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Comparative study of caffeine content in beans and leaves of Coffea arabica using UV/Vis spectrophotometer

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Caffeine is one of the compounds found in coffee beans and other parts of coffee plant. However, the relative caffeine content in different coffee plant parts is least researched. Therefore, this study was designed to determine the content of caffeine in green coffee leaves and beans. In this work, we report content of caffeine in green coffee beans and leaves of Coffea arabica using UV/Vis spectrophotometer with liquid-liquid extraction method. Obtained results indicated that the content of caffeine in green coffee beans and leaves are in the range of 9.94±0.20 to 11.46±0.10 mg/g and 2.53±0.07 to 2.73±0.16 mg/g, respectively. On top of that, the percentage of caffeine content in green coffee beans is more than in leaves in the range of 73.44 to 77.31%. These results show that there is statistically significant different content of caffeine in green coffee beans than its green leaves in a given coffee plant.

Key words: Caffeine, green coffee beans, green coffee leaves, UV/Vis spectrometer.

INTRODUCTION

Due to its pleasant taste, aroma, stimulant effect and health benefits (Gebeyehu and Bikila., 2015), coffee is one of the most widely consumed beverages throughout the world. Coffee plant is categorized among medicinal plants (Eva et al., 2016). Beans and leaves of the coffee plant 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 antiinflammatory effects on humans (Campa et al., 2012; Delaroza et al., 2017).

Literature has revealed that caffeine is distributed in different parts of the coffee seedlings (Zheng et al., 2004). Caffeine content of green coffee beans varies widely with species and as well as within a given species due to other factors such as on type of coffee bean, degree of maturation, roasting (Ky et al., 2001; Silvarolla et al., 2004). Coffea canephora coffee beans have higher caffeine content

with an overall mean value of 2.2%, where as that of C. arabica is about 1.2% (Belay, 2010; Belay et al., 2008; Franca et al., 2005). Alpdogan et al. (2002) have shown that percentage of caffeine in coffee beans is 1.36 ± 0.03%. Study of Farah and Donangelo (2006) has

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reported that percentage of caffeine content in C. arabica beans is in the range of 0.96 ± 0.01% to1.23 ± 0.06 %. Different researches have been conducted to determine the concentration content of caffeine in coffee beans. Belay et al. (2008) have reported that the percentage of caffeine content in *C. arabica* is 1.01 ± 0.04% to 1.19 ± 0.02% while Meareg and Shimelis (2012) have developed polymer modified glassy carbon electrode for the electrochemical for the determination of caffeine in coffee. Xiu-Min et al. (2018) showed that the processing and age of the leave of coffee plant have effect on phytochemical profilesand bioactivity of coffee leaves. Wondimkun et al. (2016) have measured that the content of caffeine in *C. arabica* beans beverage is determined to be 501.97 to 564.07 mg/L in different parts of Ethiopia. Gebeyehu and Bikila (2015) have also revealed that the percentage of caffeine content of roasted coffee arabica beans is 0.97 ± 0.049 to 1.53 ± 0.0 03%. According to Demissie et al. (2016), the caffeine content of green coffee beans ranges from 0.601 to 0.903 %. Another research in Ethiopia has revealed that the caffeine content in roasted coffee beans is 1.10% (Shiferaw et al., 2018).

Zheng et al. (2004) have studied that although caffeine is distributed mainly in leaves and cotyledons, essentially no caffeine was detected in roots and older brown parts of shoots. However, study done by Ashihara et al. (1996) has shown that the caffeine content in coffee leaves (fresh weight) depends on their age's level. This research has revealed that the caffeine content in buds, young leaves, matured leaves and aged leaves is 5.7, 7.1, 2.1 and 2.4 mg/g, respectively. Another study has said that the caffeine content is 3.2 mg/g in fresh young coffee leaves and 1.8 mg/g in matured coffee leaves (Ratanamarno and Surbkaral, 2017). Gustavo et al. (2019) have studied the effect of mixture of solvent on pigment extraction and antioxidant activity from *C. arabica* leaves.

