West African Journal of Pharmacy (2022) 33 (1) 99 - 113

HPLC and chemical determination of caffeine content in selected tea samples sourced from supermarkets in Sagamu, Nigeria

Olufunmilayo E. Adejumo, Temitope D. Ogbonyenitan, Olatunde A. Ayodele

Department of Pharmaceutical and Medicinal Chemistry, Olabisi Onabanjo University, Sagamu, Nigeria.

Corresponding author: Olufunmilayo E. Adejumo
Email: funmijumo@yahoo.co.uk; funmijumo@oouagoiwoye.edu.ng;
Tel: +234 8052705549

ABSTRACT

Background: There are reports of abuse and toxicity resulting from taking caffeine, a psychoactive stimulant derived from various products like tea, which is known to increase alertness and elevate mood but prone to addiction.

Objectives: This study was aimed at determination of the caffeine content of green, herbal, and black tea samples sourced from supermarkets in Sagamu, south-west Nigeria by chemical and HPLC technique.

Methods: Chemical analysis was by precipitation, filtration followed by organic solvent extraction while the HPLC is equipped with a reverse phase C18 column, detection wavelength of 272 nm and run with a mobile phase of water: methanol (30:70% v/v).

Results: Caffeine eluted at about 1.58 mins to 1.65 mins. The calibration graph for the HPLC method was linear within the range of 10-100 µg/ml (R^2 = 0.9997) with the regression equation of y=73.88x-25.96. Percentage (%) caffeine by chemical analysis ranged from 0.13±0.01 to 1.72±0.13; and by h.p.l.c from 0.14±0.192 to 0.85±0.025 while caffeine content was below detection limit in two samples, (D, and I); where sample D had the lowest caffeine content by chemical method but undetectable by the h.p.l.c method. However, sample F had 0.66±0.20 by the chemical method but gave the lowest caffeine content of 0.14±0.192 by the h.p.l.c method. There was statistically significant difference between the mean results of the two methods ($t=4.931$, $p < 0.05$).

Conclusion: All tea samples contained caffeine in amounts that are low and safe, and that meets with regulatory specifications such as the US FDA. This has positive implications for public health and quality control of the tea samples.

Keywords: Caffeine, chemical analysis, HPLC, herbal tea, green tea, black tea.

Détermination par CLHP et par voie chimique de la teneur en caféine dans des échantillons de thé sélectionnés provenant de supermarchés à Sagamu, Nigeria

Olufunmilayo E. Adejumo[1], Temitope D. Ogbonyenitan[1], Olatunde A. Ayodele[1]

[1]Département de chimie pharmaceutique et médicinale, Université Olabisi Onabanjo, Sagamu, Nigeria.

Auteur correspondant : Olufunmilayo E. Adejumo
Courriel : funmijumo@yahoo.co.uk; funmijumo@oouagoiwoye.edu.ng;
Téléphone : +234 8052705549

RÉSUMÉ

Contexte : Il existe des rapports d'abus et de toxicité résultant de la prise de caféine, un stimulant psychoactif dérivé de divers produits comme le thé, qui est connu pour sa capacité augmenter la vigilance et améliorer l'humeur, mais qui est sujet à la dépendance.

Objectifs : Cette étude visait à déterminer la teneur en caféine d'échantillons de thé vert, de thé aux herbes et de thé noir provenant de supermarchés de Sagamu, dans le sud-ouest du Nigeria, par des techniques chimiques et CLHP.

Méthodes : L'analyse chimique a été effectuée par précipitation, filtration suivie d'une extraction par solvant organique, tandis que la CLHP est équipée d'une colonne C18 en phase inverse, d'une longueur d'onde de détection de 272 nm et d'une phase mobile d'eau et de méthanol (30:70% v/v).

Résultats : La caféine a été éluée à environ 1,58 min à 1,65 min. Le graphique d'étalonnage de la méthode CLHP était linéaire dans la gamme de 10-100 µg/ml (R^2 = 0,9997) avec l'équation de régression y=73,88x-25,96. Le pourcentage (%) de caféine par analyse chimique variait de 0,13±0,01 à 1,72±0,13 ; et par HPLC de 0,14±0,192 à 0,85±0,025 tandis que la teneur en caféine était inférieure à la limite de détection dans deux échantillons, (D, et I) ; où l'échantillon D avait la plus faible teneur en caféine par la méthode chimique mais indétectable par la méthode CLHP. Cependant, l'échantillon F avait une teneur de 0,66±0,20 par la méthode chimique mais donnait la plus faible teneur en caféine de 0,14±0,192 par la méthode CLHP. Il y avait une différence statistiquement significative entre les résultats moyens des deux méthodes ($t=4,931$, $p < 0,05$).

Conclusion : Tous les échantillons de thé contenaient de la caféine à des quantités faibles et sûres, conformes aux spécifications réglementaires telles que la FDA américaine. Cela a des implications positives pour la santé publique et le contrôle de la qualité des échantillons de thé.

Mots clés : Caféine, analyse chimique, CLHP, tisanes, thé vert, thé noir.

INTRODUCTION

Herbs, both wild and cultivated ones have found wide acceptance in the past and present for the preparation of refreshing drinks, such as teas which can be used for therapeutic or nutritional purposes, depending on the chemical constituents present. Tea is produced from the Camellia sinensis plant and is presumed to be the most widely consumed beverage in the world, that has been well investigated for its constituents and benefits for over 30 years.[1,2] Consumption of tea, particularly green tea (GT), has been correlated with low incidence of chronic pathologies that is linked with oxidative stress, such as cancer[3,4] and cardiovascular diseases (CVDs).[5,6,7] The many health benefits reported from studies on green tea (Camellia sinensis) include prevention of cancer, use as stimulant, diuretic, astringent (for controlling bleeding and help heal wounds) and to improve heart health,[6,7] treating flatulence, regulating body temperature and blood sugar, promoting digestion, and improving mental processes.[8] Other varieties of tea aside green, include black, white and oolong tea which differs only in their processing.

Green tea is made from unfermented leaves and reportedly contains the highest concentration of powerful antioxidants called polyphenols.[9] The health benefits ascribed to the consumption of teas may be related to the high content of bioactive ingredients such as polyphenols which have been reported to possess antioxidant, antiviral, and anti-inflammatory activities; modulate detoxification enzymes; stimulate immune function and decrease platelet aggregation.[10,11] Among all tea polyphenols which are flavan-3-ols and flavonols, epigallocatechin gallate (EGCG) has been found to be responsible for much of the health-promoting ability of green tea.[12] In general, green tea has been found to be superior to black tea in terms of health effects[13] owing to the higher content of EGCG, although the role of thearubigins and theaflavins contained in black tea requires additional investigation.

