RESEARCH ARTICLE

Determination and comparison of caffeine and other chemical constituents in *Coffea arabica* varieties grown in Sri Lanka

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Abstract: Coffee is one of the most widely consumed hot beverages all over the world. The coffee variety *Coffea arabica* accounts for 80% of the world coffee trade because of its distinct flavor and aroma. At present, coffee is considered as a functional food, primarily due to its high content of antioxidant and other beneficial biological properties. Most of the coffee varieties grown in Sri Lanka can be found at experimental fields in the Central Research Station, Department of Export Agriculture, Matale. Hybrido-de-Timor (HDT), HDT x Takari (S9) and HDT x Catura (Catimor) are considered as high yielding varieties of coffee in Sri Lanka. The objective of this study was to investigate the phytochemical analysis such as, alkaloids, flavonoids, carbohydrates, saponin and steroids, proximate composition, mineral content and antioxidant activity of coffee beans from three varieties of *Coffea arabica* grown in Matale plantation. Standard analytical methods were used to analyze phytochemical, nutritional composition, mineral content and total anti-oxidant activity. Findings revealed that S9 variety had the highest caffeine content, acid insoluble ash and total ash content, phosphorous, zinc and potassium content and antioxidant activity compared to other two varieties. According to the sensory evaluation, coffee brewed from S9 variety was the most preferred in terms of color, taste, aroma and overall acceptance compared to other varieties.

Keywords: Antioxidant activity, Caffeine content, Coffee, Nutritional composition.

INTRODUCTION

Coffee was introduced to Sri Lanka by Arabs in 1503, and in 1658 the Dutch started its cultivation. Two coffee species namely *Coffea arabica* and *Coffea canephora* are commonly cultivated in Sri Lanka. Total coffee extent in Sri Lanka is about 6000ha, out of which about 50% is in central province. Annual export volume of coffee is 157.5tons. *Coffea arabica* is the most important species and that occupies 80% of the world coffee trade because of its distinct flavor and aroma. Arabica coffee is grown in cooler high elevation regions and robusta coffee in warmer, humid low elevation regions in Sri Lanka (FAO, 2013).

Coffee contains diverse compounds that are reported in recent times, which are associated with beneficial health effects. It is a complex mixture of chemicals provides significant amounts of chlorogenic acid and caffeine. It is the main source of caffeine in many populations. Moreover, it also contains thousands of different chemicals like carbohydrates, lipids, nitrogenous compounds, vitamins, minerals, alkaloids, and phenol compounds (Higdon and Frei 2006). Chlorogenic acid (the most prominent), caffeic acid, melanoidin are all antioxidants found in coffee. N-methyl pyridinium, one of identified as an antioxidant that found in coffee. Major important chemical constituents in coffee are caffeine, chlorogenic acid, cafestol, kahweol and other micronutrients. Coffee is a composite combination of a thousand chemicals containing possible bioactive molecules such as chlorogenic acid, caffeine, and two diterpenes including cafestol and kahweol.

Caffeine is an odorless and slightly bitter methylxanthine derivative that can be naturally found in the coffee and other plant species (Amare and Admassie, 2012). Moreover, it is considered to be one of the most commonly consumed drugs with more than 80 percent of the world's population consuming caffeine daily (Norton *et al.*, 2011). The Europeans are found to be the world's largest consumers of caffeine intake of approximately 4.6 kg/person/year. Caffeine is quickly absorbed by the body. The human salivary level, which indicates the extent of absorption, peaks around 40 minutes after caffeine consumption (Chou and Bell, 2007). This pharmacologically active substance stimulates the central nervous system, increases heartbeat rate, dilates blood vessels and works as a weak diuretic. However, the high amounts of caffeine can cause trembling, nausea, nervousness and seizure. A fatal dose of caffeine has been evaluated to be more than 10 g (about 170 mg/kg of body weight). It is also considered to be a risk species for cardiovascular diseases, kidney malfunction, asthma, and may also cause hyperactivity (Abebe *et al*., 2008).

