# Assessment of The Level of Caffeine in Some Tea Leaves Marketed in Dutse: Jigawa State 

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#### Abstract

The use of caffeine as a psychoactive stimulant in tea has been observed to have serious negative effects in humans' systems such as respiratory, nervous, cardiovascular, renal and skeletal systems. This study was carried out to assess the levels of caffeine in 10 different tea brands available in local market in Dutse, Jigawa State, Nigeria. Quantitative analysis of caffeine was performed by a simple and fast UV-Vis spectrophotometric methods using different solvents for extraction. The caffeine content in all the tea samples analyzed in this study were below the maximum allowable limits set by the USFDA. Tea have been associated with adverse health effects and the claims made by manufacturers about the benefits of tea do not highlight risks associated with excessive consumption of a combination of the ingredients contained in tea. Long term effects of tea consumption of children and young people have not been adequately studied. Therefore, it is recommended that further research be carried out on the adverse effects of energy drinks on children. Research is also needed to be done on the effects of the combination of ingredients on health and excessive consumption of those ingredients to children and adolescents. People need to be educated and given proper awareness on the health risks associated with caffeine containing beverages


Keywords: Caffeine, Tea Leaves, Assessment, Chemistry
Major classification: Health Science.

## 1. Introduction

A beverage is a drink specially prepared for human consumption either at meal or leisure times. There are a variety of beverages which can be broadly classified into alcoholic and non-alcoholic. Alcoholic beverages contain alcohol in varying concentrations while non-alcoholic beverages comprise soft drinks, fruit juices and hot beverages. Soft drinks and some fruit juices may contain caffeine arising from the raw materials used for its preparation or from deliberate addition. Hot beverages often contain caffeine and are termed 'hot' because they are usually served hot by addition of hot water or milk. This group consists of cocoa, tea and coffee-based products which are commercially available in Nigerian markets (Casimir, Stephen, Nurudeen, \& Igelige, 2014).
Tea refers to the agricultural products of the leaves, leaf buds and internodes of the Camellia sinensis_plant. It has been consumed as a beverage for almost 2,000 years starting in China. It is the most widely consumed beverage after water (Alan \& Iris, 2004). Teas from many areas may be blended. The aim is to obtain better taste, higher price or both, as a more expensive, better tasting tea may cover the inferior taste of cheaper varieties. There are two major kinds of tea, black tea and green tea. Both contain caffeine (1 to 5) \% of its dry weight (Amra, Mojca, Zeljko, Bernd, Frank, \& Sabine, 2006) depending on type, brand (Bennett \& Bonnie, 2001) and brewing method (Hicks, Hsieh, \& Bell, 1996). This is why the reported values in the literature are so variable.

Caffeine is a bitter in taste, white crystalline xanthine alkaloid that acts as a psychoactive stimulant drug and a mild diuretic. Almost sixty plant species are known to contain caffeine (Palatini, Ceolotto, Ragazzo, Dorigatti, \& Saladini, 2009). Methylxanthines, such as caffeine ( $1,3,7$-trimethylxanthine) and theobromine ( 3,7 dimethylxanthine), and methyluric acids are classified as purine alkaloids. Purine alkaloids are secondary metabolites derived from purine nucleotides that have been found in nearly 100 species in 13 orders of plant kingdom (Ashihara \& Crozier, 1999). They occur in tea, coffee and a number of other non-alcoholic beverages. Caffeine was isolated from tea and coffee in the early 1820s, but the main biosynthetic and catabolic pathways of caffeine were not fully established until 2000 . Highly purified caffeine synthase was obtained from tea leaves after which a gene encoding the enzyme was cloned (Kato, Mizuno, Crozier, Fujimura, \& Ashihara, 2000; Kato, Mizuno, Fujimura, Iwama, Irie, Crozier, \& Ashihara, 1999).