These compounds are present in lower amounts in black tea and are converted by oxidation to theaflavins and thearubigins.[14,15] Quercetin and kaempferol conjugates are the main flavonols in tea, with lower levels of myricetin. The conjugating moiety has been reported to vary from mono- to di- and triglycosides.[16,17] Other related compounds found in tea are Gallic acid and Quinic esters of gallic, coumaric, and caffeic acids, together with the purine alkaloids, xanthic bases (caffeine, theophylline, and theobromine), proanthocyanidins, and trace levels of flavones.[18,19] Hence, tea components exhibit antioxidant, anticarcinogenic, and antimutagenic activities that could protect humans against the risk of cancer by environmental agents.[20] Figure 1 gives the structures of some of these tea polyphenols.

Caffeine (1,3,7-trimethylxanthine) is a plant alkaloid which was first isolated from coffee in 1820 by a German chemist, Friedlieb Ferdinand Runge.[21] It is the most important naturally occurring xanthine derivative, which is found in many herbs such as extracts from guarana leaves, tea leaves, coffee beans, kola nuts, cocoa beans and thus in chocolate.[22] Therefore, natural caffeine is a psychoactive stimulant known to increase alertness, elevate mood and give temporary energy boost thereby easing fatigue. Synthetic caffeine is also added to products to promote arousal, alertness, energy, and elevated mood. Caffeine (mg/serving) found in green tea, black tea, and oolong tea are reported to be 20-45 mg/8oz, 47 mg/oz and 50 mg/190 ml respectively.[23,24] Caffeine in pure form, is a bitter white powder and structurally, it resembles the purines. In healthy individuals, the mean half-life of caffeine in plasma is about 5 hours. However, caffeine's elimination half-life may range between 1.5 and 9.5 hours, while its total plasma clearance rate is estimated to be 0.078 L/h/kg.[25], This wide range existing in the plasma mean half-life of caffeine can be attributed to both innate individual variations, and a variety of physiological factors and environmental characteristics that influence caffeine metabolism (e.g., obesity, oral contraceptives use, pregnancy, smoking, and altitude). It has been reported that the metabolism of caffeine in human cells produces three primary metabolites, paraxanthine (84%), theobromine (12%) and theophylline (4%)[27] occurring rapidly in the liver by demethylation within 1-3 h of exposure to millimolar concentrations.[28]

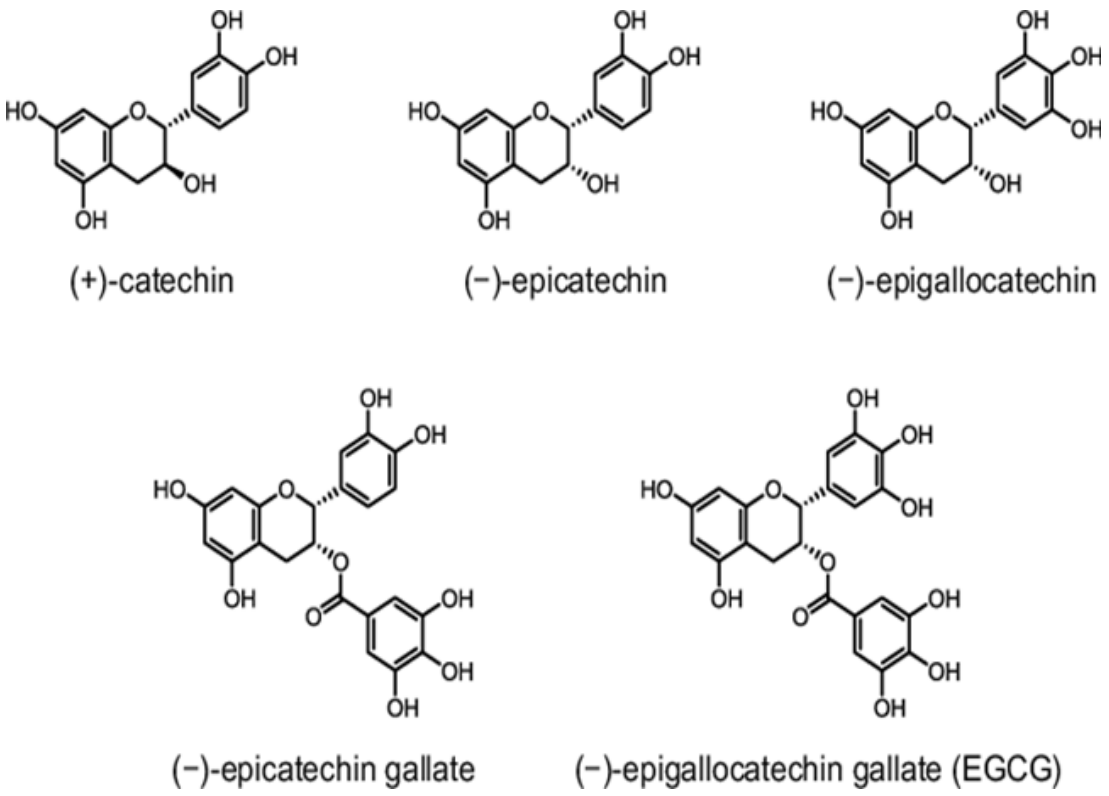

Fig 1. Chemical structures of (+)-catechin and four major green tea catechins. [29]

However, caffeine elicits pharmacological effects such as mild CNS stimulation and wakefulness, ability to sustain intellectual activity, and decreased reaction times like those of other methylxanthines.

Some studies show that consuming caffeine causes increase in blood pressure, diuresis, increase in blood sugar, increase in gastric acid and pepsin secretion, increased plasma levels of fatty acids, cortisol, and epinephrine, raised intraocular pressure and loss of calcium leading to bone loss.[30-34] Caffeine can cause addiction and thus present with adverse effects; and consumption of more than 1 g of caffeine can lead to death.[35] The US Food and Drugs Administration classification of caffeine consumption presents caffeine as safe, where caffeine intake of 130 - 300 mg/day is classified as low/moderate, and above 400 mg/day is high.[36] Determination of caffeine content in different brand of teas sold in Nigeria would give information and knowledge about the level of caffeine present in the tea samples.

Several chemical and physical methods have been reported for the determination of caffeine in tea leaves and other beverages that include chemical, ultraviolet spectroscopy, high-pressure liquid chromatography, Fourier Transform infrared spectroscopy, near infrared reflectance spectrometry, Raman spectroscopy and capillary electrophoresis.[37-40] The maximum spectrophotometric absorption of caffeine is in the ultraviolet range of 250-280 nm[37], which had resulted in the development of several methods for the determination of caffeine in samples using this absorption range. The method of choice with highest popularity and effectiveness is the high-performance liquid chromatography (HPLC).

