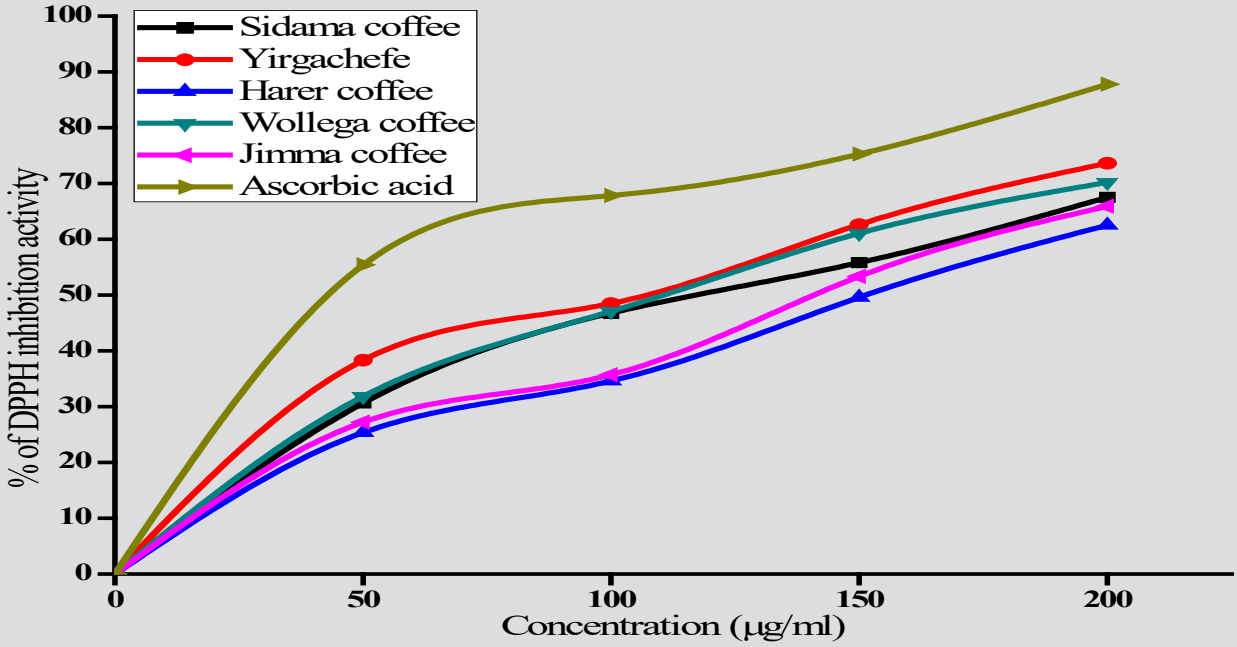

Figure. 4. Scavenging effect of ethanolic extract on DPPH radicals. Values are expressed as mean ± standard deviation (n = 3). Ascorbic acid was used as the standard.

3. Results and Discussion

3.1 Determination of Total Phenolic Content

The colour of Folin-Ciocalteau reagent changes from yellow to blue upon the detection of phenolics in the extracts which is normally due to the chemical reduction of tungsten and molybdenum oxide mixture in the reagent. In the present study, total phenolic content of five branded Ethiopian coffee namely, Sidama, Yirgachefe, Harare,Jimma and Wollega coffee. Yirgachefe coffee extract showed the highest amount of total phenolics content (80.51± 0.32 mg GAE/g, whereas the Wollega, Jimma and Sidama Coffee varieties had almost similar total phenolic contents. However, Harer coffee variety had the lowest total phenolics content (54.87 ± 0.24 mg GAE/g.) among other varieties **figure 2**. The deviation in the total phenolic contents might be attributed to the geographical factors as well as the different cultivation methods.

There were no statistical significant differences ($p > 0.05$) in TPC values observed among Sidama,Wollega and Jimma coffee varieties. and also there were no significant difference ($p < 0.05$) between aqueous and ethanolic extracts in almost all samples. A previous study had reported that phenolic content was influenced by the origin of the coffee beans and extracting solvents (Złotek *et al*., 2016; Sultana *et al*., 2007).

3.2 Determination of Antioxidant Activities

3.2.1 DPPH radical scavenging activity

The proton radical scavenging action is known to be one of the various mechanisms for measuring antioxidant activity. DPPH is one of the compounds that possess a proton free radical and shows a maximum absorption at 517 nm. When DPPH encounters proton radical scavengers, its purple colour fades rapidly. This assay determines the scavenging of stable radical species of DPPH by antioxidants.

