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Influence of altitudes of coffee plants on the alkaloids contents of green coffee beans

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ABSTRACT

The influence of altitude of coffee plant and soil pH and organic carbon on alkaloid contents of green coffee beans was evaluated. The alkaloids content of 54 green coffee beans samples collected from coffee plants in Sidama, Illubabor, Jimma, Wellega and Gedeo grown at different altitudes (1515-2220 masl) was determined by high performance liquid chromatography. The caffeine and trigonelline were found in the range of 0.68-1.74% (w/w) and 0.68-1.44% (w/w), respectively. Theobromine was detected in only 18 samples and ranged 0.0186-0.32% (w/w). Theophylline was not detected in any of the green coffee beans samples. A weak negative correlation (R = -0.222) was found between the caffeine contents and the altitude of the coffee plants while a very weak positive correlation (R = 0.072) was found between the trigonelline contents and the altitude of the coffee plants. A strong negative correlation (R = -0.775) was found between the trigonelline contents and the caffeine content. A weak negative correlation was found between the caffeine contents and the soil organic carbon (R = -0.279) and between the trigonelline contents and the soil organic carbon (R = -0.101) while a weak positive correlation was found between the caffeine contents and the soil pH (R = 0.173) and between the trigonelline contents and the soil pH (R = 0.358) at which the coffee plants were grown.

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Capsule Summary: The influence of altitude of coffee plant and soil pH and organic carbon on alkaloid contents of green coffee beans was studied and the possible correlation among each other was evaluated.

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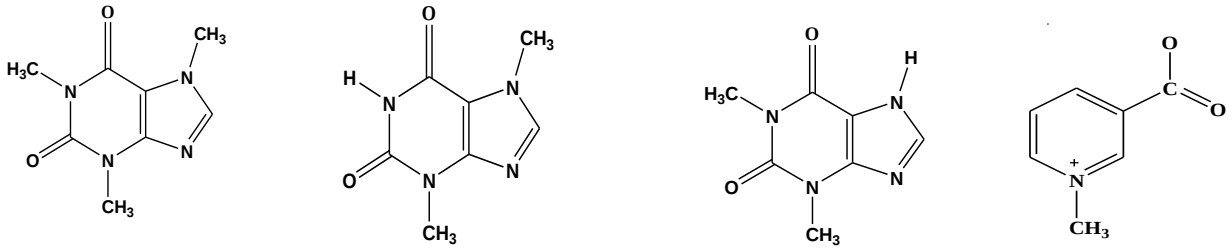
INTRODUCTION

Coffee is the most widely consumed beverage in the world due to its pleasant taste, aroma and stimulant effect and health benefits. The name coffee was originated from the name of the place 'Kaffa' where shepherds from Ethiopia discovered the coffee beans in the 6th century (Weldegebreal et al., 2016; Shiferaw et al., 2018; Hagos et al., 2018). The two main species of coffee cultivated on an industrial scale are Arabica coffee and Robusta coffee, which account for about 98-99% of the world coffee production.



(A) (B) (C) (D)

Fig. 1: The chemical structure (A) caffeine, (B) theobromine, (C) theophylline and (D) trigonelline

Coffee grows at various altitudes, ranging from 550 to 2750 meters above sea level (Hagos et al., 2018). However, Arabica coffee best thrives and is produced between altitudes of 1300 and 1800 meters, with annual rainfall amount ranging from 1500 to 2500 mm with an ideal minimum and maximum air temperatures of 15 and 25 ^{o}C, respectively. This prevails in most of the country's coffee growing areas (Mekonen, 2009).

The recognition and worldwide demand of coffee, which stems from its distinctive flavor, made it currently one of the most desirable and commonly consumed beverages. Furthermore, it has a strong historical, cultural, social and economic value in each and every society in Ethiopia (Shiferaw et al., 2018, Yisak et at., 2018a; Yisak et al., 2018b). It also plays the leading role in the national economy being the primary source of foreign exchange earnings (Mehari et al., 2016a; Mehari et al., 2016b; Shiferaw et al., 2018; Hagos et al., 2018). It is also a source of revenue of millions of Ethiopians. The major and most known coffee producing regions in the country are Oromiya and Southern Nations Nationalities and Peoples Region (Mehari et al., 2016a; Mehari et al., 2016b; Shiferaw et al., 2018).

Coffee is a rich source of alkaloids such as caffeine, trigonelline, theobromine and theophylline (Fig. 1) (Mehari et al., 2016c; Yasak et al., 2018a, Yisak et al., 2018b) and their profile in the green coffee beans is important and helpful to know the quality of coffee brew. The content of alkaloids (caffeine, theobromine and trigonelline) in green coffee beans is influenced by numerous factors such as coffee variety, genetic properties of the cultivars, environmental factors (soil, altitude, sun exposure), climatic parameters (rainfall, temperature), maturity of the beans at harvest, harvesting method and agricultural practices (shade, pruning, fertilization) (Alonso et al., 2009, Mehari et al., 2016c, Hagos et al., 2018).

The altitude and soil characteristic at which coffee plant is grown plays a major role in determining the quality of the green coffee beans. Coffee cherries from plants grown at higher altitudes take longer time to mature than plants grown at lower altitudes because there is less oxygen. High altitude grown coffee beans usually have a higher density than low altitude grown beans. Longer maturation times and increased bean sizes are usually observed for plants grown at high altitude (Sridevi and Giridhar, 2014).