This study sought to determine the amount of caffeine present in the selected tea samples in Nigeria. Caffeine is the most widely consumed psychoactive drug in the world[41-43] which is consumed for different purposes and is obtained from different sources such as beverages and medication. Caffeine is also a constituent of many over-the-counter pain relievers and prescription drugs consequent of its vasoconstricting and anti-inflammatory effects, which act as a compliment to analgesics, in some cases increasing the effectiveness of pain relievers by up to 40%.[44-48] It is reported to increase the effectiveness of these over-the-counter drugs for the treatment of conditions such as migraine and cluster headaches.[36]

Therefore, unintended over dosage or elevated padding of caffeine can occur. The main objective of the study was to ascertain the amount of caffeine present in different brands of tea readily available for sale in supermarkets located in southwest Nigeria. Consequently, the study aimed to comparatively determine caffeine content in such selected tea brands using chemical and HPLC analysis.

MATERIALS AND METHODS

Sample collection

Fifteen tea brands comprising five (5) Green teas (GT), two (2) Black teas (BT) and eight (8) herbal teas (HT) were randomly sourced for the study and purchased from various supermarkets within the Sagamu town, Ogun state, Nigeria. All analysis was done before the expiry date of the samples.

Equipment and reagents

Equipment used include a reversed phase high performance liquid chromatography (RP-HPLC) (Agilent 1100 HPLC series) equipped with a main controlling unit, quartenary pump, online degasser, waters x-bridge C18 column (Zorbax Eclipse XDB C18: 150 x 4.6mm, 5 µm particle size), with 20 µL injector loop and UV-Vis chem. station software detector. Reagents included Lead acetate, Chromic acid solution, Chloroform, Dil. HCl, and methanol all analar grade. Distilled water was used in the study. Caffeine anhydrous LR standard (ID 3812952; Batch 112A/0812/2307/13) was sourced from SDFCL Fine Chem Ltd. Mumbai, India.

Chemical analysis

Chemical analysis was according to the method of Parvathy et al. (2014).[49] Exactly 10 gm of tea leaves/coffee powder were mixed with 100 mL distilled water and heated up to extreme boiling. The solution was filtered, and lead acetate was added to the filtrate for the purpose of precipitation until a curdy brown precipitate was obtained. This was filtered again, and the filtrate was boiled until it was reduced to 25 mL. The solution was allowed to cool, and caffeine was extracted using chloroform in a separatory funnel. Chloroform was evaporated from the extract and the residue obtained which was caffeine was dried and weighed. The procedure was repeated for all tea samples and results were obtained in duplicate.[49] The following formulae was used to determine caffeine concentration.

$$\textit{Percentage yield of caffeine} = \frac{\textit{\% Dry Weight of caffeine}}{\textit{Weight of tea sample}} \text{ X } 100$$

Sample preparation for HPLC analysis

All the glassware were soaked overnight with chromic acid solution as a cleaning reagent and washed thoroughly with water and detergent, then rinsed with deionized water before use.

Cold Extraction of tea samples

1 g of the tea sample was weighed and dissolved with 10 mL of methanol which was allowed to stay for 72 hours. 1 mL of the filtrate was taken and made up to 10 mL with methanol.

Preparation of stock solution from standard caffeine

Caffeine stock solution (1000 µg/ml) was prepared by accurately weighing 100 mg of caffeine standard and transferring it quantitatively into a 100 mL volumetric flask, then making it up to the mark with the mobile phase. Working standards of 10, 20, 40, 60, 80 µg/ml were prepared by serial dilutions of the stock solution with the mobile phase.

HPLC analysis of caffeine

HPLC conditions for running the standard and samples comprised of a reversed phase Zorbax Eclipse XDB C18, 150 x 4.6 mm, 5µm, UV detector at 272 nm, and a flow rate of 1.00 ml /minute. Sample injection volume was 20 µL and run with a mobile phase of water: methanol (30:70 v/v). A calibration curve of peak areas versus concentration of the standard was plotted. The caffeine level of the various samples was calculated using regression equation of the best line of fit.

Validation parameters

The developed analytical method was validated according to the International Conference on Harmonization (ICH) guidelines.[50] Calibration curve was plotted for readings of peak area taken at five (5) serial dilutions at concentrations ranging from 10 µg/ml to 100 µg/ml. Concentration was then plotted against peak area and the concentration of caffeine in tea samples was calculated from the linear plot with equation y=73.88x-25.96; where y is the average peak area and x is the concentration.

Statistical analysis

Data were expressed as mean ± SD. Readings were taken in duplicate. The statistical significance of differences was conducted using analysis of variance (two-tailed). Values less than 0.05 were considered to indicate statistical significance.

RESULTS

In the current study, a comparative analysis to determine the caffeine content of selected tea samples using chemical and HPLC methods of analysis was carried out. Table 1 gives a description of samples characteristics used in the study.

Table 1: Description of samples purchased from Sagamu supermarkets in Nigeria

S/N	TEA SAMPLE	CODE NO.	MANUFACTURERS' NAME	MANUFACTURE DATE	EXPIRY DATE	BATCH NUMBER	TEA TYPE
1	**Hibiscus tea**	HTA	Al Rahab healthy herbal	7-2018	6-2021	Nil	Herbal tea
2	**Rheumatism tea**	HTB	Shanghal Bozheng tea co., LTD	10-2018	10-2021	Nil	Herbal tea
3	**Lazmi green tea**	GTC	Ayusri health product limited	2-2019	1-2021	E60108	Green tea
4	**Living Nature green tea**	GTD	Top Sprint Investment limited	31-07-2017	30-07-2021	N.LN.GT.H.G .B.17/19	Green tea
5	**Magic Antithyroid tea**	HTE	Magic tea life @163.com	1-8-2017	1-9-2020	NU2017/01	Herbal tea
6	**Detox tummy fat reducing herbal tea**	HTF	Doynik Ventures	10-2018	10-2021	0012	Herbal tea
7	**Magic Rheumatism tea**	HTG	Magic tea life @163.com	1-8-2017	1-9-2020	NU2017/01	Herbal tea
8	**Organic Green tea**	GTH	Michaels	15-08-2017	15-08-2022	M08017	Green tea
9	**Loyd Green tea**	GTI	Mokate S. A	24-04-2019	24-04-2021	Nil	Green tea
10	**Top tea**	BTJ	Promasidor Nigeria, Ltd.	6-2019	12-2020	425	Black tea
11	**All natural antithyroid tea**	HTK	Malshuni tea	2018	2021	Nil	Herbal tea
12	**Slimming tea**	HTL	Huian YibieiXiang tea factory	1-11-18	1-11-2013	2123	Herbal tea
13	**Natural Green tea**	GTM	Qualitea Ceylon	10-2018	10-2021	D18288	Green tea
14	**Lipton tea**	BTN	Unilever	18-06-19	17-12-2020	19252	Black tea
15	**Antimalaria tea**	HTO	Malshuni tea	2018	2021	Nil	Herbal tea

With the chemical method, after precipitation and filtration, caffeine was extracted with chloroform from the tea samples, and a polar organic solvent has a high tendency of extracting caffeine. Mean caffeine concentration was derived by adding the two values from the duplicate analysis (a+b)/2.

$$e.g.,\ Mean\ of\ sample\ A = \frac{(1.42\%+1.31\%)}{2}$$
$$= 1.37\%w/w$$

Table 2 gives the results of caffeine content determined by the chemical method.