As can be seen in **figure 3 and 4**, all the extracts were capable of scavenging DPPH free radicals and the activity were increased with increasing concentration of the samples. Among different coffee extracts, Yirgachefe coffee extracts showed the maximum inhibition activity. At 200 µg/ml, the DPPH free radical scavenging activities of the coffee extracts and the standard ascorbic acid increased in the following order: Harer (64.69 ± 0.26 %) < Jimma (77.23 ± 0.70 %) < Sidama (82.40 ± 0.74 %) < Wollega (83.51 ± 0.46) < Yirgachefe (95.26 ± 0.22 %) < ascorbic acid (97.81± 0.22 %). This difference is strongly related to the phenolic content and also to the type of the active compound present in each variety.

3.2.2 Ferric Ion Reducing Power

Reducing power measures the ability of the extract to donate electron to Fe(III) and it is evaluated by the transformation of Fe^{3+}/ ferricyanide complex to ferrous ions (Fe^{2+}) in the presence of the sample extracts (Gulcin *et al*.,2003). The higher absorbance, the higher the concentration of Fe^{2+} and the higher the ability of that particular extract to donate electrons (Siddhuraju and Becker, 2003).

Reducing power of both aqueous and ethanolic extracts of different brands of Ethiopian coffee and the standard ascorbic acid using the potassium ferricyanide reduction method were described in **figure 5 and 6**. Like the DPPH free radical inhibition activity, the reducing power of all extracts increased with increasing in their concentration. The strongest reducing power was observed in Yiragefe coffee extract and the lowest reducing power exhibited by Harare coffee extract. However, the reduction power of the standard ascorbic acid relatively more pronounced than that of the different extracts of the coffee.

At 200 µg/mL, the reducing power of aqueous extracts decreased in the following order: Yirgachefe (0.887 ± 0.14 nm) > Wollega (0.866 ± 0.37 nm) > Sidama (0.817 ± 0.09 nm) > Jimma (0.753 ± 0.13 nm) > Harare (0.634 ± 0.62 nm). Analysis of variance showed that water extracts of coffee had significantly stronger ($p < 0.05$) reducing power than ethanolic extracts. This could be due to the high amount of total phenolic content in aqueous extracts, compared to ethanolic extracts.