The different types of coffee have diverse tastes depending on their geographical origins (Mehari et al., 2016c, Mehari et al., 2019). Due to the immense variety of genetic properties, soil types, climate change and altitude of the coffee growing areas, there is an extensive ranges of variability in the quality of green coffee beans. These factors are supposed to influence coffee characteristics (flavor, aroma and chemical content) (Hagos et al., 2018). Altitude and the parameter of the soil, such as pH value and organic carbon are very important factors. High altitudes are critical for the successful production of high quality Arabica coffee in equatorial region.

The amount of alkaloids (caffeine, theobromine, trigonelline and theophylline) in green coffee bean samples is determined by various analytical methods. Quantitative determination of alkaloids in large number of samples needs an accurate and rapid method. The complete extraction of alkaloids from test samples and removal of interfering substances varies from method to method. Therefore, choosing the right analytical method is essential for the accurate simultaneous determination of alkaloids (caffeine, theobromine, trigonelline, theophylline) in test samples (Gopinandhan et al., 2014, Mehari et al., 2016c; Hagos et al., 2018).

Many analytical methods have been reported for the determination of alkaloids in green coffee beans such as ultraviolet visible spectroscopy (Belay et al., 2008; Zewdu et al., 2016; Demissie et al., 2016), fluorescence spectroscopy (Yisak et al., 2018a), FT-IR-ATR (Weldegebreal et al., 2016; Yisak et al., 2018b), high performance liquid chromatography (Belitz et al., 2009; Gopinandhan et al., 2014; Mehari et al., 2016c; Hagos et al., 2018), gas chromatography (McCusker et al., 2003; McCusker et al., 2006; Sereshti and Samadi, 2014). However, the literature survey also revealed that there is limited reports available on study of the simultaneous determination of alkaloids in green coffee beans for a given set of samples and effect of altitude and soil composite (Belitz et al., 2009; Mehari et al., 2016c; Hagos et al., 2018).

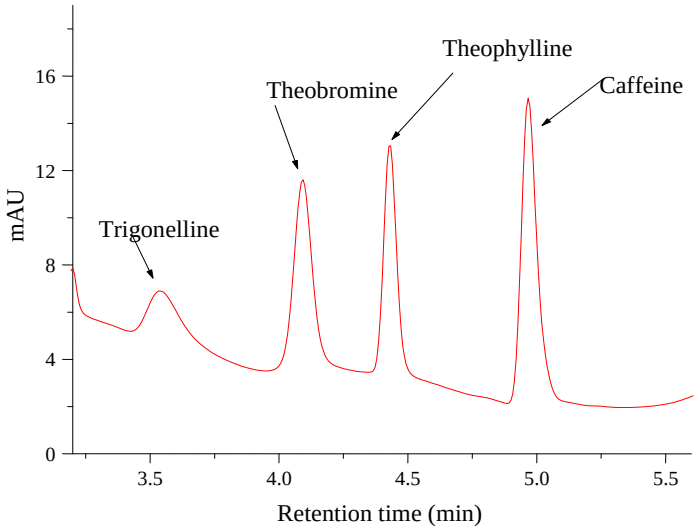


Fig. 2: HPLC chromatogram of a (10 µL) mixture (of equal volume) of standard solutions (30 mg/L of each) of the four alkaloids (trigonelline, theobromine, theophylline and caffeine)

Therefore, the aim of this study was the simultaneous determination of amount of alkaloids (caffeine, theobromine, trigonelline, theophylline) in green coffee bean samples from South east region (Sidama) and South west region (Illubabor, Jimma, Wellega and Gedeo) of Ethiopia by high performance liquid chromatography and to investigate the influence of altitude and the soil pH and organic carbon on alkaloids content and to compare the results of this study with the results reported in the literature.

MATERIAL AND METHODS

Chemicals and reagents

Standard caffeine (Merck J.T. Baker Chemical Company, USA), theobromine (Sigma-Aldrich, Italy), trigonelline hydrochloride (Sigma-Aldrich, Switzerland) and theophylline (Sigma-Aldrich, Switzerland) were used as received. HPLC grade acetonitrile, formic acid and lead acetate (BDH Chemicals, Poole, England), standard buffers (pH 4, 7, 10), concentrated sulfuric acid, potassium dichromate, ferrous sulfate, ammonium ferrous sulfate and *o*-phenanthroline monohydrate were used as received. Distilled and deionized water was used throughout the study.

Instruments

A traditional grinder (iron mortar and pestle), shaker (KS125 basic, Germany), electronic balance (SP 1500, USA), centrifuge (Janetzki, model T32c, Olympus, Japan), pH meter (CP-505, Poland), high performance liquid chromatograph (HPLC) (Agilent 1260 Infinity, Germany) coupled to a diode array detector (DAD) were used.

Soil samples

Soil samples were collected from Jimma and Gedeo, southern region of Ethiopia, at different altitudes. A total of 10 soil samples were taken from 20-30 cm depth around the coffee plants grown at different altitudes from different places. From each site 1 kg of soil samples were collected. All the samples were collected in March 2018. Each sample were stored in plastic bags under airtight conditions and transported to the laboratory for chemical analysis. All the soil samples were air-dried, ground, and sieved (2 mm) for the determination of soil pH and soil organic carbon.