Table 2: Caffeine content in tea samples by chemical analysis

Tea sample	Dry weight of Tea sample (g)	Weight of extracted caffeine (g)	Caffeine (% w/w)	Mean±SD (% caffeine)
HTA1	10.021	0.142	1.42	
HTA 2	10.018	0.131	1.31	1.37±0.08
HTB 1	10.012	0.055	0.55	
HTB 2	10.01	0.051	0.51	0.53±0.03
GTC 1	10.003	0.086	0.86	
GTC 2	10.005	0.078	0.78	0.82±0.06
GTD 1	10.023	0.012	0.12	
GTD 2	10.019	0.014	0.14	0.13±0.01
HTE 1	10.022	0.181	1.81	
HTE 2	10.014	0.163	1.63	1.72±0.13
HTF 1	10.001	0.080	0.80	
HTF 2	10.013	0.052	0.52	0.66±0.20
HTG 1	10.009	0.154	1.54	
HTG 2	10.022	0.130	1.30	1.42±0.17
GTH 1	10.016	0.092	0.92	
GTH 2	10.019	0.110	1.10	1.01±0.13
GTI 1	10.017	0.094	0.94	
GTI 2	10.013	0.086	0.86	0.90±0.06
BTJ 1	10.018	0.127	1.27	
BTJ 2	10.014	0.107	1.07	1.17±0.14
HTK 1	10.00	0.073	0.73	
HTK 2	10.003	0.096	0.96	0.85±0.16
HTL 1	10.021	0.092	0.92	
HTL 2	10.017	0.118	1.18	1.05±0.18
GTM 1	10	0.043	0.43	
GTM 2	10.002	0.076	0.76	0.60±0.23
BTN 1	10.004	0.078	0.78	
BTN 2	10.006	0.105	1.05	0.92±0.19
HTO 1	10.023	0.081	0.81	
HTO 2	10.02	0.090	0.90	0.86±0.06

The amount of caffeine in the tea samples analysed by chemical method ranged from 1.72±0.13 to 0.13±0.01 %w/w as shown in Table 2. Sample HTE was observed to have the highest caffeine content with 0.172 g/10g of tea sample, while sample GTD presented with the lowest caffeine content of 0.013 g/10 g of sample.

For the instrumental analysis, at a detection wavelength of 272 nm, caffeine eluted at a retention time of 1.632 min for 10 µg/ml, 1.639 min for 20 µg/ml, 1.646 min for 40 µg/ml, 1.576 min for 80 µg/ml, 1.645 min for 100 µg/ml: approximately between 1.58 min to 1.65 min. Calibration curve was obtained by plotting the peak area (mAU) against caffeine concentration (µg/ml) as shown in Figure 2.

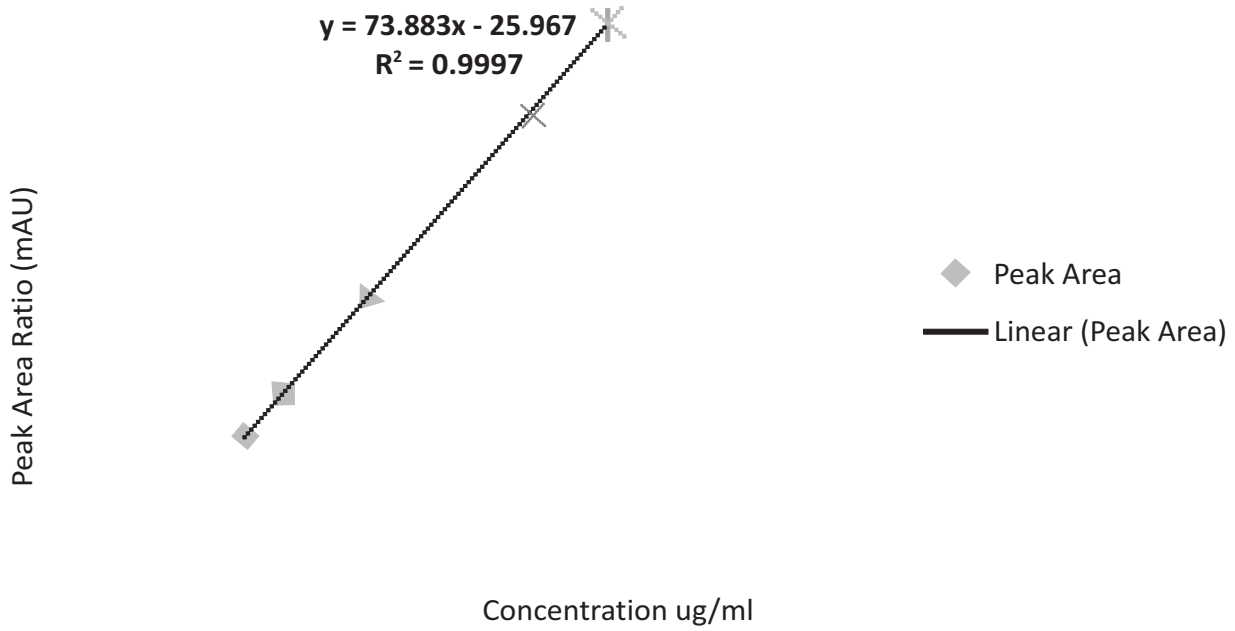

Fig.2. Calibration curve for caffeine standard over a concentration range of 10-100 µg/ml

The calibration graph was linear over the range of 10-100 µg/ml (R^2 = 0.9997) with the regression equation of y=73.88x-25.96. Results for actual samples were generated from the HPLC method through data obtained from the standard calibration curve as enumerated in Table 3.

Table 3: Caffeine content by HPLC analysis

Sample ID	Peak Area (mAU)	Concentration (µg/mL)	Caffeine Conc. in (%w/w)	Mean Caffeine conc) (% w/w
HTA1	6393.604	86.89	0.869	
HTA2	6138.692	83.44	0.834	0.85±0.025
HTB1	1384.456	19.09	0.191	
HTB2	2755.49536	37.65	0.377	0.24±0.132
GTC1	2767.94	37.81	0.378	
GTC2	4153.35	57.57	0.576	0.48±0.140
GTD1	ND	ND	ND	ND
GTD2	ND	ND	ND	ND
HTE1	3422.025	46.67	0.467	
HTE2	2098.251	28.75	0.288	0.38±0.127
HTF1	1984.89	27.22	0.272	0.14±0.192
HTF2	ND	ND	ND	ND
HTG1	4419.182	60.17	0.602	
HTG2	2595.7715	35.49	0.355	0.48±0.175
GTH1	3266.7	44.57	0.446	
GTH2	3255	44.41	0.444	0.45±0.001
GTI1	ND	ND	ND	ND
GTI2	ND	ND	ND	ND
BTJ1	2436.4	33.31	0.333	
BTJ2	2175.6	29.80	0.298	0.32±0.025
HTK1	1750	24.04	0.240	
HTK2	1752.9	24.08	0.241	0.24±0.001
HTL1	4510.513	61.40	0.614	
HTL2	4974.065	67.68	0.677	0.65±0.045
GTM1	7695.9672	104.52	1.045	0.52±0.739
GTM2	ND	ND	ND	
BTN1	2521.4	34.48	0.345	
BTN2	2236	30.62	0.306	0.33±0.028
HTO1	1617.9	22.25	0.223	
HTO2	1720.3	23.64	0.236	0.23±0.009

ND= Not Detected

Tables 4a and 4b outlined a comparison of the quantitative determination of caffeine content by the chemical and HPLC analytical methods. The amount of % caffeine in the tea samples by HPLC method ranged between 0.14±0.192 to 0.85±0.025. However. the levels of caffeine in two samples (GTD and GTI) were below the detection limit.