3.2.3 Determination of Total Antioxidant Activity (Phosphomolybdenum Method)

Total antioxidant activity of both aqueous and

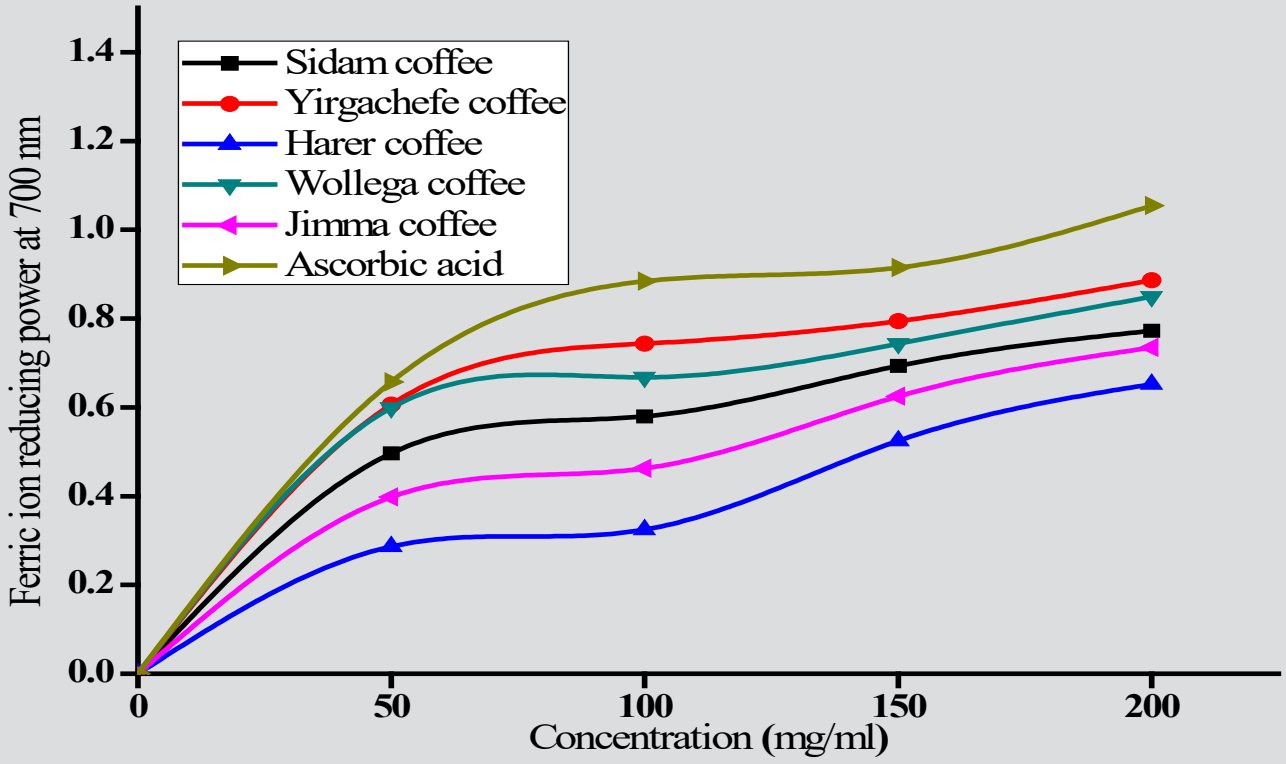

Figure 5. Reducing power of five brands of Ethiopia coffee aqueous extracts and Ascorbic acid. Each value represents the mean ± SD of triplicate experiments.

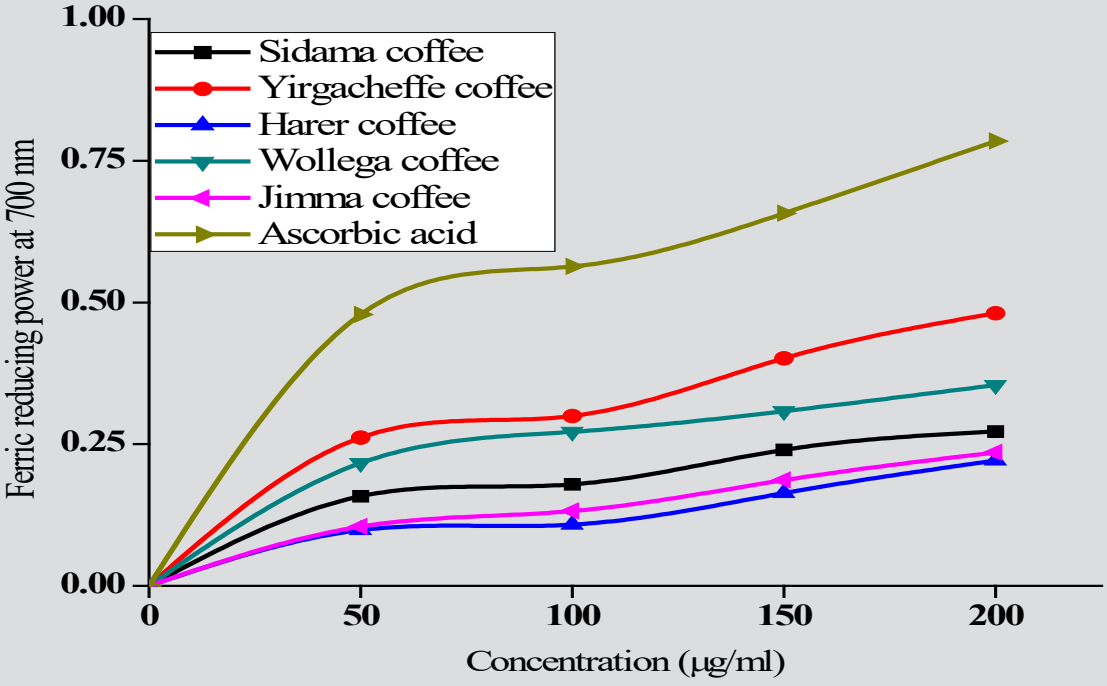

Figure 6. Reducing power of five brands of Ethiopia coffee ethanolic extracts and Ascorbic acid. Each value represents the mean ± SD of triplicates experiments.

