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Original Research Article

Comparative Study of Phytochemical Constituents, Caffeine Levels and Proximate Composition of Liven Alkaline Coffee, Nescafe Original Coffee and Nescafe Original Decaffeinated Coffee

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Abstract: Coffee is a brewed beverage which is made from the bean seeds of plants belonging to the Rubiaceae family. The consumption of coffee has grown over time for its appreciated taste and beneficial effects on health. Commercial samples of Liven alkaline coffee, Nescafe original coffee and Nescafe original decaffeinated coffee were subjected to phytochemical screening in order to detect classes of phytochemical compounds present in them. Proximate analysis was conducted as well using standard procedures. The results of the study showed that the coffee samples contained a significant number of phytochemicals which includes carbohydrates, flavonoids, saponins, alkaloids, tannins and polyphenols. The Liven alkaline coffee have highest quantity of alkaloids (3.10 ± 0.82 %) and carbohydrates (0.134 ± 0.009 mgGE/g) compared to the Nescafe original coffee; alkaloids (1.43 ± 0.80 %) and carbohydrate (0.031 ± 0.005 mgGE/g) and the Nescafe original decaffeinated coffee; alkaloids (2.57 ± 0.12 %) carbohydrate (0.041 ± 0.010 mgGE/g). The caffeine content of the three samples varied appreciably with the Nescafe original coffee containing 132.25 PPM, Liven alkaline coffee having 20.53 PPM and Nescafe original decaffeinated coffee having 8.33 PPM. The proximate analysis revealed that Liven alkaline coffee have the highest ash content with 3.3 % while Nescafé original coffee have the highest moisture content with 5.1 %.

Keyword: Proximate analysis, Coffee, Caffeine, Decaffeinated, Phytochemicals, Alkaloids.

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INTRODUCTION

Coffee is the most commercialized and widely consumed beverage in the world today (ICO, 2011). Coffee beans which are used to produce this beverage are seeds of a particular plant family called Rubiaceae (Clarke, 2003). Only two species of this family are of economic importance, they include *Coffea arabica* and *Coffea robusta* (WRI, 2010). Arabica and Robusta differ in a number of ways, including their chemical composition, physical aspects, ideal growing climates and characteristics of the brew.

High caffeine consumption produces adverse health effects, based on several comprehensive human studies (Hart & Smith, 1997). Caffeine is the major component of the coffee beverage. Other bioactive compounds include flavonoids, anthocyanins, terpenes, tannins, steroids, phenols, glycosides, gallic acid and alkaloids (Ali *et al*., 2012). Caffeine is a heat stable methyl xanthine with bitter characteristic taste which is responsible for the bitterness perceived from the coffee beverage (Bee *et al.*, 2004). Caffeine functions in stimulating the central nervous system by acting as adenosine-receptor antagonists (Kong *et al*., 2008).

In Nigeria today, coffee is a very popular household and workplace beverage, and its consumption has increased largely in recent times (Musatto *et al*., 2011). The most consumed brand of coffee in Nigeria is the Nescafé coffee, especially the original type of this brand (Mussato *et al*., 2011). A broad variety of coffee products, to which other healthy ingredients such as popular herbs or green tea are added to strengthen its effectiveness, has appeared on the

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market. One of those coffee brands is called the Liven alkaline coffee. Liven coffee is the world's first alkaline coffee and has been manufactured and sold in the Philippines, Malaysia, Japan and China. It is a high-quality product and highly regarded for its distinctive taste and aroma and is made of the finest species of *Coffea arabica* added with complete Phyto-energizer (Aim Global, 2016). There is limited qualitative and quantitative study that compares the phytochemical constituents, caffeine level and proximate compositions of the different coffee brands; Liven alkaline coffee (LAC), Nescafe original coffee (NOC) and Nescafé original decaffeinated coffee (NODC), hence the need for this research.

MATERIALS AND METHODS

The Liven alkaline coffee (LAC), Nescafe original coffee (NOC) and Nescafé original decaffeinated coffee (NODC) were gotten from a supermarket in Lagos Nigeria.

In-vitro Phytochemical Assay

Phytochemical screening was carried out using colorimetric method. For each assay, 10 mg of each coffee sample was dissolved in approximately 3.0 mL of distilled water in a test tube, obtaining test solution for each sample.