Table 4a. Comparative determination of caffeine concentration (µg/mL) in tea samples by chemical and HPLC methods

Sample ID	Caffeine conc. (Mean±SD) by chemical method	Caffeine conc. (Mean±SD) by h.p.l.c method
HTA	1.37±0.08	0.85±0.025
HTB	0.53±0.03	0.24±0.132
GTC	0.82±0.06	0.48±0.140
GTD	0.13±0.01	0.00±0.000
HTE	1.72±0.13	0.38±0.127
HTF	0.66±0.20	0.14±0.192
HTG	1.42±0.17	0.48±0.175
GTH	1.01±0.13	0.45±0.001
GTI	0.90±0.06	0.00±0.000
BTJ	1.17±0.14	0.32±0.025
HTK	0.85±0.16	0.24±0.001
HTL	1.05±0.18	0.65±0.045
GTM	0.60±0.23	0.52±0.739
BTN	0.92±0.19	0.33±0.028
HTO	0.86±0.06	0.23±0.009

Table 4b: Comparison of % of caffeine obtained from the two methods of analysis

Comparison of caffeine (%) obtained from the two methods of analysis						
Analysis	Mean±SD	Mean Diff.	95 % Confidence Interval of Difference		t- test	p-value
			Lower	Upper		
CHEMICAL	0.9323 ± 0.3912	0.5771	0.3373	0.8169	4.931	0.000
HPLC	0.3552 ± 0.2290					

The mean and standard deviation values of chemical analysis and HPLC are 0.9323 ± 0.3912 and 0.3552 ± 0.2290 respectively as shown in Table 4.1. There was statistically significant difference between the mean of the two groups ($t=4.931$, $p < 0.05$).

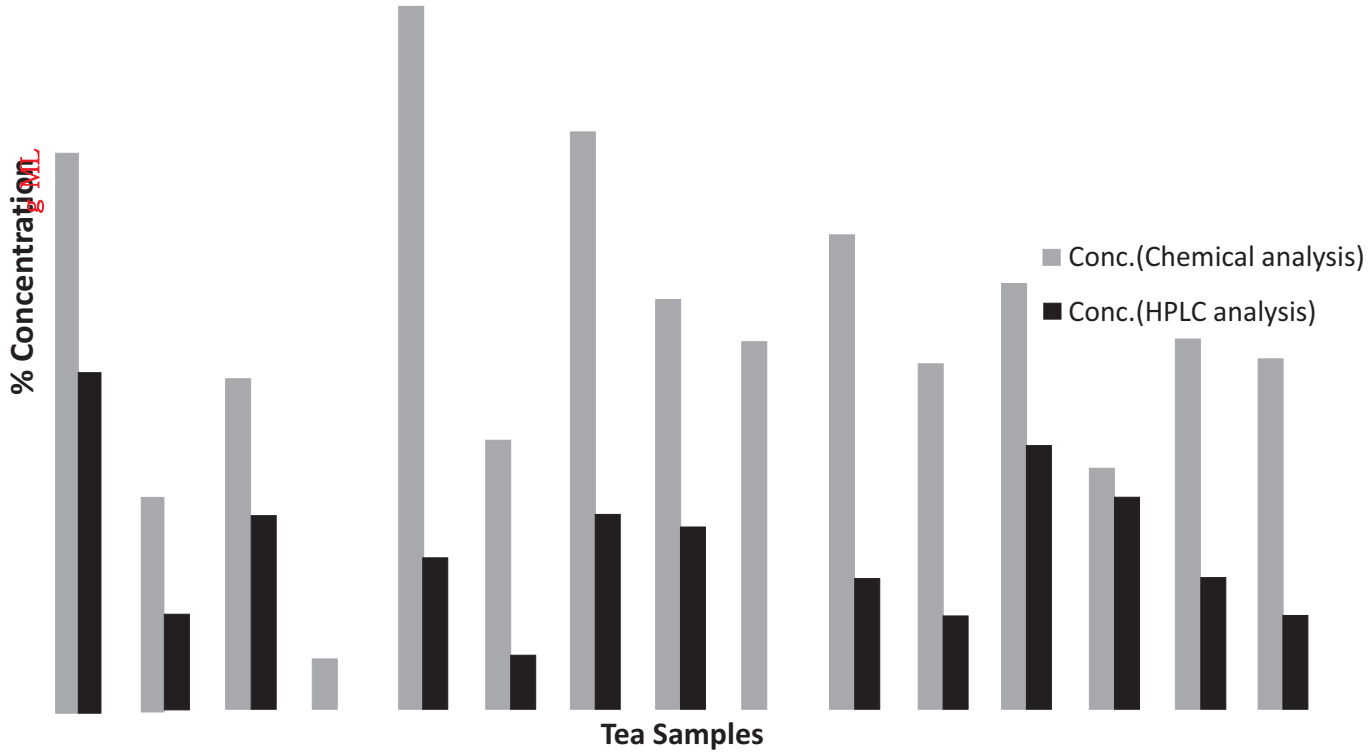

Figure 3: Graphical representation of mean caffeine concentration (µg/mL) obtained in tea samples by chemical and HPLC methodsand HPLC methods

DISCUSSION

We observed that all samples tested carried both manufacture and expiry dates, while 33% had batch numbers but none carried labelled caffeine content. This study presents higher values for samples determined by the chemical method when compared with same samples determined by the HPLC method (Table 4a). Sample HTA with approximately 85 µg/ml had the highest concentration of caffeine while sample GTD and sample GTI which did not show any detectable peak area may suggest the absence of caffeine in these two samples. This indicates that the concentration of the sample that contained the highest caffeine is 85 µg/ml (0.085 mg/ml). The value could be considered low and safe compared with the US Food and Drug Administration[51] classification of caffeine consumption. Under this classification, caffeine intake of 130-300 mg/day is low or moderate, above 400 mg/day is high while above 6,000 mg/day is heavy. This research was designed to confirm and determine the proportion of caffeine present in different selected brands of tea sold in the Nigeria market. The order of caffeine concentration in the tea samples are HTA>HTL>GTM>HTG>GTC>GTH>HTE>BTN>BTJ>HTB>HTK>HTO>HTF>GTD>GTI. The mean and standard deviation values of chemical analysis and HPLC are 0.9323 ± 0.3912 and 0.3552 ± 0.2290 respectively as shown in Table 4b. There was statistically significant difference between the mean of the two groups ($t=4.931$, $p < 0.05$). The fact that the HPLC method of analysis is known to be more sensitive, accurate and precise with minimal error as compared to the chemical method may explain the differences in our findings.