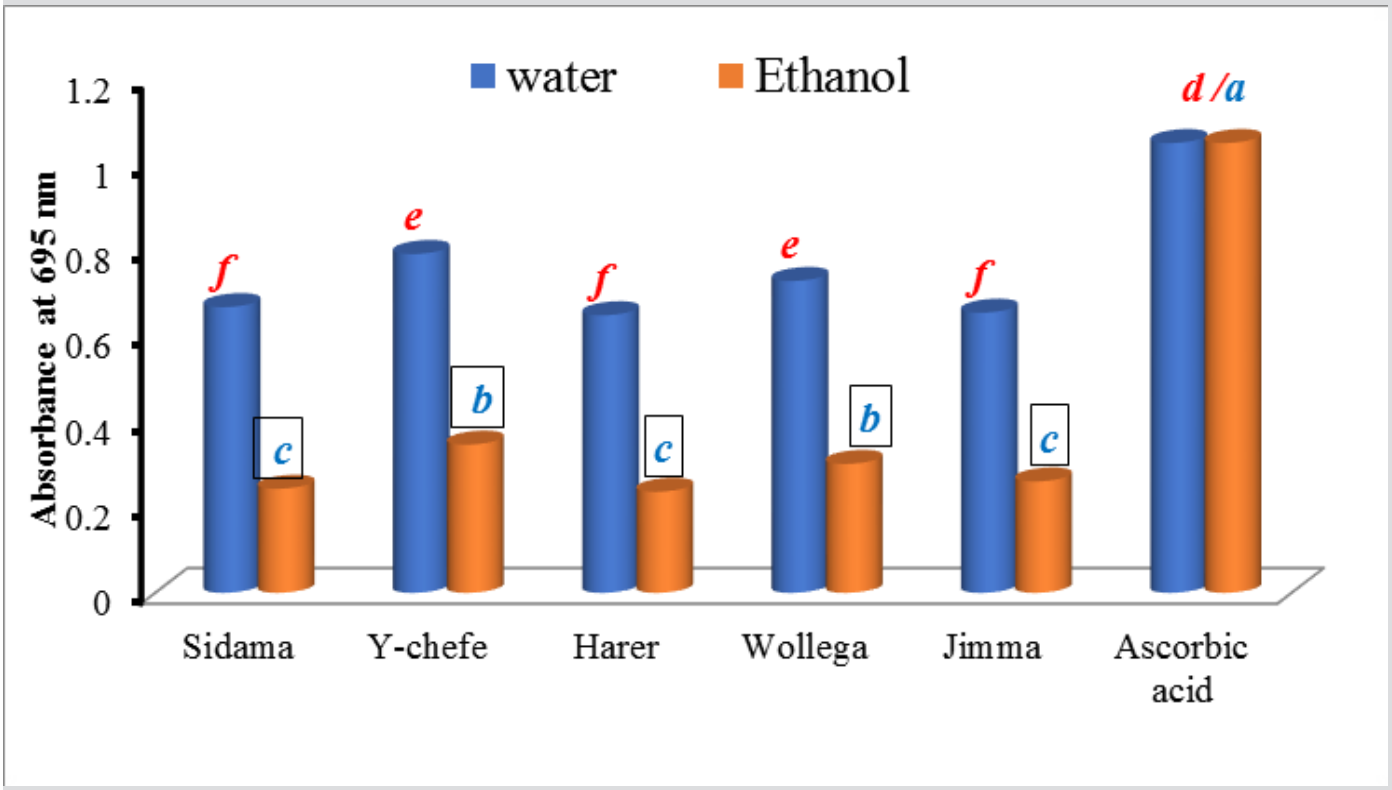

Figure 7. Total antioxidant activity of aqueous and ethanol five brands of Ethiopian coffee extracts. Each value represents the mean ± SD of triplicates experiments.