Green coffee bean samples

A total of 54 green coffee beans samples were collected from coffee plants in Sidama, south east region of Ethiopia and Illubabor, Jimma, Wellega and Gedeo, south west region of Ethiopia, grown at different altitudes (1515-2220) masl. From each site 1 kg of green coffee beans samples were collected. All of the green coffee beans samples were obtained from ripped coffee cherries processed either by washing or sun drying techniques, depending on the practices in areas from where samples were collected. All the samples were from the same harvest season and were collected throughout April 2017 and March 2018. Each sample were stored in plastic bags under airtight conditions and transported to the laboratory for the analysis of alkaloids in green coffee beans using HPLC.

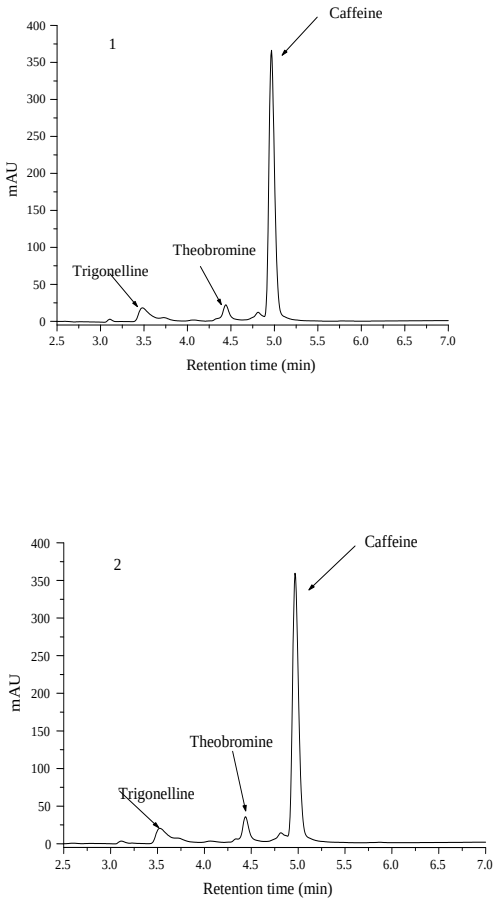


Fig. 3: HPLC chromatograms of caffeine (1) Gedeo Y. Hatursa from high altitude and (2) Jimma G. Kesosecha from low altitude

Preparation of alkaloids standard

Caffeine, theophylline, theobromine and trigonelline stock solutions (100 mg/L) were prepared separately by dissolving 2.5 mg of pure individual alkaloid in 25 mL methanol in 25 mL volumetric flask, respectively. Working standards of 1, 10, 30, 60 and 100 mg/L individual alkaloid were prepared in 10 mL volumetric flasks by serial dilution with the methanol.

Extraction of alkaloids

Alkaloids were extracted from the green coffee beans powders by using the procedure described by Mehari et al. (2016c). A 0.2 g portion of each coffee powder were weighed directly into a nylon centrifuge tube and extracted with 5.00 mL of boiling distilled water by shaking for 30 min on a plat form shaker at 200 rpm. The mixture was centrifuged for 5 min at 3600 rpm there after the supernatant was decanted carefully into a second centrifuge tube. The residue was returned to the tube and extracted a second time with 5.00 mL of boiling water. After combination of the supernatants, the volume was adjusted to 10.0 mL. A 1.50 mL portion of the extract was treated with 30 µL of 20% aqueous lead acetate solution to precipitate out polysaccharide, proteins and other colloidal material from the extract solution. After centrifuging the mixture for 5 min at 12,000 rpm, the supernatant was filtered directly into a chromatographic vial through a 0.2 µm syringe for chromatographic analysis.

Procedure for determination of soil pH

A 20 g soil was transferred to a 100 mL beaker, added 40 mL of deionized water, stirred the suspension several times and kept it for the 1 hour. The pH meter was calibrated according to the instructions of the pH meter. It was calibrated with three buffer solutions (pH 4.0, pH 7.0 and pH 10). The combined electrode (glass and calomel) of the pH meter was immersed in deep supernatant soil solution and the reading was taken during 5 min interval in which the reading was stable and not increase two digits in 30 s (Nunez et al., 2011).

Procedure for determination of soil organic carbon

Soil organic carbon was determined in the soil samples by using the procedure described by (Ryan, 2001). A 1 g of soil was transferred in to 500 mL conical flask. Exactly 10 mL of 1.0 N $K_2Cr_2O_7$ was added and the flask was swirled gently to disperse the soil in the solution. 20 mL of concentrated H_2SO_4 was added and immediately swirled the flask gently until soil and reagents were mixed. The mixture was heated on a hotplate at 150 ^oC for 1 min. The flask was allowed to stand for 30 min and 200 mL of distilled water was added. 5-6 drops of ferroin indicator was added to the mixture and titrated with 0.5 N ferrous sulfate solutions. As the end point was approached, the solution took a greenish cast and then changed to dark green.