A previous study in Nigeria reported caffeine content in green tea as 2.39%[52] but in this study, we determined mean caffeine in green tea to be 0.69% by chemical method and 0.48% by HPLC method. However, another study reported the average amount of caffeine found in different teas to be as follows: Green tea (20-45 mg/8oz); Black tea (47 mg/oz), and Oolong tea, 50 mg/190ml. [23,24] Parvathy *et al*.[49] in another study by chemical analysis found caffeine content to be 0.63 gm in 50 gm of green tea (1.26%) which is higher than that in our study. In this previous study, the caffeine content of different tea and coffee samples were studied and found to be in the range of 1-5%. Clifford *et al*, 1990[53] had determined caffeine content in coffee (Coffea spp.), tea (Camellia sinensis), guarana (Paullinia cupana), maté (Ilex para-guariensis), cola nuts (Cola vera), and cocoa (Theobroma cacao) and reported it to vary in these products - the highest

amounts being in guarana (4-7%), followed by tea leaves (3.5%), maté tea leaves (0.89-1.73%), coffee beans (1.1-2.2%), cola nuts (1.5%), and cocoa beans (0.03%), which is in agreement with our study findings. Also, the amount of caffeine obtained from liquid- liquid extraction after further recrystallization was found to be 3.37% from tea and 5.04% from coffee using an HPLC method where it was observed that coffee contained a high percentage of crude caffeine compared to tea.[54] Additionally, Nyirahabimana *et al*.[55] determined the caffeine content in tea samples from different regions of Rwanda using high-pressure liquid chromatography (HPLC) to be in the range of 476.128±97.05 to 802.927±40.04 ppm. In another study, the content of caffeine in green tea beverages, milk-flavoured tea beverages and scented tea beverages was higher than that of black tea beverages and oolong tea beverages due to the different types of raw tea. The caffeine content in all their samples met the requirements of Chinese national standard and did not exceed the prescribed limits set by the United States, Canada, Japan, and other countries. [56] We can therefore infer that our results for caffeine content agrees with these previously reported studies, i.e., the values in our study and these reported values generally agree with other literature quoted values of 2-5%. [49,53,54]

According to the British Pharmacopoeia, 1993,[57] variability of caffeine content depends on factors such as varieties of tea, location, time of plucking, age of leaves, the particle size, and other agro-chemical conditions of tea plantation. Hence, the content of caffeine has been associated with plant origin and growth conditions, as well as processing conditions.

Sapcanin *et al*.[35] reported in a previous study that caffeine is addictive and a consumption of more than 1g of caffeine can lead to death. Intake of caffeine was considered safe if the US food and drug administration [51] classification of caffeine consumption and other relevance reference books is considered.[36] Approximately 80% of the world's population consumes caffeine daily which is derived from tea, coffee, cola, and chocolate.[58] Caffeine is considered as a drug working through nervous system and not a food, hence; excessive consumption of caffeine should be avoided since this presents with adverse health effects. People suffering from conditions such as high blood pressure and those with coronary heart disease should be advised to avoid use of caffeine containing beverages because caffeine is known to increase the blood pressure as well as disrupt normal heart rhythm.[58] Consequent of these deleterious effects associated with heavy caffeine use, studies of safe doses and the effects of chronic use are paramount in understanding the implications of caffeine. The threshold of caffeine toxicity appears to be around 400 mg/day in healthy adults (19 years or older), 100 mg/day in healthy adolescents (12-18 years old), and 2.5 mg/kg/day in healthy children (less than 12 years old).[59,60] Although, the amount of caffeine needed to produce effects varies among individuals and it's based on body weight and individual sensitivity; care must be taken in children. whom consequent of their smaller weight may manifest hyperactivity and other side effects if they regularly consumed beverages with caffeine content in the safety threshold for adults.[61] Therefore, people who need caffeine restrictions (Pregnant women, hypertensive patient, diabetic patient, people suffering from insomnia, etc.) should choose product with low caffeine content and avoid regular consumption.

However, recommended safety thresholds vary. For example, the European Food and Safety Authority considers 3-mg/kg body weight/day of habitual caffeine consumption to be safe for children and adolescents. [62] Caffeine works by binding to their molecular targets, the adenosine receptors which are in the central and peripheral nervous systems as well as in various organs, such as the heart, and blood vessels; that exhibit great genetic variability.[63] There is also a wide range in the plasma mean half-life of caffeine which is due to both innate individual variations, and a variety of physiological and environmental characteristics that influence caffeine metabolism (e.g., pregnancy, obesity, use of oral contraceptives, smoking, altitude).

The pharmacological effects of caffeine typified of other methylxanthines such as found in various teas and chocolates, include mild CNS stimulation and wakefulness, ability to sustain intellectual activity, and decreased reaction times whose severity may be affected by the amount of caffeine consumed. A comprehensive review of the effects of caffeine consumption on human health concluded that for healthy adults, moderate chronic intakes of caffeine up to 400 mg/day are not associated with adverse effects on cardiovascular health, calcium balance and bone status, behavior, cancer risk, or male fertility.[59] However, the recommended intake is much lower for pregnant or nursing mothers. Agencies involved in public health education such as the National Agency for Food and Drug Administration and Control (NAFDAC) are expected to initiate programs to educate consumers, especially children and adolescents, about the dangers of highly caffeinated products as is done by the U.S. FDA.[51] Generally, manufacturers of beverages

and energy drinks with added caffeine should be required to include the caffeine content on product labels. Due to the potentially harmful health effects including adverse and developmental effects of caffeine, its dietary intake should be discouraged for all children,[59,60] in normal adults but lower levels of caffeine intake of less than 200 mg/day have been recommended by the American College of Obstetricians and Gynaecologists for pregnant women.[62] However, the European Commission's Scientific Committee of Food Safety Authority and Health Canada 62 both recommend that women consume no more than 300 mg of caffeine/day during pregnancy in consideration of the association between caffeine consumption and spontaneous abortion.[62,64] Caffeine, being a pharmacologically active substance and the most used psychoactive drug in the world 22 should have its use regulated with a requirement for its presence and labelled amount clearly stated on tea, food, and drinks and other beverages. Caffeine content in the samples used for this study could be considered low and safe when measured with the US Food and Drug Administration [51] classification of caffeine consumption, where caffeine intake of 130-300 mg/day is low or moderate, and above 400 mg/day is high while above 6,000 mg/day is heavy. Hence, these samples could be said to have passed the caffeine content standard test and meet with quality requirements where caffeine content is concerned.