Despite of the fact that caffeine exists either in coffee beans or coffee leaves, consumption of coffee beans is the common one in most part of the world (Grosso et al., 2016). In Ethiopia, besides of consumption of coffee beans, coffee leaves have been consumed for different reasons since the early times. However, the indigenous knowledge of the community about importance of coffee leaves is not the same as for coffee beans. Still today, people cook coffee leaves to prepare local beverages and drink it with some other spices. In contrast to the considerable amount of study on green beans for determination of caffeine content, there are relatively few studies concerned with the metabolite content in leaves. The leaves of the coffee plants which are believed to have high medical effects are overlooked as attention is given to beans.

Therefore, in this work, attempt is made to conduct comprehensive study of determination of caffeine content in green coffee leaves and beans for their comparative study.

MATERIALS AND METHODS

Data collection technique

Ripen green coffee beans and matured coffee leaves were taken from the same branches of a coffee tree. Simultaneously one coffee bean and coffee leave samples were collected from each woreda. All samples were collected from Southern part of Ethiopia, Hadiya Zone, of five different Woredas, specifically, Gibe, Soro, Gombora, Misrak Bedawachew and Mirab Bedawachew. Figure 1 displays the administrative map of the study area.

The sampling Woredas were selected purposively based on their productivity. 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. Figure 2 presents green coffee beans and leaves collected for sample preparations. The samples were collected in the month August within three day across all sample areas.

Standard solution preparations

Standard stock solution of 1000 ppm (parts per million) of caffeine (Evan, England) was prepared for all sample measurements. The solution was stirred and heated gently for an hour using magnetic stirrer for more homogeneity. Series of standard solutions were prepared from the stock solution (2.5, 5, 7.5, 10 and 12.5) ppm for caffeine from which calibration curve was drafted. All measurements were carried out in short period of time after preparation.

Sample preparations

Samples collected from the model farmers were dried at open air and shaded room temperature for 15 days. Each room temperature dried sample of green coffee beans and leaves was ground and sieved through 500 µm sieve to get a uniform texture granulometry. Accurately weighed 120 mg amount of sieved samples was dissolved in de-ionized water in a volumetric flask up to mark of 25 mL. Solutions were stirred for half an hour using magnetic stirrer and heated gently (85°C). In addition, the solutions were filtered through glass filter to get rid of particles from solution.

Liquid-Liquid extraction of caffeine

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 (25 mL solution) were mixed with 25 mL dichloromethane (Aldrich-Sigma, Germany) giving total of 50 mL solution of samples.

The solution was stirred for 10 min where a layer was formed with caffeine making upper layer and CGA making the lower layer. Caffeine was extracted from coffee solution and measured to get measured volume (22.5 mL) of the sample. This process was repeated three times to exhaustively extract caffeine. Either of the extraction for beans and leaves, samples at each round was stored in separate volumetric flasks. All glass wares and curette used were thoroughly cleaned, rinsed with de-ionized water and dried before use. From the extracted and measured caffeine sample solution, absorption was measured using double beam UV/Vis spectrophotometer (spectral 50 analytic Jenna, Germany) with