HPLC analysis

HPLC system, coupled to a diode array detector (DAD), was used to determine the caffeine, theophylline, theobromine and trigonelline in the green coffee beans extracts. After introduction of the sample (10 µL), separation was achieved on a reversed phase C_{18} column (Supelco, 15 cm × 4.6 mm × 5 µm, USA) maintained at 25 ^oC in a column thermostat. The analysis was carried out under isocratic conditions using 90% deionized water (acidified as 0.1% aqueous formic acid) and 10% acetonitrile at a flow rate of 0.3 mL/min. The mobile phase was allowed to flow for 3 min between each analysis to wash and recondition the column. Chromatographic data for caffeine, theophylline and theobromine were collected at 280 nm and trigonelline were collected at 264 nm. In addition, the DAD was set to collect UV-Vis spectral data in the wavelength range 200-400 nm. All samples were analyzed in triplicate from three separate extracts, and each of the triplicate samples was analyzed by HPLC.

Table 1: The mean concentrations (%w/w±SD) of alkaloids determined in green coffee beans by HPLC

No.	Green coffee beans sample	Altitude (masl)	Caffeine %w/w±SD	Trigonelline %w/w±SD	Theobromine %w/w±SD
1	Bunobedele	2012	1.09±0.02	0.78±0.01	ND
2	Jimma	1818	1.09±0.02	0.89±0.09	ND
3	Beadle (Flora)	Not known	1.21±0.02	0.91±0.01	ND
4	Illubabor	1605	1.11±0.01	0.84±0.01	ND
5	Jimma (Denbero)	1780	1.18±0.01	0.83±0.01	ND
6	Agaro Jimma (Goma)	1900	1.08±0.02	0.81±0.01	ND
7	Illubabor (Metu)	1605	1.56±0.02	1.00±0.05	ND
8	Bolo Wolega Nekemt	2088	1.74±0.02	1.31±0.03	ND
9	Gumay Jimma	1763	0.97±0.01	1.00±0.04	ND
10	Illubabure Dildilaba	1665	1.36±0.02	1.05±0.01	0.176±0.001
11	Yachi Jimma	1780	1.69±0.03	0.92±0.03	ND
12	Orubetela	1952	1.05±0.01	0.77±0.03	ND
13	74158	1808	1.05±0.001	0.68±0.01	ND
14	Tulisa	1818	1.08±0.01	0.77±0.00	ND
15	Gedeo Konga	1898	0.83±0.01	1.09±0.03	ND
16	Aposto Sidama	Not known	0.87±0.02	1.12±0.01	ND
17	Konga Ulaulla Gedeo	1998	0.85±0.04	1.20±0.02	ND
18	74158 Gedeo Chito	Not known	0.87±0.01	1.05±0.02	0.0356±0.0001
19	Orubetela	1952	0.83±0.02	0.93±0.01	0.0227±0.0012
20	74110 Gedeo Chito	Not known	0.97±0.03	0.88±0.11	ND
21	1377Angafa Gedeo	1871	0.79±0.02	0.80±0.02	ND
22	Sidama W. Sample 3	1723	0.92±0.03	0.96±0.06	ND
23	Sidama W. Sample 4	Not known	0.70±0.05	0.82±0.01	0.0186±0.0025
24	Sidama W. Sample 5	1723	0.97±0.01	1.09±0.04	0.0405±0.0075
25	Sidama Wendogent	Not known	0.93±0.03	1.22±0.01	ND
26	Sidama W. Sample 4	1723	0.95±0.05	1.15±0.03	0.125±0.0029
27	Kurme Orubetela	1952	0.68±0.01	1.09±0.01	0.121±0.0125
28	Sidama W. Sample 1	1723	0.79±0.03	1.00±0.04	ND
29	Degga Variety	1808	0.87±0.02	0.84±0.00	ND
30	Gedeo Dumersa	1867	1.03±0.06	1.15±0.04	0.199±0.0097
31	Mike Gedeo	2210	0.79±0.04	0.79±0.03	ND
32	Gedeo 74112	Not known	0.88±0.07	1.08±0.08	ND
33	Gedeo Tulusa	1909	1.00±0.05	1.34±0.07	0.057±0.002
34	Gedeo Kange	Not known	0.99±0.13	0.78±0.00	ND
35	Gedeo Chito	1852	0.95±0.01	0.97±0.05	0.236±0.012
36	Gedeo Yirgacheffe Kurme	Not known	0.90±0.01	1.12±0.05	ND
37	Gedeo Yirgacheffe 74158	1909	0.92±0.01	1.11±0.04	0.193±0.011
38	Gedeo Y. Sample 1	Not known	0.99±0.02	0.88±0.08	0.320±0.0076
39	Gedeo Y. Afurca	Not known	0.86±0.04	0.90±0.03	0.153±0.056
40	Gedeo Y. 74158	1909	0.79±0.05	1.00±0.09	ND
41	Gedeo Y. Washo	2000	0.78±0.01	0.92±0.03	ND
42	Gedeo Yirgacheffe	Not known	0.90±0.003	1.17±0.01	ND
43	Gedeo Y. Yurme	2210	0.94±0.002	0.97±0.03	ND
44	Gedeo Y. Kurme	1960	1.04±0.05	0.99±0.02	ND
45	Jimma G. Chochie	1515	1.11±0.06	1.44±0.04	ND
46	Gedeo Y. Kokie	1918	0.92±0.09	0.98±0.04	ND
47	Jimma Iruche	1627	0.91±0.03	1.05±0.05	ND
48	Jimma G. Omogurdie	1770	1.23±0.03	1.03±0.02	0.121±0.013
49	Jimma G. Achareta	1782	1.17±0.06	1.18±0.07	ND
50	Jimma G. Kesosecha	1579	1.01±0.05	1.21±0.01	0.192±0.011
51	Gedeo Y. Hatursa	1900	1.41±0.04	1.13±0.05	0.320±0.0076
52	Jimma Omogurdie	1972	1.02±0.04	0.92±0.04	0.176±0.001
53	Gedeo Y. Wegide	2037	1.07±0.02	1.19±0.05	ND
54	Gedeo Y. Wetie	2220	0.86±0.02	0.94±0.02	0.176±0.001

