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Determination of chlorogenic acid content in beans and leaves of *coffea arabica* using UV/Vis spectrometer

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Chlorogenic acid (CGA) is one of the compounds found in coffee beans and other parts of coffee plant. However, its relative content in different coffee plant parts is least researched. Therefore, this study was designed to determine the contents of CGA in coffee leaves and beans. Green coffee beans and leaves were collected from South part of Ethiopia to study the content of CGA using UV/Vis spectrometer with liquid-liquid extraction method in dichloromethane. Results indicated that mean percentage of CGA in green coffee beans and leaves are in the range of 5.96±0.01 to 6.40±0.02 % and 1.94±0.01 to 2.31±0.01%, respectively. On top of that, the percentage difference of CGA between green coffee beans and leaves was at least about 63%, with beans taking the upper hands. These results showed that there is statistically significant content of CGA in green coffee beans than green coffee leaves.

Keywords: CGA, *Coffea arabica*, coffee leaves, concentration, green coffee beans.

INTRODUCTION

Coffee is one of the most widely consumed beverages throughout the world due to its pleasant taste, aroma, stimulant effect and health benefits (Gebeyehu and Bikila, 2015). Coffee plant is categorized among the medicinal plants because studies on beans and fleshy organs of the coffee plant including leaves have revealed that these organs are found to have generous amounts of secondary metabolites such as phenolic compounds, esters of hydroxycinamic acids and mangiferin which have high level of antioxidant properties and anti-inflammatory effects on humans (Campa et al., 2012).

Chlorogenic acid (CGA) is one of the compounds found in coffee beans and other parts of coffee plant. CGA is the main phenolic compound in coffee beans and its concentration is the highest in coffee beans than other coffee parts (Farah et al., 2005). Chlorogenic acids are esters of quinic acid and various class of hydroxycinnamic acids, chiefly caffeic acid (3, 4-dihydroxycinnamic acid), ferulic acid (3-methoxy-4-hydroxycinnamic acid), p-coumaric (4-hydroxycinnamic acid), and sinapic acid (3, 5-dimethoxy-4-hydroxycinnamic acid) (Manach et al., 2004). CGA comprises major class of phenolic compounds. The most abundant are the caffeoylquinic acids (Clifford et al., 2003). They account for approximately 80% of the total chlorogenic acid content (Farah et al., 2005). The total CGA content of green coffee beans varies according to genetic species, degree of maturation and less importantly agricultural practices,

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climate and soil (Farah et al., 2006).

Most of the researches have targeted their journey at studying *Coffea arabica* and *Coffea canephora*. Studies have revealed that the percentage of CGA for green coffee beans on the dry matter varies from 4 to 8.4% for *C. arabica* and 7 to 14.4% for *C. canephora* with some hybrids containing intermediate levels (Farah et al., 2005). Campa et al. (2005) have reported that the CGA level of green coffee beans from Cameroon and Congo is in the range of 0.8 to 11.9% on dry matter basis. However, as reported by Farah et al. (2006b) and Ky et al. (2001), the level of CGA in green coffee beans is about 4 to 8.4% for *C. arabica*. In addition, Perrone et al. (2008) have reported a similar result with a total CGA content of 6.3 and 5.5 g/100 g for *C. arabica* using LC-MS. Budiman et al. (2017) have reported that CGA concentration of Arabica coffee before and after decaffeination has been measured to be 4.16±0.16 and 3.02±0.14, respectively. As reported by hall et al. (2018), decaffeinated coffee beans contain 13,005.32 to 46,048.63 mg/kg while caffeinated one possesses 30,171.02 to 49,488.03 mg/kg of CGA content.