CONCLUSION

The highest amount of caffeine was found in herbal teas followed by black tea and then green tea. It can be deduced that people who need caffeine restrictions (pregnant women, elderly, etc.) can opt for green tea which contains antioxidants for additional health benefits with carers' advice. This study presents tea samples which all conform with caffeine content specifications by regulatory authorities such as European Commission's Scientific Committee of Food Safety Authority and Health Canada, as well the U.S. FDA. Additionally, agencies involved in public health education such as the NAFDAC are expected to initiate programmes to educate consumers, especially adolescents, pregnant women, and children, about the dangers of highly caffeinated products as is done by the U.S. FDA. Samples should also bear batch numbers and corresponding labelled caffeine content.

ACKNOWLEDGEMENT

We appreciate the contributions and support of the analyst, Mr. Taiwo in terms of HPLC method development and running, and supplying the caffeine standard. The authors also thank Olabisi Onabanjo University for providing the laboratory facilities and HPLC instrument.

REFERENCES

1. McKay DL, Blumberg JB (2002). The role of tea in human health: an update. *Journal of American College Nutrition* 21: 1-13.
2. Gardner EJ, Ruxton CH, Leeds AR (2007). Black tea-helpful or harmful? A review of the evidence. *European Journal of Clinical Nutrition* 61(1): 3-18.
3. Chung FL, Schwartz J, Herzog CR, Yang YM (2003). Journal of Nutrition 133(10): 3268S-74S.
4. Butt MS, Sultan MT (2009). Green tea: Nature's defense against malignancies. Critical Review of Food Science and Nutrition 49(5): 463-473.
5. Stangl V, Dreger H, Stangl K editors. (2007). Molecular targets of tea polyphenols in the cardiovascular system. Cardiovascular Research 73(2): 348-58.
6. Babu PV, Sabitha KE, Shyamaladevi CS (2006). Therapeutic effect of green tea extract on oxidative stress in aorta and heart of streptozotocin diabetic rats. Chemico-Biological Interactions 162: 114.
7. Babu PV, Liu D (2008). Green tea catechins and cardiovascular health: An update. Current Medicinal Chemistry 15(18): 1840-1850.
8. Varnam AH, Sutherland JP (1994). "Beverages: Technology, Chemistry and Microbiology." London: Chapman &Hall.
9. Graham HN (1992). Green tea composition, consumption, and polyphenol chemistry. Preventive Medicine 21: 334-350.
10. Lampe JW (2003). Spicing up a vegetarian diet: Chemopreventive effects of phytochemicals. *American Journal of Clinical Nutrition* 78: 579S-83S.
11. Frankel EN, Finley JW (2008). How to standardize the multiplicity of methods to evaluate natural antioxidants. Journal of Agricultural Food Chemistry 56(13): 4901-4908.
12. Khan N, Afaq F, Saleem M, Ahmad N, Mukhtar H (2006). Targeting multiple signaling pathways by green tea polyphenol (-)-epigallocatechin-3-gallate. Cancer Research 66(5): 2500-2505.
13. Tan LC, Koh WP, Yuan JM, Wang R, Au W-L, Tan JH, Tan E-K, Yu MC (2008). Differential effects of black versus green tea on risk of Parkinson's disease in the Singapore Chinese Health Study. *American Journal of Epidemiology* 167: 553-560.
14. Finger A, Kuhr S, Engelhardt UH (1992). Chromatography of tea constituents. *Journal of*

Chromatography 624: 293-315.
15. Balentine DA, Wiseman SA, Bouwens LCM (1997). The chemistry of tea flavonoids. Critical Reviews in Food Science and Nutrition 37: 693-704.
16. Wang J, Sporns P (2000). MALDI-TOF analysis of food flavonols glycoside. *Journal of Agricultural and Food Chemistry* 48: 1657-1662.
17. Del Rio D, Stewart AJ, Mullen W, Burns J, Lean MEJ, Brighenti F, Crozier A (2004). HPLC-MSn analysis of phenolic compounds and purine alkaloids in green and black tea. *Journal of Agricultural and Food Chemistry* 52: 2807-2815.
18. Del Rio D, Calani L, Cordero C, Salvatore S, Pellegrini N, Brighenti F (2010). Bioavailability and catabolism of green tea flavan-3-ols in humans. Nutrition 26(11-12): 1110-1116.
19. Crozier A, Jaganath IB, Clifford MN (2009). Dietary phenolics: Chemistry, bioavailability, and effects on health. Natural Products Reports 26(8): 1001-1043.
20. Mukhtar H, Wang ZY, Katlya SK, Agarwal R (1992). Tea components: antimutagenic and anticarcinogenic effects. Preventive Medicine 21: 351-360.
21. Lovett R (2005). "Coffee: The demon drink?". New Scientist (2518).
22. Verma R, Kumar L (2010). Characterization of Caffeine Isolated from Camellia Sinensis Leaves of Sikkim Himalayan Region. *Journal of Chemical and Pharmaceutical Research* 2(4): 194-198
23. Chen ZP, Schell JB, Ho CT, Chen KY (1998). Green Tea epigallocatechin gallate shows a pronounced growth inhibitory effect on cancerous cells but not on their normal counterparts. Cancer Letters 129: 173-179.
24. Ahmad N, Gupta S, Mukhtar H (2000). Green tea epigallocatechin-3-gallate differentially modulates nuclear factor -B in cancer cells versus normal cells. Archives of Biochemistry and Biophysics 376: 338-346
25. Busto U, Bendayan R, Sellers EM (1989). Clinical pharmacokinetics of non-opiate abuse drugs. Clinical Pharmacokinetics 16: 1-26.
26. Brachtel D, Richter E (1992). Absolute bioavailability of caffeine from tablet formulation. *Journal of Hepatology* 16: 385.
27. Aurnaud MJ (1987). The pharmacology of caffeine. Progress in Drug Research 31: 273.
28. Goth R, Cleaver JT (1976). Metabolism of caffeine to nucleic acid precursors in mammalian cells. Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis 36(1): 105-113
29. Suzuki T, Pervin M, Goto S, Isemura M, Nakamura Y (2016). Beneficial effects of tea and the green tea catechin epigallocatechin-3-gallate on obesity. Molecules 21(10): 1305
30. Klang MJ, Wang N, Meoni LA (2002). Coffee intake and Risk of Hypertension: The John Hopkins Precursors Study. Archives of Internal Medicine 162: 657-662
31. Conde SV, da Silva TN, Gonzalez C, Carmo MM, Monteiro EC, Guarino MP (2012). Chronic caffeine intake decreases circulating catecholamines and prevents diet-induced insulin resistance and hypertension in rats. *British Journal of Nutrition* 107: 86-95
32. Guarino MP, Ribeiro MJ, Sacramento JF, Conde SV (2013). Chronic caffeine intake reverses age-induced insulin resistance in the rat: effect on skeletal muscle Glut4 transporters and AMPK activity. Age (Dordr) 5: 1755-1765
33. Panchal SK, Wong WY, Kauter K, Ward LC, Brown L (2012). Caffeine attenuates metabolic syndrome in diet-induced obese rats. Nutrition 28: 1055-1062
34. Yeh T-C, Liu C-P, Cheng W-H, Chen B-R, Lu P-J, Cheng P-W, Ho W-Y, Sun G-C, Liou J-C, Tseng C-J (2014). Caffeine intake improves fructose-induced hypertension and insulin resistance by enhancing central insulin signaling. Hypertension 63: 535-541
35. Šap?anin A, Uzunovi? A, Jancan G, Pehli? E (2013). Determination of caffeine, theophylline, and theobromine content in energy drinks from Bosnian markets. *Balkan Journal of Health Science* 1: 35-38
36. Ogah CO, Obebe OT (2012). Caffeine Content of Cocoa and Coffee Beverages in Lagos, Nigeria. *Journal of Innovative Research in Engineering and Sciences*. 3(1): 404-411
37. Tarka MS, Hurst WJ (1998). Introduction to the Chemistry isolation, and biosynthesis of methylxanthines. In Caffeine; Spiller, GA Eds. CRC Press: Boca Raton, 9-16.
38. Bispo MS, Veloso MCC, Pinheiro HLC, Oliveira RFSD, Reis JON, Andrade JBD (2002). Simultaneous determination of caffeine, theobromine, and theophylline by high-performance liquid chromatography. *Journal of Chromatography Science* 40: 45-48.
39. Belay A, Ture K, Redi M, Asfaw A (2008). Measurement of caffeine in coffee beans with UV/vis spectrometer. Food Chemistry. 108: 310-315.
40. He Q, Lv Y, Zhou L, Shi B (2010). Simultaneous determination of caffeine and catechins in tea extracts by HPLC. *Journal of Liquid Chromatography and Related Techniques* 33: 491-498.
41. Nehlig A (1999). Are we dependent upon coffee and caffeine? A review on human and animal data.