There are some high yielding coffee selections belonging to *Coffea arabica* species; Hybrido-De-Timor (HDT), HDT x Takari (S9) and HDT x Catura (Catimor)

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which are grown at Central Research Station, Department of Export Agriculture, Matale. HDT is a Hybrid variety which bears large berries and more resistant to leaf rust disease. Average dry yield is more than 2000 kg/ha. S9 is a hybrid variety (HDT x Takari) which has superior flavor and aroma, berries are in medium size and resistant to rust disease. The average yield is more than 2000 kg/ha. Catimor is a high adaptable variety resulting of HTD and Catura and has medium berries. It is more resistant to rust disease and yield more than 2500 kg/ha. Well matured coffee beans should be used in order to obtain accurate results and wet process or dry process helps to separate beans from berries. Parchment layer should be removed before further processes and can be achieved using machine or motor and pestle. There are variety of machines available for roasting of coffee beans. The roasting process provides unique flavor and aroma which enhance the consumer complacence. Grinding is followed after roasting and sizes of particles are important.

Therefore, the objectives of this study were to determine and differentiate the caffeine and other chemical constituents found in coffee varieties of S9, HDT and Catimor and to select the suitable coffee variety for the industries in order to fulfill the consumer demand.

MATERIALS AND METHODS

Qualitative phytochemical analysis

Qualitative chemical tests were conducted on extracts to establish the chemical composition profile of the *Coffea arabica* extracts (T_1: S9, T_2: Catimor and T_3: HDT). The following tests were carried out to detect various phytochemicals in the samples.

Preparation of samples

A sample of 10 g of the *Coffea arabica* powder was extracted successively with 150 mL of petroleum ether using a Soxhlet extractor for 16 h. All the extracts were concentrated under reduced pressure in a rotary evaporator. The extracts obtained were filtered separately using Whatman No. 42 filter papers and stored in airtight bottles for further use.

Dragendorff's test for alkaloids

Alkaloid was qualitatively tested according to the method described by Chandrashekar *et al*. (2010). 2 mL of Dragendorff's reagent was added to few milliliters of filtrate. A prominent yellow precipitate indicated the presence of alkaloids in samples.

Shinoda's test for flavonoids

Flavonoids were qualitatively tested according to the method described by Korwar *et al.* (2010). The extracts were treated with few fragments of magnesium metal separately followed by drop wise addition of concentrated hydrochloric acid. The formation of magenta color indicated the presence of flavonoid.

Benedict's test for carbohydrates

Alkaloid was qualitatively tested according to the method described by Chandrashekar *et al*. (2010). 2 mL of extract was shaken with 10 mL of water, filtered and the filtrate was concentrated. To this 5 mL of Benedict's solution was added and boiled for 5 minutes. Formation of brick red colored precipitation indicates the presence of carbohydrates.

Detection of saponin by foam test

Saponin was qualitatively tested according to the method described by Kokate (1999). 2 mL of distilled water was added to 1 mL of extract. Mixture was shaken vigorously and allowed to stand for 10 minutes. Development of a foam on the surface of the mixture indicated the presence of saponin in samples.

Detection of steroids by sulphuric test

Steroids were qualitatively tested according to the method described by Chandrashekar *et al*. (2010). Chloroform (10 mL) was added to 1 mL of the filtrate. Then, 10 mL of sulphuric acid was added slowly by the sides of the test tube. Upper layer turned red and sulphuric acid layer showed the yellow color with green fluorescent, indicating the presence of steroids in the filtrate.