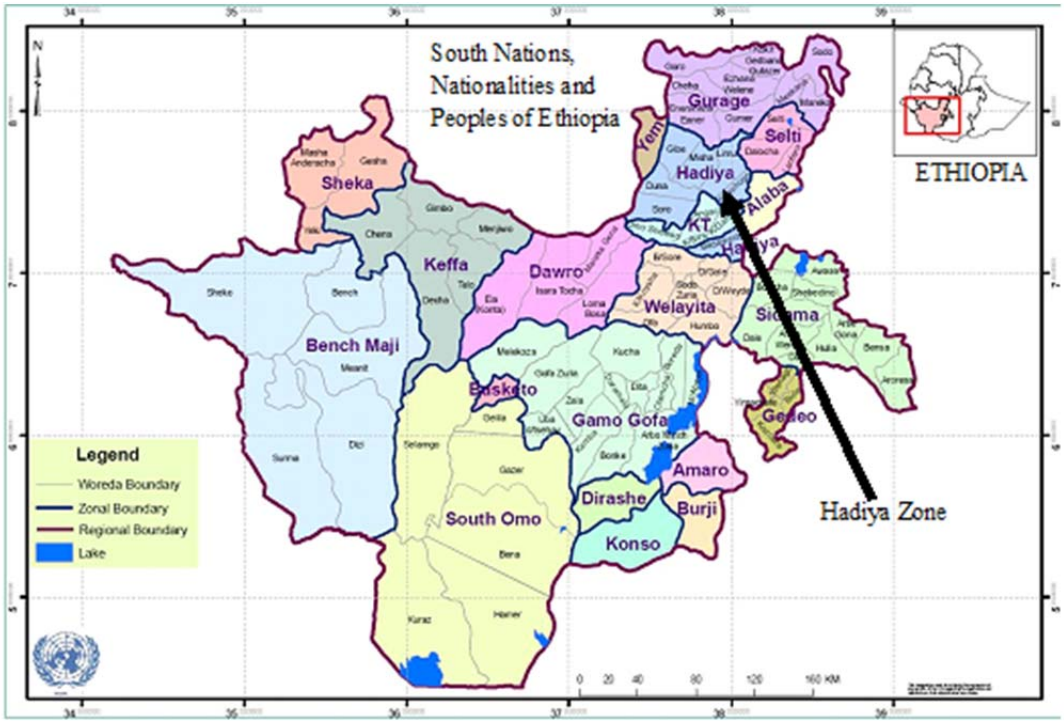

Figure 1. Map of study area (Hadiya Zone) as indicated by an arrow (Retrieved from http://www.ethiodemographyandhealth.org/SNNPRAdmMap.jpg)

Figure 2. Collected coffee samples (a) beans and (b) matured leaves.

Wave length ranges of 190 to 1100 nm from which caffeine 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 caffeine content

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

$$\text{Caffeine content (mg)} = \frac{\text{conc}(\text{mgL}^{-1}) * (\text{total sample volume}(\text{mL}))^2}{\text{measured sample volume}(\text{mL}) * 1000}$$

Statistical analysis

Data entry management and preliminary summaries were done on Microsoft Office Excel spread sheet. All analyses were carried out

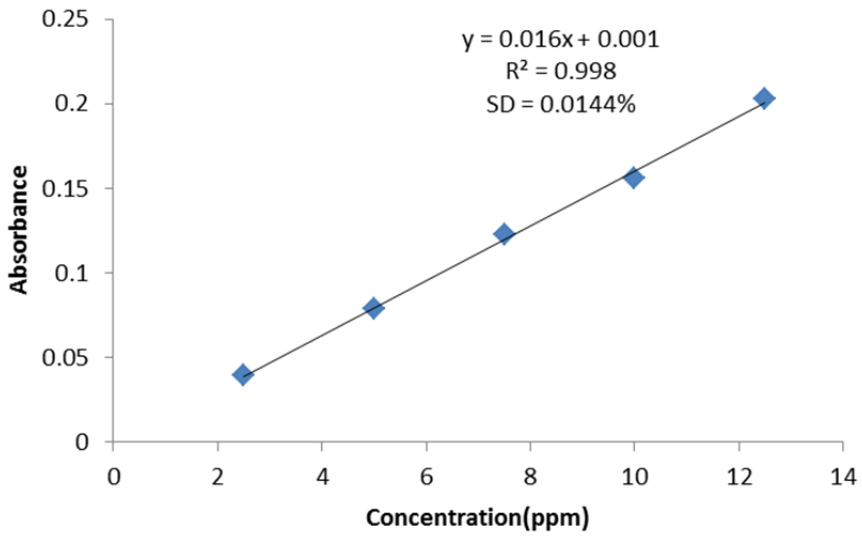

Figure 3. Calibration curve of standard caffeine solution.