Ethiopia is the mother and diversification land of *C. arabica* (Mekuria et al., 2004) with Oromia and Southern Nations, Nationalities and Peoples' Regions are the leading producers. Report from West Ethiopia by Belay and Gholap have indicated that the percentage of chlorogenic acid determined by UV/Vis spectrometry is from (6.05± 0.33) - (6.25±0.23) % (Belay and Gholap, 2009). HPLC analysis by Ayelign and Sabally (2013) has unveiled that the CGA concentration of coffee beans collected from different regions of Ethioipa is in the range of 0.981 to 46.155 mg/g. Although chlorogenic acid is found in coffee leaves, researches tilted to coffee beans (Claudine et al., 2012). However, Kristiningrum (2015) reported that concentration percentage of CGA in old and young coffee leaves of C. arabica are 2.79±1.87 and 1.89±2.15, respectively while for *C. canephora* are 1.46±0.83 and 1.05±1.19, respectively.

The most biologically active compounds exist either in coffee beans or coffee leaves are caffeine and chlorogenic acids (Farah et al., 2006b; Rodrigues et al., 2013). Consumption of coffee beans is common in most part of the world (Pohl et al., 2013). In Ethiopia, besides of coffee beans consumption, coffee leaves have been consumed in different parts of Ethiopia for different reasons since the early times. However, the indigenous knowledge community about leaves is not the same as for beans. Inhabitants of Wolaita in Southern part of Ethiopia cook leaves at matured growing stage to prepare beverages like 'Hytatuke' and drink it with some other spices for usual drink as well as for medicinal purposes. In contrast to the considerable amount of study on green beans, there are relatively few studies concerned with the metabolite content of other parts of coffee plant, such as leaves, the outer fleshy layers of the and other organs. The leaves from the coffee plant which are suspected to have high medical effects are being undermined by many people. Therefore, this was designed to determine contents of bioactive compound CGA in matured growing stage coffee leaves and green coffee beans for their comparisons.

MATERIALS AND METHODS

Data collection technique

Green coffee beans and matured coffee leaves were taken from the same branches. Simultaneously one coffee bean and coffee leave samples were collected from each woreda. Samples were collected from Southern part of Ethiopia, Hadiya zone, of five different major coffee growing woredas, specifically, Gibe, Soro, Gombora, Misrak Bedawachew and Mirab Bedawachew. In total, ten samples (five for leaves and five for beans) were collected and analyzed. The coffee samples were collected from the model farmers of the selected woredas by considering their productivity without considering their varieties.

Standard solution preparation

For the standard solutions preparation, a commercially bought pure CGA (Aldrich-Sigma, Germany) of 1000 mg was accurately weighted and dissolved in one liter of de-ionozed water to prepare stock standard CGA solution. The solution was uniformly dissolved using magnetic stirrer in dark room to avoid light interaction. Series of standard solutions were prepared from the stock solution (5, 10, 15, 20 and 25) mgL^{-1} for CGA in de-ionized water and all measurements were carried out in short period of time after preparation and absorbance of each series was measured immediately. The series solutions were prepared for method validation against Beer-Lambert's law.

Sample preparations

To prepare CGA samples, the same sample preparation method has been followed for both green coffee beans and coffee leaves. Each sample of green coffee beans and room temperature dried coffee leaves were ground and sieved through 500 µm sieve to get a uniform texture. Accurately weighed 40 mg amount of sieved coffee was dissolved in de-ionized water in a volumetric flask up mark of 30 mL. The solutions were stirred for half an hour using magnetic stirrer and heated gently to increase the solubility of CGA in solution. In addition the solutions were filtered through glass filter to get rid of particles from solution. After filtration, extraction of CGA was done by following liquid-liquid method (Belay et al., 2008).

Liquid-Liquid extraction and absorption measurement procedures

Dichloromethane liquid-liquid extraction was deployed in order to avoid caffeine and CGA spectral overlapping in 200 to 500 nm wavelength range. The same procedure was followed for both beans and leaves samples. The procedure developed by Belay et al. (2008) was deployed to extract caffeine from the solution. Sample solutions prepared above (30 mL solution) were mixed with 30 mL dichloromethane giving total of 60 mL solution of samples. The solution was stirred for 10 min where a layer was formed with