Not known: sample was bought from the local market, ND: not detect

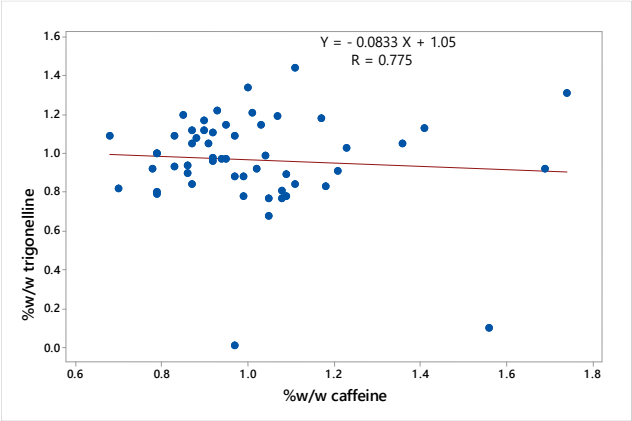


Fig. 4: Correlation between trigonelline and caffeine in the green coffee beans

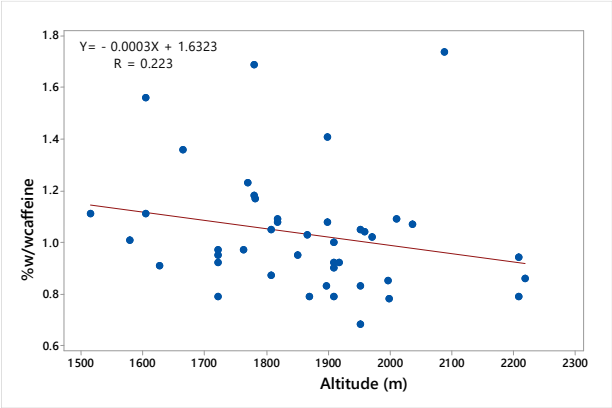


Fig. 5: Correlation between % w/w caffeine content of the green coffee beans and altitude of the coffee plants.

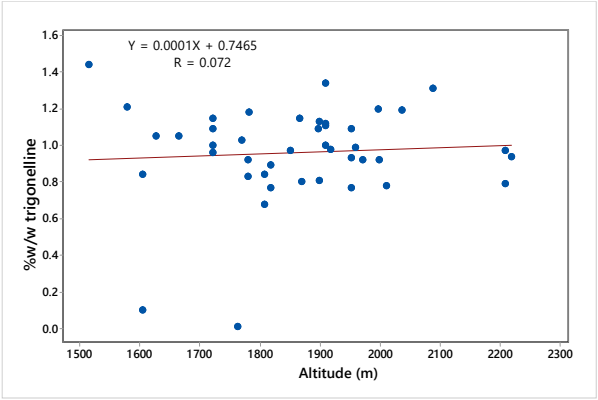


Fig. 6: Correlation between % w/w trigonelline content of the green coffee beans and the altitude of the coffee plants

Statistical analysis

The correlations among the parameters were assessed by Pearson correlation methods (SPSS Version 21). The graphical expression was done using Origin 6. One way analysis of variance (ANOVA) was used for test of significance at $p = 0.05$.

RESULTS AND DISCUSSION

Determination of alkaloids in green coffee beans by HPLC method

The aqueous extracts of the green coffee beans samples were analyzed to determine the concentrations of caffeine, theophylline, theobromine and trigonelline. Analysis of a 10 µL standard mixture (prepared by mixing 2.5 µL each, of concentration 30 mg/L each) of the four alkaloids by HPLC provided well separated peaks within 7 min (Fig. 2). Mehari et al. (2016c) and Hagos et al. (2018) have reported a HPLC method for the separation of caffeine, trigonelline, theobromine, and theophylline within 7 min. Quantitative determination of caffeine, theophylline, theobromine and trigonelline was done after constructing calibration curves from the chromatographic peak areas obtained after analysis of standard solutions of caffeine, trigonelline, theophylline and theobromine.

Calibration curves

Calibration curves were obtained using five different concentrations of caffeine, theophylline, theobromine and trigonelline separately in the range of (1-100) mg/L for the determination of the individual alkaloid content of aqueous extracts of green coffee beans. The equations of the calibration curves were y = 7.3208x – 21.037, R^2 = 0.9959 for caffeine, y = 8.0827x – 17.063, R^2 = 0.9936 for theophylline, y = 8.0827x – 17.063, R^2 = 0.9936 for theobromine and y = 3.167x – 16.748, R^2 = 0.9994 for trigonelline, where y is peak area of the alkaloid, x is concentration of the alkaloid in mg/L and R is the linear regression coefficient. The levels of the individual alkaloids in the samples were calculated from the regression equation of the standards. Samples were analyzed in triplicates from three separate extracts, and each individual triplicate sample was analyzed by the HPLC.