Caffeine is a most common ingredient of energy drinks. It is added as a flavouring agent and to make the drinks addictive (Andrews, Schweitzer, Zhao, Holden, \& Roseland, 2007). Common sources of caffeine are the "bean" (seed) of the coffee plant; in the leaves of the tea bush; and in kola nuts. Some other sources include yaupon holly leaves, South American holly yerba mate leaves, seeds from Amazonian maple guarana berries (Nathanson, 1984).
In 1819, the German chemist Friedrich Ferdinand Runge first time isolated pure caffeine in laboratory (Jarvis, 2002). Caffeine is one of the world most widely used drugs. Many anthropologists believe people used caffeine start from Stone Age. Caffeine was first extracted from coffee in 1821 (Anna \& Kurek, 2013). Caffeine is a naturally occurring substance found in the leaves, seeds or fruits of over 63 plants species worldwide and is part of a group of compounds known as methyl xanthine's. The most commonly known sources of caffeine are: coffee, cocoa beans, cola nuts and tea leave (Runge \& Friedlieb, 2014).
Caffeine is a naturally occurring substance found in humans, caffeine is a central nervous system (CNS) stimulant (Nehlig, Daval, \& Debry, 1992). It has the effect of temporarily warding off drowsiness and restoring alertness. Beverages containing caffeine, such as coffee, tea, soft drinks and energy drinks, enjoy great popularity (Torres \& Francis, 2009). Caffeine is the world most widely consumed psychoactive substance. Adults receive nearly three quarters of their daily caffeine from coffee. Children receive one half of their caffeine from soft drinks. Energy drinks represent a fast-growing beverage market. Different energy drinks having different amount of caffeine and its range is from $50-300 \mathrm{mg}$. Most people experience no behavioral effects with less than 300 mg caffeine. Sleep is more sensitive and can be disrupted by 200 mg caffeine (Frary, Johnson, \& Wang, 2005).
The caffeine content in the average cup of coffee is around 100 mg . Decaffeinated coffee isn't actually caffeine-free and can contain up to 12 mg of caffeine. Your average cup of tea contains 85 mg of caffeine. A single can of commercially available energy drink can have anywhere between 80 and 280 mg of caffeine depending on the can size. Green tea is close behind with 60 mg of caffeine, followed by white tea with 55 mg . Slim fast chocolate drinks come in at 20 mg of caffeine in a single serving. Caffeine is metabolized in the liver into three primary metabolites: Para xanthine (84\%), Theo bromine (12\%), and theophylline (4\%) (David, Bizgan, Popa, Buleandra, \& Moldovan, 2015). Caffeine is metabolized in the liver by the cytochrome P450 oxidase enzyme system (specifically, the 1A2 isoezyme) into three metabolic dimethyl xanthine's (Figure 1) which each have their own effects in the body (Bolton \& Null, 1981). An acute overdose of caffeine, usually in excess of 250 milligrams (more than 2-3 cups of brewed coffee), can result in a state of central nervous system overstimulation called caffeine intoxication (Sfectu, 2006). The effects of caffeine in the body may begin as early as 15 minutes after ingesting and last up to hours (Kamijo, Soma, Asari, \& Ohwada, 1999). Caffeine is highly addictive, caffeine decreases stress level. Caffeine accelerates aging and wrinkles (Mrvos, Reilly, Dean, \& Krenzelok, 1989).
Caffeine intake of $150-300 \mathrm{mg}$ after a 10 hrs fast increased urinary calcium excretion 2 -3hrs after exposure in adolescent men and women (Thelle, 1993). Dehydration is a major drawback of caffeine consumption, and results from the drugs ability to increase urine production. In addition to dehydration, caffeine causes some people to get jittery stomachs or "coffee stomach" which can be quite uncomfortable and mask any potential benefits (La, Luk, Cheng, \& Chiu, 1992). 100-200 mg dose of caffeine result in increased alertness and wakefulness, faster and clearer flow of thought, increased focus, and better general body coordination (Stanton \& Gray, 1995).
Caffeine makes people more alert, less drowsy, and improves coordination when combined with certain pain relievers or medicines for treating migraine headache (Robert, Crowford, \& Atkins, 2014).
In a large 217, 883. person study, those that consumed caffeine from any source had less kidney stone formation than those that did not consume caffeine (Ferraro, Taylor, Gambaro, \& Curhan, 2013). Caffeine is the world's most widely consumed psychoactive substance, but, unlike many other psychoactive substances, is legal and unregulated in nearly all jurisdictions.
Beverages containing caffeine, such as coffee, tea, soft drinks, and energy drinks, enjoy great popularity; in North America, $90 \%$ of adults consume caffeine daily. The U.S. Food and Drug Administration (2007) lists caffeine as a "multiple purpose generally recognized as safe food substance" Caffeine has diuretic properties when administered
in sufficient doses to subjects who do not have a tolerance for it. Regular users, however, develop a strong tolerance to this effect and studies have generally failed to support the common notion that ordinary consumption of caffeinated beverages contributes significantly to dehydration. Caffeine does not accumulate in the body over the course of time and is normally excreted within several hours of consumption (Barone \& Roberts, 1996).
