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Phytochemical and physiochemical characterization of compounds of different coffee genotypes seeds

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Abstract

The experiment was conducted on coffee seed imported from different countries i.e., Rwanda and Vietnam during 2015-16 to find out the physico-chemical and phytochemical properties. Phytochemical analysis revealed that certain phytochemicals i.e. flavonoids, saponins, carbohydrates, glycosides, carotenoids were found positive both in seed varieties while proteins were found negative. Amino acid was observed in 0.0063% Rwandan and 0.0065% in Catimor. The polyphenol was found 5.2% in Rwandan and 6.5% in Catimor while 22.4% caffeine in Rwandan and 16.9% in Catimor seeds were detected. Physiochemical analysis revealed that 12% moisture content was observed in Rwandan while 8.3% in Catimor. In addition, 6.66% ash content was recorded in Rwandan and 4.33% in Catimore. It proved that Rwandan variety better than the catimor in all respectively.

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Introduction

Coffee is one of the most popular and widely consumed beverages throughout the world due to its pleasant taste and aroma and its stimulant effect. More than 80 species of the genus *Coffea* L. (Rubiaceae) are known. The most important are *Coffea arabica* and *Coffea canephora*, which account, respectively, for about 75% and 24% of the world production. Coffee is an expensive raw material, especially *Arabica* coffee, and over the years many fraudsters have been tempted to falsify the product declaration due to the increasing practice of selling coffees on the basis of their botanical and/or geographic origin (Prodolliet, 1996).

The main difference between the varieties of coffee beans seems to be the composition of the unsaponifiable matter (Folstar, 1989). Epidemiologic surveys have shown that high coffee consumption is associated with lower incidences of obesity (Rajpathak *et al.* 2006) and type 2 diabetes (Van Dam, 2002; Bhuparthiraju *et al*., 2013). These beneficial effects of coffee are also observed with decaffeinated coffee. Of the known active compounds in coffee, polyphenols have been shown to protect against oxidative stress (Vitaglione, 2010) and reduce risks for cardiovascular disorders and diabetes (Goya, 2007).

Coffee consumption is also associated with lower incidences of nonalcoholic fatty liver disease and the progression of nonalcoholic fatty liver disease to nonalcoholic steatohepatitis or hepatocellular carcinoma (Molloy, 2012). Furthermore, it has been observed that coffee polyphenols could down regulate lipogenic pathways and reduce liver fat accumulation in high-fat fed mice (Vitaglione, 2010; Murase, 2011; Murase, 2012). These findings have suggested that coffee polyphenols may specifically prevent tissue diet-induced ectopic lipid deposition and insulin resistance. Keeping in view the therapeutic significance of Coffee seeds, the present study developed with the aims and objectives were .i) evaluated the phyto-chemical parameters i.e. Caffeine, amino acid, polyphenols ii) Physicochemical analysis of seeds i.e. moisture contents, ash contents.

Materials and methods

Location/Site

The study was conducted at National Tea & High Value Crops Research Institute, Shinkiari, Mansehra during the period 2015-16. Two varieties of coffee seeds i.e. “Rwandan and Catimor H-528” were imported from Rwanda and Vietnam from coffee research institute of both the countries.

Dormancy Test

Initially upon arrival of the seed from abroad both the exotic varieties were tested for dormancy and found to utilize for experimental trial.

Selection for pre-treatment of seeds

Coffee seeds of two varieties (Rawanda and Catimor) were selected randomly for analysis both the varietal seeds were put in fine plastic bags duly labeled with number and date of selection of the samples. Standard botanical methods were followed for the Selection of the seeds. Multiple specimens of the seeds were selected for broad spectrum of analysis and to analyze statistically. Before analysis, sample was inspected for any visible dirt and insect parts were removed and were tested for physicochemical properties and phytochemical properties like polyphenol, amino acid and caffeine. (Limyati *et al.,* 1998; Kudva *et al*., 1998, USP-30, Lim and Murtijaya, 2007, Hazra *et al*., 2008).

Determination of proximate composition

Moisture content

Proximate composition of seeds was determined as follows: Moisture was determined by Standard Official Methods of Analysis of the AOAC (1990) (method 14:004).

Ash content

Total ash was determined by Furnace Incineration described by AOAC (1990) (method 14:006) using about 1.0 g of finely grounded dried samples.