Determination of alkaloids in green coffee beans

The aqueous extract (10 µL) of 54 green coffee beans were injected separately to the HPLC system under the same chromatographic conditions as used for the standard alkaloids described in the experimental section and their respective chromatograms were recorded. Two representative HPLC chromatograms of the green coffee beans samples from (1) Gedeo Y. Hatursa from high altitude and (2) Jimma G. Kesosecha from low altitude are shown in Fig. 3. The alkaloid contents of the green coffee beans samples were calculated by using the calibrations equations of the standard alkaloids. The data on alkaloids contents in the 54 green coffee beans samples obtained by HPLC are presented in Table 1.

Table 2: Comparison of results of the present study with the results reported in literature

Methods	Caffeine % (w/w)	Trigonelline % (w/w)	Country of coffee origin	Reference
HPLC	0.60 - 1.09 (n = 9)	0.049 - 0.093 (n = 9)	Ethiopia	Hagos et al. (2018)
HPLC	0.66 - 2.52 (n = 4)		University of Zagreb	Hecimovic (2011)
HPLC	1.32 - 1.36 (n = 6)		Ethiopia	Shiferaw et al. (2018)
HPLC	0.96 - 1.23 (n = 42)		Ethiopia	Yigzaw et al. (2007)
HPLC	0.87 - 1.4 (n = 99)	0.98 - 1.32 (n = 99)	Ethiopia	Mehari et al. (2016c)
HPLC	1.6 - 1.3 (n = 3)		Kenya	Wanyika et al. (2010)
HPLC	0.89 - 1.5 (n = 8)		India	Gopinandhan et al. (2014)
HPLC	0.68 - 1.74 (n = 54)	0.68 - 1.44 (n = 54)	Ethiopia	Present work

Table 3: Soil pH and soil organic carbon of the coffee sampling areas

Samples	Altitude (masl)	pH value	Soil organic carbon (% w/w)
Jimma Goma Chochie	1515	5.8	3.84
Jimma Goma Kesosecha	1579	5.4	3.31
Jimma Iruche	1627	4.8	0.47
Jimma Goma Omogurdie	1770	5.3	2.56
Jimma Goma Achareta	1782	5.4	0.51
Gedeo Yirgacheffe Kokie	1918	5.7	0.44
Gedeo Yirgacheffe Hatursa	1900	5.3	2.33
Jimma Omogurdie	1972	4.3	3.44
Gedeo Yirgacheffe Wegide	2037	4.5	2.60
Gedeo Yirgacheffe Wetie	2220	5.8	3.20

Levels of caffeine in green coffee beans

The data on caffeine level in the 54 green coffee beans samples obtained by HPLC method are presented in Table 1. The caffeine content in all the green coffee bean samples was observed in the range 0.68-1.74% (w/w). The ANOVA results revealed that there was a significant difference ($p < 0.05$) in the mean value of caffeine contents among all the green coffee bean samples. The data obtained in this study is compared with the data reported in the literature (Table 2). An average value of 1.10% (w/w) (Yigzaw et al., 2007), in the range 0.87-1.38% (w/w) (Mehari et al., 2016c) and 0.6-1.09% (w/w) (Hagos et al., 2018) caffeine in Ethiopian green coffee beans were reported. Therefore, these values are in reasonable degree of agreement with the findings of the present work. The variation in caffeine level of green coffee beans samples may be due to geographical origins which might have different altitude, soil composition, soil pH, rain fall and other agricultural as well as environmental conditions.

Trigonelline in green coffee beans

The data on trigonelline level in the 54 green coffee beans samples obtained by HPLC method are presented in Table 1. The trigonelline content in the green coffee bean samples was observed in the range 0.68-1.44% (w/w). The ANOVA results revealed that there is a significant difference ($p < 0.05$) in the mean value of trigonelline contents among all the green coffee bean samples. The data obtained in this study is compared with the data reported in the literature (Table 2). The trigonelline contents in Arabica green coffee beans were reported in the range of 0.6-1.2% (w/w) (Belitz et al., 2009), 0.74-1.12% (w/w) (Duarte et al., 2010), 0.98-1.32% (w/w) (Mehari et al., 2016c) and 1.00-1.10% (w/w) (Hagos et al., 2018). Therefore, these values are in reasonable degree of agreement with the findings of the present work. The variation in trigonelline level of green coffee beans samples may be due to geographical origins which might have different altitude, soil composition, soil pH, rain fall and other agricultural as well as environmental conditions. Furthermore, the correlation between trigonelline and caffeine contents of the green coffee beans has been assessed (Fig. 4). The study revealed that there is strong negative correlation ($R = -0.775$) between trigonelline and caffeine contents in the green coffee beans.

Theobromine and theophylline in green coffee beans

The data on theobromine in the 54 green coffee beans obtained by HPLC are presented in Table 1. However, theobromine was detected only in 18 samples and not detected in the remaining 36 samples. The theobromine content in the 18 green coffee bean samples was observed in the range 0.0186-0.320% (w/w). The data obtained in this study is compared with the data reported in the literature. The theobromine contents in the green coffee beans have been reported in the range of 0.0036-0.0040 % (Belitz et al., 2009) and 0.048-0.094% w/w (Mehari et al., 2016c), which are in line with present work.