Quantitative chemical analysis

Determination of caffeine content

The caffeine amount in the *Coffea arabica* was determined by High Performance Liquid Chromatographic-DAD method (Mesfine *et al*., 2018). Caffeine stock solution of 1000 ppm was prepared by dissolving 100 mg of standard caffeine powder with 50 mL of warm water in a 100 mL volumetric flask and filled to the final volume with distilled water after cooling down to room temperature. 100 ppm standard solution was prepared by pipetting 10 mL of stock solution in to a 100 mL volumetric flask and brought up to volume with distilled water. The caffeine working standard solutions of different concentrations 0, 5, 10 and 25 ppm were prepared from the above prepared solution in 10 mL volumetric flasks with distilled water. Each roasted coffee bean sample was ground and screened through 250 µm sieve to get a uniform texture. Exactly 0.50 g of each ground and sieved coffee sample was put into 250 mL beakers, and were boiled in 100 mL of distilled water in a temperature range of 80-90°C with continuous stirring using magnetic stirrer for 30 minutes. Coffee solutions were cooled to room temperature, and then filtered into conical flasks with 0.45 µm filter papers using suction filtration. Small amount of hot water was added on the scum left over the filter paper to wash caffeine remains.

To determine the caffeine content in the coffee samples, the method was validated using the standard solution prepared for the experiment. The standard solutions used in experiment were 0, 5, 10 and 25 ppm. These solutions were then injected into the HPLC machine (Agilent 1100 Series, US) following the chosen chromatographic conditions. A calibration curve for peak area against concentration of working caffeine standards was constructed to validate

the HPLC quantification of caffeine in terms of linearity, sensitivity, precision and for calibration purpose. The curve showed good linear relationship between the peak area and concentrations of the standard solutions. Its equation was derived as $Y = 52.8x + 22.9$ and calibration curve of standard ($R_2 = 1.0000$) where Y is peak area, X is concentration of caffeine ($mg\ L^{-1}$) and R is the linear correlation factor.

Determination of total antioxidant activity (Phosphomolybdenum assay)

The total antioxidant capacities of extracts were evaluated using phosphomolybdenum method (Matthias *et al.*, 2015). Coffee extracts ($0.5\text{-}1\ mg\ mL^{-1}$) 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) were added to all the tubes and incubated at 95 °C for 90 min. After cooling the sample to room temperature, the absorbance of the solutions was measured at 695 nm against blank using a UV Visible spectrophotometer (Thermo Fisher Orion AquaMate, India). Ascorbic acid was used as the reference. Total antioxidant activity (TAA) was expressed as mmol Trolox/g coffee beans.

Analysis of proximate composition

Moisture, ash, fat, acid-insoluble ash and water soluble matter contents were analyzed according to the AOAC method (2000).

Analysis of mineral content

Finely ground roasted coffee sample of 0.5 g was taken and kept at the muffle furnace at 475°C for 6 hours for ashing. Ash samples were treated with 2.5 mL of concentrated hydrochloric acid and diluted with distilled water. Mixture was filtered through Whatman No.42 filter paper into a 50 mL standard flask. The final volume was made up to 50 mL with double distilled water. Phosphorus content in the digested sample was estimated by colorimetric method (McKie and MccleAry, 2016). Potassium content in the digested sample was estimated by a Jenway Clinical PFP7/C Flame Photometer (Boraste *et al.*, 2009). Magnesium, calcium, ferrous and zinc contents in the digested samples were estimated by a PinAAcle 900H Atomic Absorption Spectrophotometer - (Zafar *et al.*, 2010).

Sensory evaluation

Sensory evaluation was done using 30 untrained panelists at the Central Research Station, Department of Export Agriculture. These panelists are from different age groups between 23-55 years and with different positions. Hedonic test was conducted using 5-point scale with five different sensory attributes; color, taste, aroma, mouth feel and overall preference. Coffee brew was made from 12 g of roasted coffee. Coffee powder was added into filter bag and 150 mL hot water at 90°C was added into beaker and was kept for 30 seconds. Filter bag was removed and 30 mL of coffee brew was poured into test glasses. Glasses were coded with three-digit number code and three glasses of coffee brew were given to each panelist with five ballots.

Data analysis

The experimental design was the Complete Randomized Design (CRD). The mean values of replicates were determined using DMRT (Duncan Multiple Range Test) at $p = 0.05$. Difference among different variety was tested with one–way analysis of variance (ANOVA) by using SAS statistical software.