Table 1. Mean concentration of CGA content in green coffee beans (n = 3)**.**

Woredas	Total sample volume (mL)	Measured sample volume (mL)	[a]CGA content (mg)	CGA content (mg/g)	[b]CGA content (% w/w)
Gombora	60	28	2.48	62.10±0.13^{D}	6.21±0.01
Gibe	60	28	2.38	59.58±0.12^{E}	5.96±0.01
Misrak Bedawacho	60	28	2.53	63.25±0.09^{B}	6.33±0.01
Mirab Bedawacho	60	28	2.49	62.33±0.15^{C}	6.233±0.02
Soro	60	28	2.56	64.04±0.17^{A}	6.40±0.02

Means with the same letter in the fifth column are not statistically significantly different.
[a]Equation (1) was used.
[b]Equation (2) was used.

caffeine making upper layer and CGA making the lower layer. Caffeine was extracted from coffee solution. After caffeine extraction from the solution the remaining residue of CGA containing solution of the sample were collected and measured to get measured volume (28 mL) of the sample. This process was repeated three times to exhaustively extract caffeine. Either of the extraction of beans or leaves, samples at each round were stored in separate volumetric flasks. All glass wares and curette were thoroughly cleaned, rinsed with de-ionized water and dried before use. From the residue of CGA collected and measured, absorption of CGA were measured using double beam UV/Vis spectrophotometer (spectral 50 analytic Jenna, Germany) with wavelength ranges of 190 to 1100 nm from which CGA concentration were calculated against the standard solution by Beer Lambert's Law at the maximum wavelength. The same extraction procedure was repeated for all the five areas samples for both beans and leaves of the coffee.

Determination of CGA content

Once the CGA concentration was calculated from the absorbance of the measured sample solution through Beer Lambert's law at maximum wavelength, the CGA contents in coffee beans as well as coffee leaves were calculated using Equation 1 (Zewdu et al., 2016).

$$\text{CGA content (mg)} = \frac{\text{conc}\left(\text{mg L}^{-1}\right) * (\text{total sample volume (mL)})^2}{\text{measured sample volume (mL)} * 1000} \quad (1)$$

The percentages of CGA content in the samples analyzed were calculated through Equation 2.

$$\text{Percentage of CGA (w/w \%)} = \frac{\text{calculated mass of CGA (mg)}}{\text{mass of coffee sample measured (mg)}} * 100\ \% \quad (2)$$

Statistical analysis

Data entry management and preliminary summaries were done on Microsoft Office Excel spread sheet.. Means of data obtained from quantitative measurement of spectrophotometer were determined. One-way analysis of variance (ANOVA) at $p<0.05$ was used to determine statistically significant differences in the mean concentrations of CGA in leaves and beans as well as across study areas. For comparison of the means, the Fisher's least significant difference (LSD) test was used to check the significance level. Data were presented in mean ± (SD) as well as in percentages ± (SD).

RESULTS

Validation of the method was carried out in the linearity property of Beer-Lambert's law from calibration graph correlating the absorption intensity with the corresponding concentration which was constructed for CGA at the highest peak of intensity. The calibration curve facilitated measurement of the content of CGA and validation of the method is displayed in Figure 1. The calibration equation is (Y = 0.028 + 0.013X, R = 0.998, S.D = 0.073%, N = 5) where Y, represents the peak height at maximum wavelength and X is concentration in mgL^{-1}.

In this research, the percentage of chlorogenic acid determined at maximum wavelength of 324 nm in various green coffee beans collected from five district woredas of Hadiya zone are presented in Table 1. The results in percentage from UV-Vis spectroscopy method range from 5.96±0.01 to 6.40±0.02 %. Relatively the highest percentage of CGA was observed in Soro while the lower one goes to Gibe study area. The average percentage of the five distinctive areas of study was 6.23%. The CGA concentrations of green coffee beans were statistically different when considered in all the study areas.

In the same way, results obtained in this work for green coffee leaves are presented in Table 2.. Maximum absorbance was obtained at 322 nm wavelength.