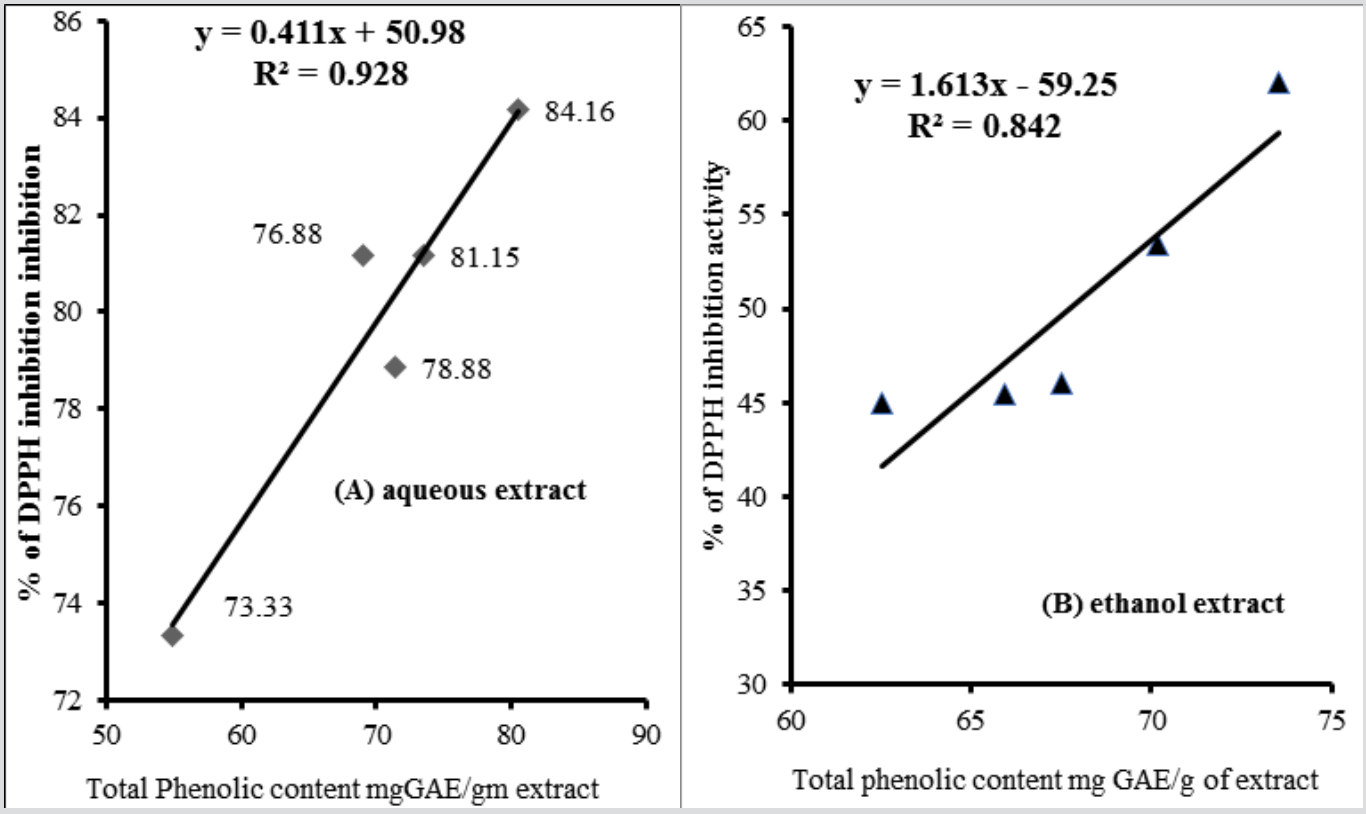

Figure. 8 Correlation between total phenolic content and DPPH scavenging capacity of aqueous (A) and ethanol (B) extract

ethanolic extracts of five brands of Ethiopian coffee was estimated by the phosphomolybdenum complex formation method at acidic medium, which is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/Mo (V) complex with a maximum absorption at 695 nm.

Figure 7 showed that, Yirgachefe coffee showed the highest total antioxidant activity as compare to other extracts but this result was lower than the standard ascorbic acid. At 200 µg/mL, the total antioxidant activity of the aqueous extracts was found to increase in the following order: Harare coffee (0.648 ± 0.13 > Jimma coffee (0.654 ± 0.25 > Sidama coffee (0.667 ± 0.32) > Wollega coffee (0.728 ± 0.19) > Yirgachefe coffee (0.791 ± 0.41). The results also showed that no significant difference ($p > 0.05$) with Harare, Sidama and Jimma coffee but these values were significantly different ($p < 0.05$) from Wollega and Yirgachefe coffee extracts.

3.3 Correlation Analysis

The results presented in this study demonstrated that total phenolics played an important role in the antioxidant activity. Aqueous extracts of coffee showed a positive and very high correlation ($r^2 = 0.928$) between total phenolic content and DPPH free radical scavenging inhibition activity value with a significant difference. Also, a positive correlation ($r^2 = 0.842$) with a significant relationship was found for the ethanolic extract (**Figure 8**). The strong correlation between total phenolic content and DPPH free radical scavenging inhibition activity are in agreement with other reports (Xu *et al*., 2010*;* Cheng *et al*., 2012*).*

The present study also showed a positive and high correlation between total phenolic content and antioxidant potential based on ferric reducing assay with a significant relationship for both water ($r^2 = 0.902$) and ethanolic extracts ($r^2 = 0.800$) (**Figure 9**). This result was in agreement with Othman *et al*. (2007), who found a strong correlation between total phenolic content and ferric reducing power assay.