RESULTS AND DISCUSSION

Qualitative analysis of phytochemicals for different varieties of *Coffea arabica*

In the addition of the Dragendroff reagent and petroleum ether extracts of S9 (T_1), Catimor (T_2) and HDT (T_3) varieties gave orange yellow solution with pale orange precipitation. In the addition of the NaOH few drops, petroleum ether extracts gave intense yellow color with slight precipitation. After adding Benedict's reagent and boiling of the extracts, layer separation was resulted in petroleum ether extracts. The lower layer turned into bluish green. After adding distilled water and shaking, layer separation was occurred in petroleum ether extracts. Foam was developed on the surface of the mixture. After adding chloroform and sulphuric acid, layer separation was observed. In petroleum ether extract, upper layer turned into dark red and bottom layer turned into pale yellow with green fluorescent.

+++=Present in higher amounts, ++ = moderately present, + = Present, - = absent

The qualitative phytochemical analysis of three different varieties of *Coffea arabica* in petroleum ether extract is shown in Table 1. Three different varieties contain high amounts of carbohydrate, flavonoids, alkaloid, steroid and saponin. Different solvents with different polarities extract

Table 1: Qualitative phytochemical screening of *Coffea arabica* in petroleum ether extract.

Compound	Coffee Varieties		
	T_1	T_2	T_3
Alkaloid	+++	++	++
Flavonoid	+++	+++	+++
Carbohydrate	+++	+++	+++
Saponin	+++	+++	+++
Steroid	++	++	+

specific phytochemicals in plants. But, here it seems to be that alkaloid like phytochemicals had been highly extracted into non-polar solvents like petroleum ether (Chen *et al.*, 2014).
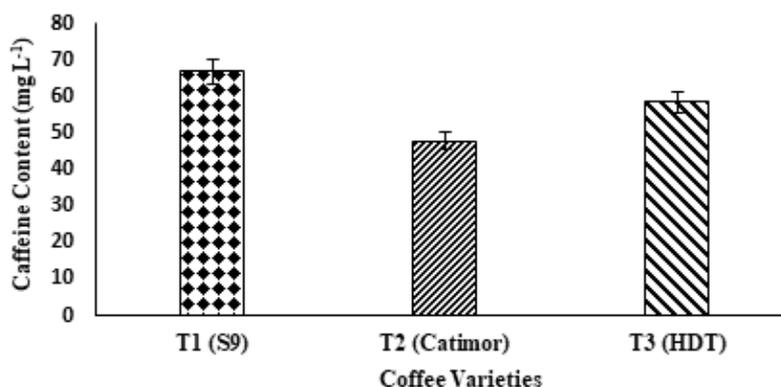
Comparative phytochemical characteristic analysis of three different varieties of *Coffea arabica*

Caffeine content in three different varieties of Coffea arabica

The caffeine content in three different varieties of roasted *Coffea arabica* powder is shown in Figure 1. Caffeine Content of S9, Catimor and HDT varieties were 66.71 mg L^{-1}, 47.63 mg L^{-1} and 58.43 mg L^{-1} respectively. Caffeine content was significantly different ($p<0.05$) among varieties. The S9 variety showed the highest caffeine content while the Catimor variety showed lowest caffeine content. According to the Kebena *et al.* (2017), caffeine content of *Coffea arabica* in South West Ethiopia was 57.23 to 64.61 mg L^{-1}

**Total antioxidant activity (Phosphomolybdenum assay)**

Coffee is a rich source of dietary antioxidants, and this property, coupled with the fact that coffee is one of the world's most popular beverages, has led to the understanding that coffee is a major contributor to dietary antioxidant intake. As shown in Figure 2, variety S9 coffee extract had significantly high ($p<0.05$) total antioxidant activity compared to that in extracts from other two varieties. These differences are due to the genetic variation among the varieties. In accordance with literature (Daglia *et al.*, 2000), a rise of total anti-oxidant activity from the green coffee beans to the light roasting degree was detected. It is due to a release of highly active low molecular weight phenols from the green coffee constituents by moderate heating (Montavon *et al.*, 2003). Antioxidant content of *Coffea arabica* in Brazil, Columbia, Ethiopia and India (according to region of origin) were 0.95, 1.01, 1.03, 1.17 mmol Trolox/g respectively (Ewa *et al.*, 2017).