In this study, the percentage of CGA contents in coffee leaves is ranged from 1.94±0.01 to 2.31±0.01%. The highest concentration was found in Gombora while the lowest was identified in Gibe. The average concentration of CGA in coffee leave samples in all the five district areas of study in this Zone was found to be 2.05%.

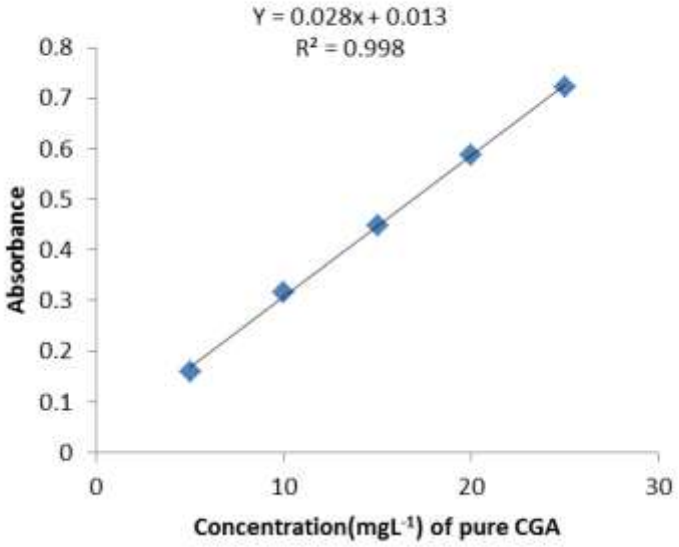

Figure 1. Absorbance versus concentration of pure CGA.

Table 1. Mean concentration of CGA content in green coffee beans (n = 3)**.**

Woredas	Total sample volume (mL)	Measured sample volume (mL)	[a]CGA content (mg)	CGA content (mg/g)	[b]CGA content (% w/w)
Gombora	60	28	2.48	62.10±0.13^{D}	6.21±0.01
Gibe	60	28	2.38	59.58±0.12^{E}	5.96±0.01
Misrak Bedawacho	60	28	2.53	63.25±0.09^{B}	6.33±0.01
Mirab Bedawacho	60	28	2.49	62.33±0.15^{C}	6.233±0.02
Soro	60	28	2.56	64.04±0.17^{A}	6.40±0.02

Means with the same letter in the fifth column are not statistically significantly different.
[a]Equation (1) was used.
[b]Equation (2) was used.

Table 2. Mean concentration of CGA in green coffee leaves (n = 3).

Woredas	Total Sample volume(mL)	Measured Sample Volume(mL)	[a]CGA content (mg)	CGA content (mg/g)	[b]CGA content (% w/w)
Gombora	60	28	0.92	23.08±0.10^{A}	2.31±0.01
Gibe	60	28	0.78	19.40±0.11^{E}	1.94±0.01
Misrak Bedawacho	60	28	0.79	19.75±0.09^{C}	1.98±0.01
Mirab Bedawacho	60	28	0.78	19.51±0.08^{D}	1.95±0.01
Soro	60	28	0.82	20.55±0.14^{B}	2.06±0.01

Means with the same letter in the fifth column are not statistically significantly different.
[a]Equation (1) was used.
[b]Equation (2) was used.

The analysis of one way ANOVA indicated that CGA concentration of all the studied areas were statistically significant.

CGA contents in green coffee beans and leaves were measured by following the same procedures and by using the same computational and experimental methods. However, as can be seen from Tables 1 and 2, the contents of CGA in coffee beans and coffee leaves are significantly different. The measured CGA contents in each coffee bean samples were greater than that of

Table 3. Percentage difference of CGA between green coffee beans and leaves (n = 3).

Woredas	CGA content in coffee beans (% w/w)	CGA content in coffee leaves (% w/w)	CGA difference in beans and leaves in percentage
Gombora	6.21±0.01	2.31±0.01	62.85±0.96
Gibe	5.96±0.01	1.94±0.01	67.44±1.03
Misrak Bedawacho	6.33±0.01	1.96±0.01	68.78±1.36
Mirab Bedawacho	6.23±0.02	1.95±0.01	68.68±0.88
Soro	6.40±0.02	2.06±0.01	67.91±1.33

coffee leave samples.