Qualitative Phytochemical Screening

Test for Carbohydrates

Fehling's test for carbohydrates was employed. 5 mL of Fehling's solution was added to 0.5 mg of the coffee samples and boiled in a water bath. The mixtures were then observed for colour change in the test tubes. A red or yellow precipitation indicates the presence of carbohydrates (Brain & Turner, 1975).

Test for Saponins

0.5 g of the coffee samples was boiled with 2 mL of distilled water in test tubes, allowed to cool and shaken well to mix thoroughly. The mixtures were then observed for frothing or foaming (Sofowora, 1993).

Test for Flavonoids (Shinoda Test)

1 mL of 2 N sodium hydroxide was added to 2 mL of the coffee samples. The mixtures were then observed for colour change in the test tubes. A yellow coloration indicates the presence of flavonoids (Brain & Turner, 1975).

Test for Tannins (Ferric Chloride Test)

2 ml of 5 % ferric chloride was added to 1 mL of the coffee samples. The mixtures were then observed for colour change in the test tubes. A greenish coloration indicates the presence of tannins (Harborne, 1973).

Test for Alkaloids (Mayer's Test)

To 2 mL of coffee samples, 2 mL of concentrated hydrochloric acid was added. Then a few drops of Mayer's reagent were added. The mixtures were then observed for colour change in the test tubes. A cloudy yellow precipitation indicates the presence of alkaloids (Harborne, 1973).

Test for Phenols (Ferric Chloride Test)

1 mL of the coffee samples was added into test tubes followed by 2 mL of distilled water and a few drops of 10 % ferric chloride. The mixtures were then observed for colour change in the test tubes. A greenish coloration indicates the presence of phenols (Brain & Turner, 1975).

Quantitative Phytochemical Screening

Determination of Carbohydrate

The carbohydrate content was determined using Anthrone colorimetric method for determining the concentration of total sugars in a sample. Sugars react with the anthrone reagent uder acidic conditions to yield blue green colour (Morris, 1999).

Procedure: 10 µL of the coffee samples and glucose standard at different concentrations were mixed with sulfuric acid and the anthrone reagent and then boiled for about 4 minutes. The solutions were then allowed to cool, and their absorbance measured at 620 nm (Morris, 1999).

There is a linear relationship between the absorbance and the amount of sugar that was present in the original sample. This method determines both reducing and non-reducing sugars because of the presence of the strongly oxidizing sulfuric acid.

Saponin Determination

Determination of total Saponin content was determined by the method describes by Makkar *et al.* based on vanillin-sulfuric acid colorimetric reaction with some modifications.

Procedure: 50 µL of the coffee samples and different dilutions of standard was added with 250 µL of distilled water. To this, about 250 µL of vanillin reagent (800 mg of vanillin in 10 mL of 99.5% ethanol) was added. Then 2.5 mL of conc. Sulphuric acid was added, and it was mixed well. The solutions were kept in a water bath at 60 ^{0}C for 10 min. after 10 min, they were cooled in ice cold water and the absorbance values were read at 544 nm. The values were expressed as diosgenin equivalents derived from a standard curve (Makkar *et al*., 2007).

Flavonoid Determination

Aluminium chloride colorimetric method was used for the determination of total flavonoids in the coffee samples.

Preparation of quercetin: 10% quercetin was prepared by dissolving 0.5 g of quercetin in 50 mL of distilled water.

Preparation of Aluminium chloride: 10% aluminium chloride was prepared by dissolving 10 g of aluminium chloride in 100 mL of distilled water. Preparation of potassium acetate: 28.615 g of potassium acetate was dissolved in 100 mL of distilled water.

Procedure: 1 mL of the dissolved coffee samples and different dilutions of standard quercetin were mixed with 0.2 mL of 1 M potassium acetate, 3 mL of methanol, 0.2 mL of 10% aluminium chloride and 5.6 mL of distilled water. The mixtures were maintained at room temperature for about 30 minutes. The absorbance of the mixtures was measured at 415 nm (Chang *et al*., 2002).

A standard curve for quercetin in methanol was prepared using different concentrations. The total phenolic content was expressed in terms of quercetin equivalents (Chang *et al*., 2002).