Table 1. Concentration of caffeine content in green coffee beans in this work (n = 3).

Study area	Total sample volume (mL)	Measured sample volume(ml)	Caffeine content (mg)	Caffeine content (mg/g)
Gombora	50	22.5	1.248	10.41^{B}±0.21
Gibe	50	22.5	1.372	11.44^{A}±0.04
Misrak Bedawacho	50	22.5	1.259	10.50^{BB}±0.11
Mirab Bedawacho	50	22.5	1.375	11.46^{A}±0.12
Soro	50	22.5	1.193	9.94^{C}±0.20
Critical value	-	-	-	2.23
LSD	-	-	-	0.27

Means with different letters are statistically different ($p < 0.05$) in the fifth column.

in triplicates and data were presented as means ± standard deviation. Means of data obtained from quantitative measurement of spectrophotometer were determined. One-way analysis of variance (ANOVA) at $p < 0.05$ and SAS 9.1.3 statistical software were used to determine statistically significant differences in the mean concentrations of caffeine 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.

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 standard caffeine at the highest peak of intensity. The calibration curve facilitated measurement of the content of caffeine samples and validation of the method is displayed in Figure 3. The calibration equation is ($Y = 0.016x + 0.001$, $R = 0.998$, $SD = 0.0144\%$, $N = 5$) where Y, represents the peak height at maximum wavelength and x is concentration in mgL^{-1}. Results obtained in this work for green coffee beans are presented in Table 1. Maximum absorbance was obtained at 276 nm wavelength.

The percentage of caffeine content in coffee beans calculated for five independent measurements ranges from 0.99±0.01% to 1.15±0.01%. As can be seen from Table 1, high concentration value of caffeine is recorded in Mirab Bedawacho (11.46 mg/g) study area followed by Gibe (11.44 mg/g).

On the other hand, Soro showed low concentration recording (9.94 mg/g). One-way and SAS analysis of variance (ANOVA) at $p < 0.05$ show that all the mean concentrations are statistically significant different across the study areas, except in case of Gibe nad Mirab Bedawacho which are statically insignificant figures (Table 1).

In the same way, results obtained in this work for green coffee leaves are presented in Table 2. Maximum absorbance was obtained at 272 nm wavelength. The percentage of caffeine content in green coffee leaves

Table 2. Concentration content of caffeine in green coffee leaves in this work (n = 3).

Study area	Total sample volume (mL)	Measured sample volume (mL)	Caffeine content (mg)	Caffeine content (mg/g)
Gombora	50	22.5	0.304	2.53^{B}±0.07
Gibe	50	22.5	0.328	2.73^{A}±0.16
Misrak Bedawacho	50	22.5	0.314	2.62^{AA}±0.07
Mirab Bedawacho	50	22.5	0.312	2.60^{AA}±0.15
Soro	50	22.5	0.317	2.64^{AA}±0.08
Critical value	-	-	-	2.23
LSD	-	-	-	0.20

Means with different letters are statistically different ($p < 0.05$) in the fifth column.

Table 3. Percentage difference of caffeine content between green coffee beans and leaves in this work (n = 3).