As can be seen from Table 3, the percentage difference of CGA in green coffee beans and leaves in each study area signifies that green coffee beans carry high content of CGA. CGA in green coffee beans was at least 62.85% higher than its counterpart CGA in leaves. Table 3 displays percentage difference of CGA between each green coffee bean and coffee leave samples.

DISCUSSION

Percentage concentration of chlorogenic acid in this work for green coffee beans ranged from 5.96±0.012 to 6.40±0.02 % with average of 6.23%. The level of chlorogenic acid in green coffee beans reported by (Farah et al., 2006b; Ky et al., 2001) were about 4 to 8.4% for Arabica coffee which agrees with results of this work. CAG concentration found in this work also fits in the rage of results obtained in Cameroon and Congo (0.8 to 11.9%) (Campa et al, 2005). According to Belay and Gholap (2009), the percentage of CGA in various green coffee beans collected from south west of Ethiopia is in the range of ((6.05± 0.33 to 6.25±0.23) %) which agrees with the results of this work. However CGA results found in this work were found to be slightly lower in the upper range than the one found by Ky et al. (2001) for Arabic coffee (3.5 to 7.5%).

Moreover, concentration of chlorogenic acid in green coffee leaves in matured ages in this study ranged from 1.94±0.01 to 2.317±0.01% with average percentage of 2.05%. Results of the study by Kristiningrum (2015) by validated TLC-Densitometry method showed that CGA concentration percentage of old (aged) and young leaves of Arabica coffee were 2.79±1.87% and 1.89±2.15%, respectively. It can be seen that result of this work is in agreement with results of Kristiningrum (2015) leaves as it falls between the values for young and old (aged) As the age of leaves at matured growing stage is in between young and aged or old (Sujitrat et al., 2017), the percentage of CGA is also in between the two growing stages.

As an be observed in Table 3, high CGA content was recorded in green coffee beans than green coffee leaves at matured growing stage. CGA in beans was at least 63 % greater than in leaves. The minimum mean CGA concentration in beans was 59.58±0.12 mg/kg recorded in Gibe while the maximum 64.04±0.12 mg/kg which was obtained in Soro. On the other hand the maximum content of CGA in green coffee leaves was 23.08±0.10 mg/kg obtained in Gombora and the minimum values was 19.40±0.11 mg/kg obtained in Gibe study area. For decaffeinated coffee beans, Hall et al. (2018) found CGA of 31,115.23 mg/kg and Budiman et al. (2017) reported that it was 3.02±0.14%. These researches also determined CGA content for caffeinated coffee beans and Hall et al. (2018) reported 38,932.81 mg/kg and Budiman et al. (2017) reported that it was 4.16±0.16%. Current results, as compared to these literatures, are found to be more than both caffeinated and decaffeinated CGA contents of coffee beans reported by Budiman et al. (2017) and Hall et al. (2018). Present results may be linked with work of Fujioka et al. (2008) which reported that diet rich in CGA compounds play a great role in preventing various diseases associated with oxidative stress, as well as cancer, aging, and cardiovascular and neurodegenerative diseases. Thus, intake of more green coffee beans may be recommended for people for such health cases considering other side effects in to account.

Conclusion

We report comparative study on determination of concentration of CGA in green coffee beans and leaves (at matured growing stage) with UV/Vis spectrophotometer though liquid–liquid extraction of dichloromethane solvent. It was observed that more CGA content was present in coffee beans than the leaves in all considered study areas. Percentage of CGA in green coffee beans is at least 63.00% greater than that of green coffee leaves. In order to recommend, intake of more green coffee beans for health benefits like oxidative stress, cancer, aging, and cardiovascular health cases, toxicity study could be recommended. On top of that, the contents of CGA in coffee beans and coffee leaves obtained in this
work in agree with results of most literature cited.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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