Caffeine analysis

Coffee liquor with a volume of 20ml was taken and infused into a 250ml volumetric flask and added 10ml 0.01N hydrogen chloride and 2ml basic lead acetate,

hereafter diluted to 250ml with distilled water and shaken vigorously, laid aside for clarification and then filtered the solution. Filtered liquor of 50ml was taken and infused into a 100ml and shaken thoroughly and evenly filtered the solution. The optical density E of the filtered solution at 274nm is obtain by UV-spectrophotometer with 10nm quartz colorimetric cup, using distilled water as blank reference Watch critically for the caffeine content from standard curve as per optical density E with the following formula has been applied for determination.

$$\text{Caffeine (\%)} \frac{C/10000x1x150/20x100/50}{MxMr} \text{ x } 100$$

C = Caffeine content (mg/ml) checked out from the standard curve as per the optical density E of the tested solution

L1 = Total volume of liquor (ml)

M = Weight of sample (mg)

Mr = Net weight of sample excluding moisture content

Polyphenol analysis

One ml of liquor was taken with pipette and put into a 25ml volumetric flask and added 4ml distilled water and 5ml iron titrate solution, afterward diluted to 25ml with pH 7.5 buffer solution and gradually shaken gently. The optical density E at 540nm was obtained by spectrophotometer with 10mm colorimetric cup. As a reference iron titrate solution was used (Yuan Long ping, 1999).

$$\text{Polyphenol} = \frac{C/Ex3.913}{1000} \text{ x } \frac{C/L1}{L2xMxMr} \times 100$$

L1 = Total volume of liquor

L2 = Pipettes volume of liquor

M = Sample Weight

Mr = Net weight of sample excluding moisture content

Amino acid analysis

One ml of liquor was taken and infused into 25ml volumetric flask and added 0.5ml of pH 8.0 buffer solution and 0.5 ml of 2% Ninhydrin solution to the volumetric flask and heated for 15 minutes in boiling water and cooled to room temperature and diluted to 25 ml with distilled water and laid aside for 10 to 15 minutes (Yuan Long ping, 1999).

The optical density E of the solution at 570 nm obtained by spectrophotometer with 5 mm cholorimetric cup by using distilled water as a blank (reference).

$$\text{Amino acid (\%)} = \frac{C/NxL1/L2x1/1000}{MxMr} x100(=9.5 \text{ x } E)$$

N= Weight of amino acid per ml obtained from the standard curve, the experimental data is 0.57 mg.

L1= Total volume of liquor (ml)

L2= Pipette volume of liquor (ml)

M= Weight of samples (gram)

Mr= Net weight of sample excluding moisture content.

Preliminary phytochemical screening of various extracts

Preliminary phytochemical screenings of various extracts and drug powder were carried out as per the standard textual procedure (Harborne, 1973).

Test for Saponins

The seed extracts was shaken well with water and observed the formation of foam which was stable for 15 minutes indicates being for positive results.

Test for Terpenoids (Salkowshi test)

Two ml of the ethanolic extract of seeds were dissolved in 2 ml of chloroform and evaporated to dryness. Two ml of concentrated sulphuric acid was added and heated for about 2 minutes. Gradually the development of a greyish colour in final stage indicates the presence of terpenoids.

Test for Flavonoids (Ferric chloride test)

The test solution was treated with few drops of ferric chloride solution would result in the formation of blackish red colour indicating the presence of flavanoids.

Test for Alkaloid (Hager's test)

The solution was treated with few drops of Hager's reagent (saturated picric acid solution). Formation of yellow colour precipitate shows a positive result for the presence of alkaloids.

Test for Proteins (Biuret Test)

Test solution with 10% sodium hydroxide and two drops of 0.1% copper sulphate and observed for the formation of violet/pink colour to determine the proteins.

Test for carbohydrate (Benedict's test)

Test solution was mixed with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled in water bath, observed for the formation of reddish brown precipitate to show a positive result for the presence of carbohydrate.

Tests for glycosides: Liebermann's test

Two ml of the organic extract was dissolved in 2 ml of chloroform and then 2 ml of acetic acidwas added in it. The solution was cooled well in ice and Sulphuric acid (H_2SO_4) was added carefully. A colour change from violet to blue to green indicates the presence of a steroidal nucleus (a glycone portion of glycoside).

Test for carotenoids

One g of each sample was extracted with 10 ml of chloroform in a test tube with vigorous shaking. The resulting mixture was filtered and 85% sulphuric acid was added. A blue colour at the interface showed the presence of carotenoids.

Results and discussion

Experimental results were carried out accordingly and analyzed statistically and observe the significance level at P>0.05 of probability.

Fresh Weight

Result in table.1 shows that no significant relation was statistically proved but mean values showed that the maximum fresh weight (1.13g) for Rwandan Variety, while minimum (0.83g) recorded for Catimor Variety.