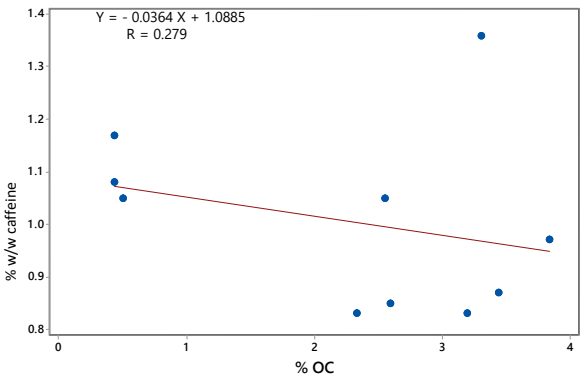


Fig. 7: Correlation between % w/w caffeine content of the green coffee beans and % organic carbon of soil of coffee plant

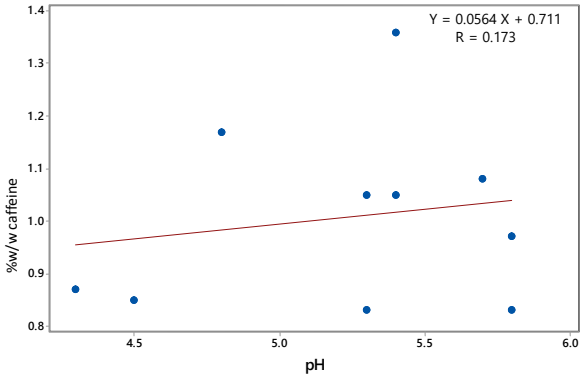


Fig. 8: Correlation between % w/w caffeine content of the green coffee beans and soil pH

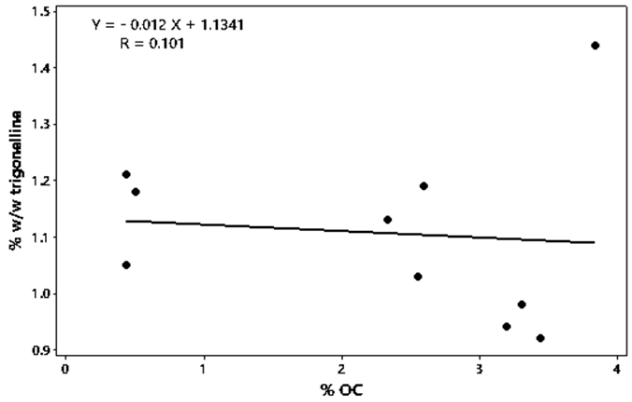


Fig. 9: Correlation between % w/w trigonelline content of the green coffee beans and % w/w organic carbon of soil

Theophylline was not detected in any of the green coffee beans samples. The theophylline was also not detected in the Arabica green coffee beans by the previous researchers, for example, in the Ethiopian Arabica green coffee beans (Mehari et al., 2016c) and Arabica green coffee beans from Africa, Asia and America (Alonso et al. 2009). Therefore, the present work is in reasonable degree of agreement with the results reported in the literature.

Effect of altitude of coffee plants on the alkaloid contents in the green coffee beans

The correlation of caffeine content of green coffee beans and the altitude of the coffee plants is shown in Fig. 5, which indicates that the altitudes at which coffee plants grown had influence on caffeine content and has weak negative correlation (R = - 0.223) between altitude of the coffee plant and its caffeine content. The correlation of trigonelline content of green coffee beans and the altitude of the coffee plants is shown in Fig. 6, which indicates that the altitudes at which coffee plants grown had very small influence on trigonelline content and has very weak positive correlation (R = 0.072) between altitude of the coffee plant and its caffeine content.

Effect of soil pH and soil organic carbon on alkaloid in the green coffee beans

Composite soil samples were randomly collected from all the sampling sites from a depth of 20-30 cm. Ten of the collected soil samples were analyzed for pH and soil organic carbon. The results showed that the highest soil pH of 5.8 was found at Jimma Goma Chochie and Gedeo Yirgacheffe Wetie while the lowest pH of 4.3 was obtained from Jimma Omogurdie soil. The soil at Jimma Goma Ghochie had the highest soil organic carbon (3.84%) content, while the lowest (0.44%) was recorded from the soil of Gedeo Yirgacheffe Kokie. The soil pH and soil organic carbon of the coffee sampling areas are presented in Table 3.

Table 3 indicates the content of soil organic carbon in ten soil samples. The content of soil organic carbon in all the soil samples was observed in the range 0.44-3.84% w/w. There was a significant difference ($p < 0.05$) in contents soil organic carbon among all the soil samples. The green coffee beans growing in different soil composite (Fig. 7) indicates that the content of soil organic carbon at which coffee plants grown had influence on concentration caffeine of green coffee beans and has weak negative correlation (R = -0.279) between content soil organic carbon of the soil samples and its caffeine content.