Tannin Determination

Preparation of HCl: 1% HCl was prepared by diluting 1 mL of HCl in 99 mL of methanol. Preparation of HCl Vanillin: 8% HCl was prepared by diluting 8 mL of concentrated HCl in 92 mL of distilled water. 1% Vanillin was prepared by dissolving 1 g of Vanillin in 100 mL of methanol.

Procedure: 0.2 g of the coffee samples was placed in conical flask followed by the addition of 10 mL 1% HCl in methanol. The flask was capped and mixed continuously for 20 min. The mixtures were centrifuged at 2,500 g for 5 min. 1 mL of the supernatant and different dilutions of the tannin standard were pipetted into test tubes containing 5 mL vanillin HCl reagent and left to stand for 30 mins at 30 ^{0}C. Absorbance of the mixtures was measured at 450 nm. A standard curve was prepared to express the result as tannic acid equivalent (Hagerman *et al*., 2000)

Alkaloid Determination

The total alkaloid in the coffee samples was determined using the Harborne method. Procedure: 5 g each of the samples was weighed into 250 mL beakers and 200 mL of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. These were filtered and the extracts were concentrated in a water bath to one quarter of the original using 100 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL, 500 µg/mL, 600 µg/mL and 700 µg/mL. The total phenolic content was expressed in terms of gallic acid equivalents (Siddhuraju & Becker, 2003).

Proximate Analysis

The analysis of the proximate composition of the NOC, NODC and LAC was carried out to determine the moisture, ash and reducing sugar contents using the official methods of analysis of the Association of Official Analytical Chemists (AOAC, 2003).

RESULTS

PHYTOCHEMICAL ANALYSIS

Qualitative Analysis

The LAC coffee shows high presence of carbohydrate with moderate presence of alkaloids and flavonoids, while NOC and NODC indicate high presence of flavonoids. Tannins is also very high in NOC coffee as seen in Table 1.

Table-1: Qualitative Phytochemical profile of the LAC, NOC, and NOD coffee samples

PHYTOCHEMICALS	Liven alkaline coffee	Nescafe original coffee	Nescafe original decaffeinated coffee
Carbohydrates	+++	+	+
Saponins	-	++	++
Flavonoids	++	+++	+++
Tannins	+	+++	+
Alkaloids	++	+	+
Phenols	+	++	++

-: Absent, +: Present, ++: moderately present and +++: highly present

LAC- Liven alkaline coffee, NOC- Nescafe original coffee, NODC- Nescafe original decaffeinated coffee

Quantitative phytochemical analysis

The total carbohydrate, saponin, flavonoid, tannins, alkaloid, and polyphenols in LAC, NOC, NODC coffee were quantified as shown in Table 2. The LAC has the highest quantity of carbohydrate (0.134 ± 0.009 mgGE/g) and alkaloid (3.100 ± 0.820 %) when compared with NOC (0.031 ± 0.005 and 1.430 ± 0.800 %) and NODC (0.041 ± 0.010 and 2.570 ± 0.120) respectively.

Table-2: Quantitative phytochemical profile of the LAC, NOC, and NODC

Phytochemicals (Mean ± SD)	Sample volume (mL)	Liven alkaline coffee	Nescafe original coffee	Nescafe original decaffeinated coffee
Total carbohydrates (mgGE/g)	0.01	0.134 ± 0.009	0.031 ± 0.005	0.041 ± 0.010
Total saponin (mgDE/g)	0.05	0.016 ± 0.001	0.016 ± 0.001	0.027 ± 0.002
Total flavonoid (mgQUE/g)	0.05	0.070 ± 0.008	0.231 ± 0.028	0.222 ± 0.019
Total tannins (mgTAE/g)	0.05	0.465 ± 0.059	0.125 ± 0.101	0.554 ± 0.123
Total alkaloid (%)	0.05	3.100 ± 0.820	1.430 ± 0.800	2.570 ± 0.120
Total polyphenol (mgGAE/g)	0.05	0.020 ± 0.004	0.030 ± 0.004	0.031 ± 0.004

LAC- Liven alkaline coffee, NOC- Nescafe original coffee, NODC- Nescafe original decaffeinated coffee; SD- standard deviation

Caffeine level determination

The concentration of caffeine in the Liven alkaline coffee, Nescafé original coffee and Nescafé original decaffeinated coffee samples were 20.53 PPM, 132.25, PPM and 8.33 PPM respectively.