The values are means of triplicates
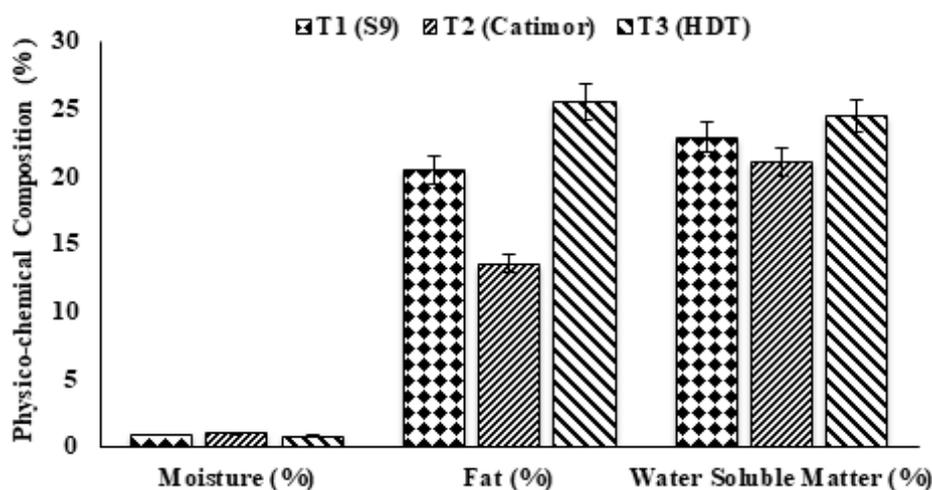The vertical bars indicate the standard errors

Figure 1: Caffeine content of three different varieties of *Coffea arabica.*
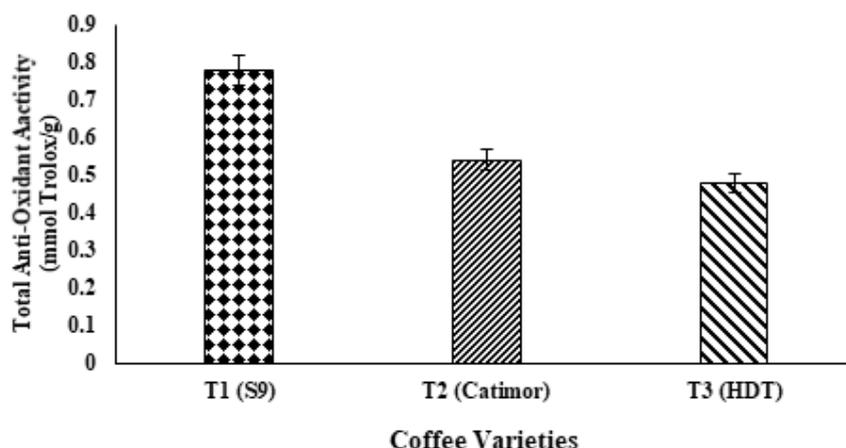
The values are means of triplicates
The vertical bars indicate the standard errors

Figure 2: Total antioxidant activity of three different varieties of *Coffea arabica.*

The values are means of triplicates
The vertical bars indicate the standard errors

Figure 3: Moisture, fat and water soluble matter of three different varieties of *Coffea arabica*.

Comparative proximate analysis of three different varieties of *Coffea arabica*

Moisture percentage of three different varieties of Coffea arabica

Figure 3 indicates the moisture content in three varieties of roasted *Coffea arabica* powder. Moisture content in the roasted *Coffea arabica* powder was significantly different among varieties (p<0.05). It was observed that Catimor variety showed highest (p<0.05) moisture content while HDT variety showed significantly lowest (p<0.05) moisture content. Corresponding values were reported by Ismail and Anuar, (2013) were 12.54% for *Coffea liberica*.