The data obtained in this study is compared with the data reported in the literature. The soil organic carbon content in soil samples observed in this study is in the range 0.44-3.84% (w/w). The soil organic carbon contents in the soil samples have been reported by the previous researchers, for example, the amount of soil organic carbon in the soil samples in Arabica green coffee beans growing area was reported in the range of 0.7-3.7% (Mekonnen, 2009), 1.37-3.63% (Bahilu et al., 2016), 1.27-2.83% (Taye, 2011), 1.51-2.83% (Taye, 2006), 0.55-2.85% (Jha et al.,

2014). Therefore, these values are in reasonable degree of agreement with the findings of the present work.

Table 3 indicates the value of soil pH in ten soils of the green coffee samples. The pH in all the soil samples was observed from 4.3-5.8. The green coffee beans growing in different soil samples (Fig. 8) indicates that the value pH at which coffee plants grown had influence on concentration caffeine of green coffee beans and has weak positive correlation (R = 0.173) between pH of the soil samples and its caffeine content.

The data obtained in this study is compared with the data reported in the literature. The soil pH in soil samples observed in this study is in the range 4.3-5.8. The soil pH in the soil samples have been reported by the previous researchers, for example, the pH of soil samples in Arabica green coffee beans growing area was reported in the range of 5.23–6.35 (Taye, 2011), 4.53-7.69 (Nunez, 2011), 5.39-6.23 (Taye, 2006), 4.27-7.33 (Orhan and Huseyin, 2009). Therefore, these values are in reasonable degree of agreement with the findings of the present work.

Table 3 indicates the content of soil organic carbon in ten soil samples. The content of soil organic carbon in all the soil samples was observed in the range 0.44-3.84% w/w. There was a significant difference (p<0.05) in contents soil organic carbon among all the soil samples. The green coffee beans growing in different soil composite (Fig. 9) indicates that the content of soil organic carbon at which coffee plants grown had influence on concentration caffeine of green coffee beans and has weak negative correlation (R = -0.101) between content soil organic carbon of the soil samples and its trigonelline content.

The data obtained in this study is compared with the data reported in the literature. The soil organic carbon content in soil samples observed in this study is in the range 0.44-3.84% (w/w). The soil organic carbon contents in the soil samples have been reported by the previous researchers, for example, the amount of soil organic carbon soil samples in Arabica green coffee beans growing area was reported in the range of 0.7-3.7% (Mekonnen, 2009), 1.37-3.63% (Bahilu et al., 2016), 1.27-2.83% (Taye, 2011), 1.51-2.83% (Taye, 2006), 0.55-2.85% (Jha et al., 2014). Therefore, these values are in reasonable degree of agreement with the findings of the present work.

Table 3 indicates the value of soil pH in ten soils of the green coffee samples. The pH in all the soil samples was observed from 4.3-5.8. The green coffee beans growing in different soil samples (Fig. 10) indicates that the value pH at which coffee plants grown had influence on concentration trigonelline of green coffee beans and has moderate positive correlation (R = 0.358) between pH of the soil samples and its trigonelline content. The data obtained in this study is compared with the data reported in the literature. The soil pH in soil samples observed in this study is in the range 4.3-5.8. The soil pH in the soil samples have been reported by the previous researchers, for example, the pH soil samples in Arabica green coffee beans growing area was reported in the range of 5.23-6.35 (Taye,

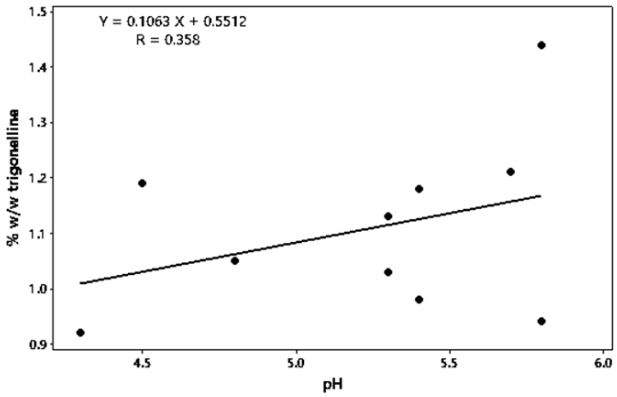


Fig. 10: Correlation between % w/w trigonelline content of the green coffee beans and pH

2011), 4.53-7.69 (Nunez, 2011), 5.39-6.23 (Taye, 2006), 4.27-7.33 (Orhan and Huseyin, 2009). Therefore, these values are in reasonable degree of agreement with the findings of the present work.

CONCLUSIONS

The alkaloids content in 54 green coffee beans samples collected from coffee plants grown in Illubabur, Sidama, Jimma and Gedeo South region of Ethiopia grown at different altitudes was determined by high performance liquid chromatography (HPLC). A significant variation in the concentration of alkaloids in the green coffee bean samples were observed depending on the level of altitudes, soil organic carbon and soil pH at which the coffee plant grows. The caffeine, trigonelline and theobromine contents were obtained in the range of 0.68-1.74% (w/w), 0.68-1.44% (w/w) and 0.0186-0.32% (w/w), respectively. Among the 54 coffee varieties from Sidama South East region of Ethiopia and Illubabor, Jimma, Wellega and Gedeo South west region of Ethiopia, Kurme Orubetela, Mike Gedeo, Gedeo Yirgacheffe 74158 and Gedeo Yirgacheffe Washo can be considered as low content of caffeine obtained from the coffee plant grown at high altitudes.

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