Table-3: Caffeine concentration of LAC, NOC, and NODC samples

Sample (0.05 ml)	Caffeine concentration (ppm)
Liven alkaline coffee	20.53
Nescafe original coffee	132.25
Nescafe original decaffeinated coffee	8.33

Proximate analysis

The results of proximate analysis of the coffee samples showing the moisture content, ash content and reducing sugar level are represented in the table below.

Table-4: Proximate analysis of LAC, NOC, and NODC samples

Sample	Parameters Moisture (%)	Ash (%)	Reducing sugar
Liven alkaline coffee	4.9	3.3	Not detected
Nescafe original coffee	5.1	3.1	Not detected
Nescafe original decaffeinated coffee	4.8	2.8	Not detected

DISCUSSION

Different physicochemical parameters are employed for standardization of products (Ahmad *et al*., 2014). The extractive values from assays are then used to determine the active constituents. Preliminary qualitative phytochemical analysis of the LAC, NOC, and NODC revealed the presence of alkaloids, flavonoids, phenols, Saponins, carbohydrates and tannins. These secondary metabolites are reported to have many biological and therapeutic properties (Narender *et al*., 2012), so these beverages are expected to have many medicinal uses.

Quantitative assays for the above listed phytochemicals were then carried out on the samples and this revealed that LAC contains the highest amount of alkaloid and carbohydrates but had the lowest concentration polyphenols, flavonoids and saponin. The NOC had the highest amount of saponin and flavonoid but had the lowest concentration of tannins, carbohydrates, and alkaloid. The NODC had the highest concentration of tannins and polyphenols. The alkaloid concentration of the LAC is in tandem with the quantitative work done on *Camellia sinensis* (green tea) by Madan *et al*. (2013). Also, analysis carried out by Gayathri *et al*. (2013) on *Coffea arabica* showed polyphenol and flavonoid concentration ranges of 0.3-0.6 mgGAE/g and 0.2-0.3 mg/g respectively which are in agreement with the results gotten from the Arabica based Nescafe coffee. The highest caffeine concentration in the coffee samples was seen in the Nescafe original coffee sample; this was followed by the NODC then the least was seen in liven alkaline coffee. The NOC having such high caffeine level is in agreement with previous work reported by Wanyika *et al*. (2010). The LAC caffeine content was almost as low as that of the decaffeinated coffee. The LAC contains a very moderate amount of caffeine which has a lot of therapeutic functions, and therefore would not cause any side effects that ordinary coffee would.

The results obtained from proximate analysis establishes that the moisture contents of the LAC, NOC, and NODC coffee samples are 4.9%, 5.1% and 4.8% respectively, which is low. The moisture content of any food is an index of its water activity and is employed as a measure of the susceptibility and stability of microbial contamination (Frazier & Westoff, 1978). The results

imply that the LAC, NOC, and NODC samples are very likely to have a long shelf life because of their low moisture contents. The ash content values recorded for the LAC, NOC, and NODC samples were in the same range, the range which is 3.3%, 3.1% and 2.8% respectively. This indicates that they all contain about the same level of mineral contents as the total cash value is a diagnostic purity index. Some of the minerals present in the three coffee samples could be assumed based on a previous proximate study carried out on *Coffea arabica* samples by Shripad *et al*. (2016), where minerals such as Na, K, N and Rb were present. No reducing sugars were detected on proximate analysis of all the coffee samples.

CONCLUSION

From the above results, it can be suggested that the three types of coffee contain a number of very essential phytochemicals. Therefore, LAC can be used as a good and easily accessible source of phytonutrients and moderate caffeine as their substances can reduce common health problems hence efficiency to live a healthy and productive life. Information from the proximate analyses reveals that the three coffee samples have long shell lives as well as a good mineral composition. *In-vitro* studies should be carried out on LAC, especially diabetic studies due to its high sugar content. LAC containing moderate amount caffeine can serve as a good source of caffeine.

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