Dry Weight

In case of the dry weight (Table .1) the maximum dry weight (0.88g) for Rwandan whereas the minimum weight (0.73g) recorded for the Catimor Variety. The results were observed non-significant statistically.

Moisture contents

Maximum average moisture content 12% was showed by Rwandan variety and 8.33% moisture content was observed in Catimor variety.

Table 1. Coffee seeds Parameters studied in situ.

Varieties	Fresh Weight (g)	Dry weight (g)	Moisture Content %	Ash Content %
Rwandan	1.13a	0.88a	12a	6.66a
Catimor	0.83a	0.73a	8.33a	4.33a
LSD (0.05)	0.74	0.32	3.4	3.4

Fig 1-2. Moisture and Ash content of both varieties of Coffee seeds.

Ash content

Average ash content 6.66% was reported in Rwandan variety and 4.33% ash content was observed in Catimor. It was examined that average ash content of both varieties were not significantly differ but mean values shows the variation as shown in Table 1.

Table 2. Coffee seeds Parameters studied in situ.

Varieties	Amino Acid %	Caffine %	Polyphenol %
Rawanda	0.0063a	22.4a	5.2a
Catimore	0.0065a	16.93b	6.5a
LSD (0.05)	0.002	1.65	5.58

Amino acid analysis

Average 0.0063% of amino acid was observed in Rwandan variety and 0.0065% was observed in Catimor variety as presented in Table 2.

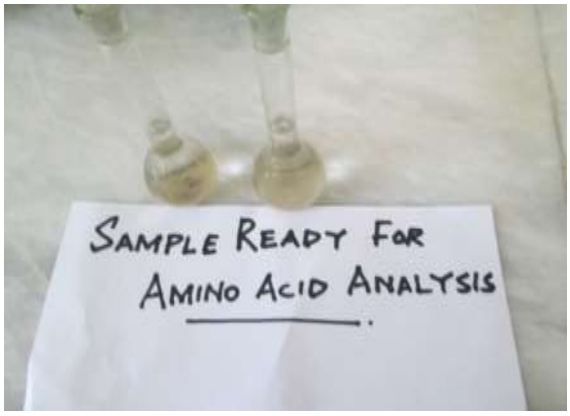

Fig 3. Amino acid analysis of both varieties of coffee seeds.

Caffiene analysis

Average 22.4% of caffeine content was reported in Rwandan variety while 16.9% in Catimor variety. It was examined that caffeine content of Rwandan variety is significantly higher than that of Catimor variety.

Fig. 4. Caffiene analysis of both varieties of coffee seeds.

Polyphenols analysis

Average 3.16% of polyphenol was recorded in Rwandan variety while 6.5 % was examined in Catimore variety.

Fig. 5. Polyphenol analysis of both varieties of coffee seeds.

Phytochemical analysis of coffee seeds

The phyrochemical analysis of coffee seeds of both exotic varieties were carried out in situ at NTHRI, Shinkiari during the period 2015-16. However, the results reviled that alcholides and saponins were found positive in both the varieties respectively. Due to the presence of saponins and showing positive results in the plant which presumed that the cytotoxic effects may also be positive which mean that it has some effects on human body due to the presence of saponins. As concerned for the research carried out on the saponins, it can only be used for externally rather internally e.g. the saponins presence in the tea seeds had very toxic effects when it was used in *animalia* kingdom (Waheed *et. al.,* 2014) while the presence of alkaloids showed that plant has analgesic, antispasmodic and bacterial properties of saponins. It has been showed antibacterial properties of the plant as the results are at par with (Chung *et al.,* 1998). Tannins were found positive in both varieties. Tannins inhibit the growth of many fungi, bacteria and viruses (Chung *et al.,* 1998).

Glycosides and carotenoids were found positive. Whereas it has been reported by David, (1983) Glycosides have the ability to increase the efficiency of heart beat and impulses.

It mean, it has a very good effects of glycosides which not only increase the heart beat but also stable the functioning of heart to normalize accordingly.

Table 3. Phytochemical analysis of both varieties of Coffee seeds.

Varieties	Fla	Sap	Alk	Tan	Pro	Carb	Gly	Cart
Rwandan	++	++	++	++	--	++	++	++
Catimor	++	++	++	++	--	++	++	++

++ indicate Presence, -- indicate Absence.

Alk, Alkaloids; Fla, Flavonoids, Sap, Saponins; Pro: Protein, Carb, Carbohydrates; Cart; carotenoids; Gly; Glycosides; Tan; tannins.

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