Fat percentage of three varieties of Coffea arabica

Figure 3 indicated the fat content in three different coffees of roasted *Coffea arabica* powder. Fat content was significantly different (p<0.05) among varieties and HDT variety had the highest fat (p<0.05) content while Catimor variety showed the lowest. The values for oil content in roasted coffees are higher than those for green beans due to the beans dry matter loss during roasting, which in turn varies with the degree of roast (Speer and Kölling-Speer, 2006).

Water soluble matter percentage of three different varieties of *Coffea arabica*

Figure 3 indicated the water soluble matter in three different varieties of roasted *Coffea arabica* powder. It was observed that the highest water soluble matter content was in HDT variety while lowest water soluble matter content was shown by Catimor variety. Water soluble matter content was significantly different (p<0.05) among varieties. Corresponding values were reported by Rajesh Kumar *et al.* (2014).

Total ash percentage of three different varieties of *Coffea arabica*

Figure 4 indicated the total ash content in three different varieties of roasted *Cofeea arabica* powder. The S9 variety showed highest total ash content and the HDT variety showed lowest total ash content. However, there was no any significant difference between S9 and Catimor, Catimor and HDT varieties (p>0.05). There was a significant difference (p< 0.05) in total ash content between S9 and HDT varieties. Corresponding values were reported by Ismail and Anuar, (2013) were 3.71% for *Coffea liberica*. Values obtained in this study for ash percentage in three *Coffea arabica* varieties were significantly higher than the comparable findings reported for other *Coffea* species.

Acid insoluble ash percentage of three different varieties of *Coffea arabica*

The acid insoluble ash content in three varieties of roasted *Coffea arabica* powder are shown in Figure 4.

The variety S9 showed the highest acid insoluble ash content and HDT variety showed the lowest acid insoluble ash content. But the S9 and Catimor, S9 and HDT varieties showed significant difference among each other (p<0.05). Corresponding values were reported by Rajesh Kumar *et al*. (2014).

Comparative mineral analysis of three different varieties of *Coffea arabica*

Coffee contains many micronutrients such as magnesium, potassium, niacin, and vitamin E. and other important minor compounds. Coffee contains many minerals and vitamins, particularly and nicotinic acid. Composition of the coffee depends on the following factors such as the quantity of ground coffee, the brewing method, the quality of the water used, and the other ingredients used in the coffee (Moreira *et al*., 2017; Tran *et al*., 2017). Generally, the concentration of twenty-seven elements are (Li, Be, B, Mg, Al, P, K, Ca, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Cd, Sn, Sb, Ba, Hg, Pb, Bi, H, and U) in green coffee (Semen *et al*., 2017). Table 2 indicated the phosphorous, potassium, magnesium, calcium, ferrous and zinc content in three different varieties of roasted *Coffea arabica* powder.
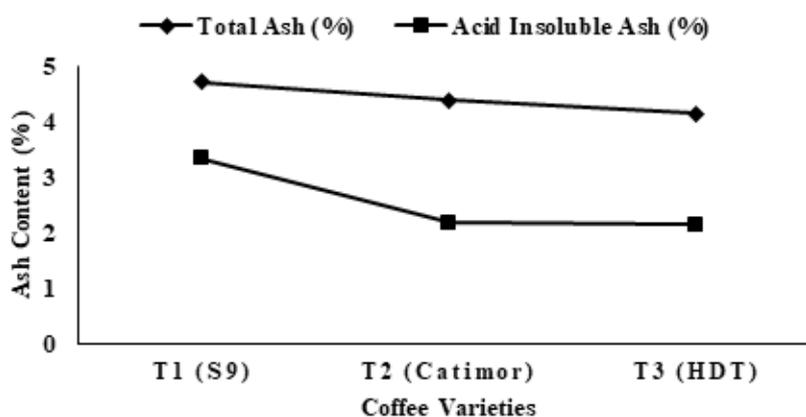
The values are means of triplicates