4. Conclusions

The present study revealed that Ethiopian coffee extracts demonstrated high total phenolic content and potent antioxidant activity, achieved by free radical scavenging and reducing power assays. Based on the results of the present investigation it can be concluded that Yirgachefe and Wollega coffee varieties had the highest antioxidant activities whereas Sidama, Jimma and Harare coffee contained moderately high antioxidant activity. The results obtained also indicated that the use of aqueous solvent gives an effective determination of antioxidant activity. It is evident from present study that the studied coffee samples have a potential source of natural antioxidant that could have great importance as therapeutic agents in the preventing oxidative stresses-related degenerative diseases.

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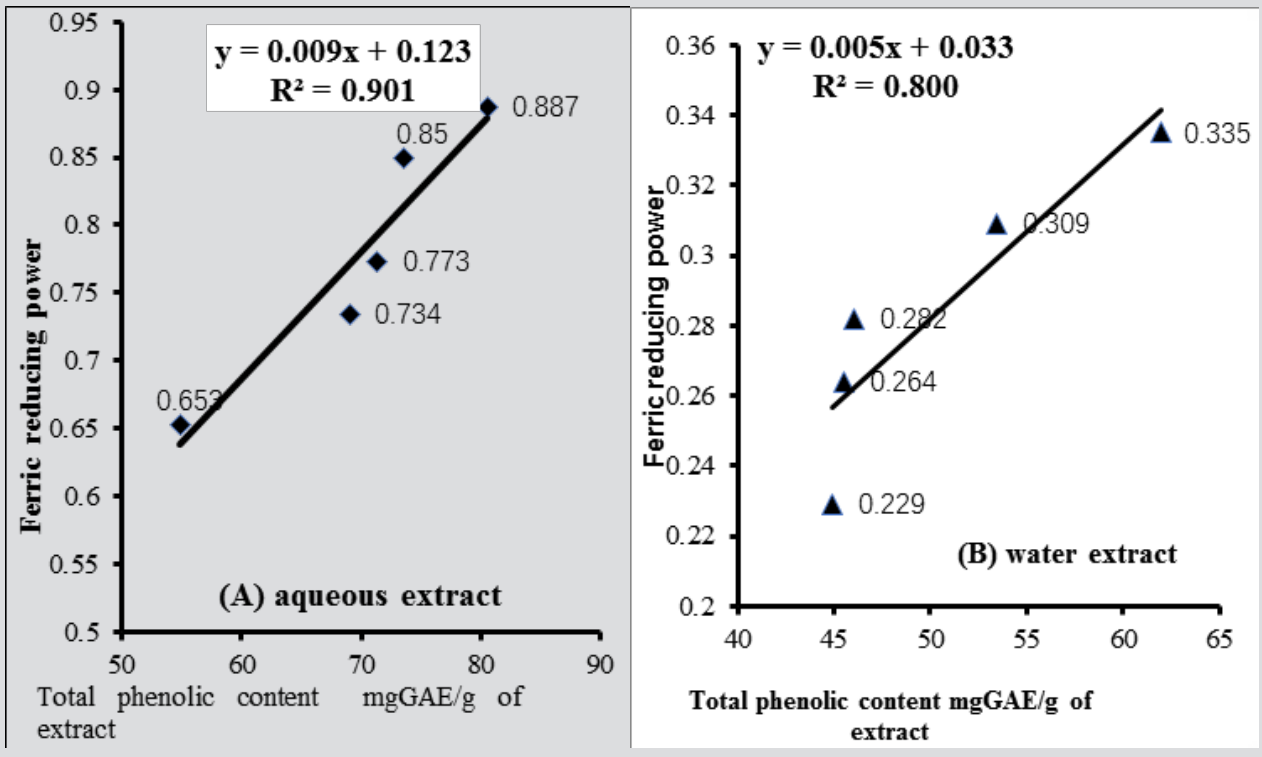

Figure. 9 Correlation between total phenolic content and ferric ion reducing power of aqueous (A) and ethanol (B) extract