Neuroscience Biobehavioral Reviews 23: 563-76.
42. Smith A (2002. Effects of caffeine on human behaviour. Food and Chemical Toxicology 40: 1243-1255
43. Ogawa N, Ueki H (2007). Clinical importance of caffeine dependence and abuse. Psychiatry and Clinical Neurosciences 61: 263-268
44. Laska EM, Sunshine A, Mueller F, Elvers WB, Siegel C, Rubin A (1984). Caffeine as an analgesic adjuvant. Journal of the American Medical Association 251(13): 1711?1718.
45. Sawynok J, Yaksh TL (1993). Caffeine as an analgesic adjuvant: A review of pharmacology and mechanisms of action. Pharmacological Reviews 45(1): 43?85.
46. Krymchantowski AV (2003). Overuse of symptomatic medications among chronic (transformed) migraine patients: profile of drug consumption. Arquivos de Neuro-Psiquiatria 61: 43-47.
47. Diener HC, Pfaffenrath V, Pageler L, Peil H, Aicher B (2005). The fixed combination of acetylsalicylic acid, paracetamol and caffeine is more effective than single substances and dual combination for the treatment of headache: a multicentre, randomized, double-blind, single-dose, placebo-controlled parallel group study. Cephalalgia 25: 776-787.
48. Renner B, Clerke G, Grattan T, Beisel A, Mueller C, Werner U (2007). Caffeine accelerates absorption and enhances the analgesic effect of acetaminophen. *Journal of Clinical Pharmacology* 47: 715-726.
49. Parvathy S, Luiz A, Varkey JT (2014). Chemical Analysis of Caffeine Content in Tea and Coffee Samples. *Asian Journal of Science and Applied Technology* 3(1): 1-4
50. ICH (Q2B) (1996). Note for Guidance on Validation of analytical Procedures: Methodology. Geneva, Switzerland: IFPMA, International Conference on Harmonization
51. US Food and Drug Administration (FDA) (2006). Food Additives Status List, Food and Drug Administration. Retrieved form: http://www.cfsan.fda.gov/dms/opa-appa.html. classification of caffeine consumption. Assessed on 12 July 2019
52. Aroyeun SO, Shittu TR, Yahaya AT, Sowunmi FA, Odumbaku LA, Okelana FA, Akoroda MO (2013). Green tea processing in Nigeria and its economic implications. *Global Advanced Research Journal of Management and Business Studies* 2(1): 50-55.
53. Clifford MN, Ramirez-Martinez JR (1990). Chlorogenic acids and purine alkaloids contents of mate (Ilex paraguariensis) leaf and beverage. Food Chemistry 35: 13-21
54. Pradeep S, Rameshaiah GN, Ashoka H (2015). Caffeine extraction and characterization. *International Journal of Current Research and Review* 7(9): 16-19
55. Nyirahabimana F, Uwimana P (2017). Quantitative Analysis of Caffeine Content from Different Tea Growing Regions of Rwanda. *International Journal of Food Science and Biotechnology* 2(2): 51-55.
56. Shao J, Zhang Y (2019). Determination of caffeine content in tea beverages IOP Conf. Ser.: Earth and Environmental Science 330: 042056
57. British Pharmacopoeia. 1993. Vol. I, 95.
58. Barone JJ, Roberts HR (1996). Caffeine consumption. Food and Chemical Toxicology 34: 119-129.
59. Nawrot P, Jordan S, Eastwood J, Rotstein J, Hugenholtz A, Feeley M (2003). Effects of caffeine on human health. Food Additives and Contaminants 20: 1-30.
60. Seifert SM, Schaechter JL, Hershorin ER, Lipshultz SE (2011). Health effects of energy drinks on children, adolescents, and young adults. *Pediatrics* 127: 511-528.
61. Benjamin LT, Rogers AM, Rosenbaum A (1991). Coca-Cola, Caffeine, and Mental Deficiency: Harry Hollingworth and the Chattanooga Trial of 1991. *Nigeria Journal of Tree Crop Research* 27: 42-45.
62. McGuire S (2014). Institute of Medicine. Caffeine in food and dietary Supplements: Examining safety: Workshop summary. Washington, DC: National Academies Press. *Advances in Nutrition* 5(5): 585-586
63. Sachse C, Brockmoller J, Bauer S, Roots I (1999). Functional significance of a C->A polymorphism in intron 1 of the cytochrome P450 CYP1A2 gene tested with caffeine. *British Journal of Clinical Pharmacology* 47: 445-449.
64. Miles-Chan JL, Charriere N, Grasser EK, Montani JP, Dulloo AG (2015). The blood pressure-elevating effect of red bull energy drink is mimicked by caffeine but through different hemodynamic pathways. Plant Physiology Reports 3:e12290.