Figure 4: Total ash and insoluble ash of three different varieties of *Coffea arabica.*

Table 2: Mineral contents of three different varieties of *Coffea arabica*

Variety	P (%)	K (%)	Mg (%)	Ca (%)	Fe (%)	Zn (%)
S9	1.89±0.02[a]	1.31±0.01[a]	0.16±0.02[a]	0.49±0.03[a]	0.45±0.01[a]	0.04±0.02[a]
Catimor	1.58±0.04[b]	1.27±0.02[a]	0.11±0.05[b]	0.65±0.02[a]	0.57±0.04[b]	0.01±0.05[ab]
HDT	1.29±0.05[c]	1.24±0.01[a]	0.18±0.01[a]	0.58±0.01[a]	0.43±0.01[a]	0.03±0.03[b]

The values are means of triplicates ± standard error
The means with the same letters are not significantly different at 5% level

Table 3: Sensory evaluations on color of three different varieties of *Coffea arabica.*

Variety	Color	Aroma	Taste	Mouth feel	Overall Acceptability
S9	4.93±0.02[a]	4.86±0.01[a]	4.44±0.02[a]	4.13±0.02[a]	4.59±0.02[a]
Catimor	4.72±0.03[ab]	4.13±0.013[c]	3.64±0.01[c]	3.75±0.03[b]	3.75±0.03[b]
HDT	4.87±0.01[a]	3.42±0.02[b]	4.29±0.01[b]	4.29±0.03[a]	4.56±0.01[a]

The values are means of 30 replicates ± standard error
The means with the same letters are not significantly different from each other at 5% level

The S9 variety showed significantly ($p<0.05$) highest phosphorous content and the HDT variety showed significantly lowest phosphorous content among other varieties. Three varieties showed significant difference among each other ($p<0.05$). The S9 showed highest potassium content compared to the other varieties. HDT variety showed lowest potassium content compared to the other varieties. There were no any significance differences among three coffee varieties ($p>0.05$).

The HDT variety showed the highest magnesium content and the Catimor variety showed the lowest magnesium content. S9 and HDT varieties did not show any significance difference between each other ($p>0.05$). The Catimor variety showed the highest calcium content compared with the other two varieties. There was no any significance difference among each variety ($p>0.05$). There was a significant difference ($p<0.05$) in ferrous content among varieties and Catimor variety showed the highest ferrous content and the HDT variety showed lowest ferrous content. There was no significant difference ($p>0.05$) in ferrous content between S9 and HDT varieties. Zinc content was significantly different($p<0.05$) among varieties and S9 variety showed the highest zinc content and the Catimor variety showed the lowest zinc content.

Sensory evaluation on aroma of three different varieties of *Coffea arabica*

The Table 3 indicated that the S9 variety showed significantly ($p<0.05$) highest value and the Catimor variety showed significantly ($p<0.05$) lowest value for aroma. A significant difference ($p<0.05$) was observed for aroma between S9 and HDT varieties. The panelists preferred S9 variety than others and according to hedonic scale panelists like S9 variety moderately based on overall acceptability.

CONCLUSIONS

This study was carried out to find out phytochemical, physico-chemical composition, mineral content and total antioxidant activity of roasted *Coffea arabica* powder collected from three different varieties such as Hybrido-De-Timor (HDT), HDT x Takari (S9), HDT x Catura (Catimor). Phytochemical screening and physico-chemical analysis revealed that the S9 variety is richest in caffeine content and total ash content, while the Catimor and HDT varieties are richest in moisture and crude fat content, respectively. In mineral analysis, phosphorus, potassium and zinc content were highest in S9, while magnesium content was highest in HDT variety. Catimor variety contains significantly (p<0.05) higher amount of ferrous and calcium compared to other two varieties. In sensory evaluation coffee brewed from S9 was the most preferred in terms of color, taste, aroma and overall acceptance.

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CONFLICT OF INTEREST

The Authors declare that there is no conflict of interest.

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