Study areas	Caffeine in coffee beans (% w/w)	Caffeine in coffee leaves (% w/w)	Caffeine difference (%)
Gombora	1.04±0.01	0.25±0.01	75.67±1.05
Gibe	1.14±0.01	0.27±0.01	76.12±1.21
Misrak Bedawacho	1.05±0.02	0.26±0.01	74.93±1.68
Mirab Bedawacho	1.15±0.01	0.26±0.00	77.31±1.54
Soro	0.99±0.01	0.26±0.01	73.44±1.34

(calculated for five independent measurements) in this work ranges from 0.25±0.01% to 0.27±0.01%. The mean concentration recorded in Gibe study area (2.73±0.16 mg/g) is observed to be higher while in Gombora area (2.53±0.07 mg/g) study area it is recorded to be less (Table 2). One-way variance and SAS analysis show that the concentrations of Misrak Bedawacho, Mirab Bedawachoand Soro samples are statistically insignificantly different while the others two samples are statistically significantly different.

The content of caffeine in green coffee beans and leaves are significantly different from each other and the measured caffeine content in each coffee bean samples is greater than its respective counterpart coffee leave samples (Table 3).

The percentage difference in each study area signifies that the caffeine content in green coffee beans is much higher than that of caffeine content in green coffee leaves in study areas. Caffeine content in green coffee beans is at least 73.44% higher than its counterpart of caffeine content in green coffee leaves. Table 3 displays percentage difference of caffeine content between each green coffee beans and coffee leaves samples.

DISCUSSION

The caffeine content calculated in this work for green coffee beans ranges from 0.99±0.01 (9.94±0.20 mg/g) to 1.15±0.01% (11.46±0.12 mg/g). Alpdogan et al. (2002) reported that the percentage of caffeine content in coffee is 1.36 ± 0.03%. The caffeine content of *C. arabica* beans reported by Farah and Donangelo (2006) is 0.96 ± 0.01 to 1.23 ± 0.06%. Moreover, Belay et al. (2008) showed that the caffeine content in *C. arabica* from different parts of Ethiopia is 1.01 ± 0.04% to 1.19 ± 0.02%. Results found in this work fall in the concentration range reported by Shiferaw et al. (2018) (0.46 to 2.82%) which were done in the same country. Similarly this work's result is in line with results reported by Demissei et al. (2016) (0.5 to 0.9%) and Belay (2010) (0.95 to 1.27%). Caffeine content determined in this work is in agreement with the caffeine content of *C. arabica* beans reported by most researchers' work in different methods. However, results in the current work are found to be less as compared with results reported in (Gebeyehu and Bikila., 2015).

The caffeine content of green coffee leaves at matured growing stage in the current work ranges from 0.25±0.01 (2.53±0.07 mg/g) to 0.27±0.01% (2.73±0.16 mg/g). Zheng et al. (2004) showed that caffeine contents for upper leaves or young, middle or matured and lower leaves or an aged leaves is 55.8 µmol/g (10.84 mg/g), 47.2 µmol/g (9.17 mg/g) and 42.6 µmol/g (8.27 mg/g), respectively. Caffeine content in fresh coffee leaves as determined by Ratanamarno and Surbkaral (2017) is 3.2 mg/g for young and 1.8 mg/g for matured leaves. The current result is less than the results obtained by Zheng et al. (2004) and Ratanamarno and Surbkaral (2017) but more than the results reported by Ashihara et al. (1996) (2.1±0.43 mg/g) for matured leaves of *C. arabica*.

Result obtained in current work shows that green coffee leaves are found to have less concentration of

caffeine content than its respective green coffee beans. Researchers have revealed that intake of caffeine lowers the level of depression (Grosso et al., 2016) and enhance endurance and sprint of runners and concentration level during tiredness feelings (Conger et al., 2011). The intake of caffeine also contributes to the completeness of the desired therapeutic effect for patients (Madhusudhan, 2013; Straube et al., 2011).

Conclusion

We report comparative study of caffeine content in green coffee beans and leaves of the same plant using double beam UV/Vis spectrophotometer. Results indicate that the concentration content of caffeine in green coffee beans is 0.99 to 1.15% while that of green coffee leaves is 0.25 to 0.27%. These results showed that the caffeine content in green coffee beans is at least 73. 44% more than the one found in matured growing stage green coffee leaves.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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