Caffeine is a common organic molecule found in many beverages such as coffee, tea and cola. It is a naturally occurring alkaloid which is found in the leaves, seeds and fruits of over 63 plants species worldwide. It is an alkaloid of methylxanthine family (Wanyika, Gatebe, Gitu, Ngumba, \& Maritim, 2010; Bispo, Veloso, Pinheiro, De Oliveira, Reis, \& De Andrade, 2002). The methylxanthines caffeine (1,3,7-trimethyxanthine), theobromine (3,7dimethylxanthine), and theophylline (1,3-dimethylxanthine) can be normally found in cola nuts, coffee beans, cocoa beans, tea leaves, mate leaves and other kinds of plants (Paradkar \& Irudayaraj, 2002). While coffee and tea beverages naturally contain caffeine and other methylxanthines, caffeine serves as an ingredient in many carbonated soft drinks including colas, pepper-type beverages, and citrus beverages. Caffeine is found in varying quantities in the beans, leaves, and fruit of some plants, where it acts as a natural pesticide that paralyzes and kill certain insects feeding on the plants. Other sources include yerba maté, guarana berries, and the yaupon holly. It is a common ingredient of energy drinks. It is deliberately added as a flavoring agent and to make the drinks addictive.
Caffeine has numerous physiological effects on major organ systems, including the nervous system, cardiovascular system, digestive system, and respiratory system. Renal function and skeletal muscles are also affected by caffeine. Numerous studies have proven caffeine to be a stimulant to human's central nervous system (Spiller, 1998). It also increases heartbeat rate, dilate blood vessels and elevate levels of free fatty acids and glucose in plasma. 1g of caffeine leads to insomnia, nervousness, nausea, ear ringing, flashing of light derillum and tremulosness. In cases of overdosing and in combination with alcohol, narcotics and some other drugs, these compounds produce a toxic effect, sometimes with lethal outcome (Mamina \& Pershin, 2002; Ben Yuhas, 2002; Wanyika et al., 2010; Tavallali \& Sheikhaei, 2009).
Caffeine facilitates the conduction velocity in the heart and directly affects the contractility of the heart and blood vessels. Nevertheless, caffeine may significantly reduce cerebral blood flow by constricting of cerebral blood vessels. Caffeine provides a diuretic effect due to elevating the blood flow and glomerular filtration rate of the kidneys. In a large study of about 217,883 persons, those that consumed caffeine from any source had less kidney stone formation than those that did not consume caffeine. Heartburn is an issue for some subjects' gastrointestinal system after consuming caffeine. The effects of caffeine to skeletal muscles are mainly the increasing occurrence of tremors (James 1991; Spiller 1998).
Its physiological effects on many body systems have been reported by researchers, including the central nervous, cardiovascular, gastrointestinal, respiratory, and renal systems (Nehlig et al., 1992). The International Olympic Committee (IOC) defined caffeine as a drug and abuse is indicated when athletes have urine caffeine concentrations higher than $12 \mu \mathrm{~g} / \mathrm{mL}$ (De Aragao, Veloso, Bispo, Ferreira, \& De Andrade, 2005). Caffeine has drawn more attention in the past decades due to its physiological effects beyond that of its stimulatory effect. The Food and Drug Administration (FDA) defines caffeine as a generally recognized substance. However, FDA specifies that the maximum amount in carbonated beverages is limited to $0.02 \%$ (FDA, 2006). Therefore, the highest legal amount of caffeine allowed in a $355 \mathrm{~mL}(12 \mathrm{Oz})$ can of soft drink is about 71 mg . Caffeine has attracted the interest of consumers and health professionals alike due to its wide consumption in the diet by a large percentage of the population and its pharmacological effects in humans (Mandel, 2002). The human's saliva caffeine level, demonstrates the extent of absorption, peaks around 40 minutes after caffeine consumption (Liguori, Hughes, \& Grass, 1997).
Caffeine (1, 3, 7-trimethylxanthine) Caffeine is an odorless, white solid that has the form of needles or powder. Caffeine has a bitter taste. It is slightly soluble in water due to its moderate polarity. Caffeine is a natural central nervous system stimulant, having the effects of reducing drowsiness and recovering alertness. Since it is widely consumed by humans, caffeine is considered the most frequently used psychoactive substance in the world (Ligouri et al., 1997).


Figure 1: Structure of caffeine
Caffeine is metabolized in the liver into three primary metabolites: Para xanthine (84\%), Theo bromine (12\%), and theophylline (4\%). Caffeine is metabolized in the liver by the cytochrome $\mathrm{P}_{450}$ oxidase enzyme system (specifically, the $1 \mathrm{~A}_{2}$ isoezyme) into three metabolic dimethyl xanthine's which each have their own effects on the body.


Figure 2: Caffeine and its main metabolic products (Wanyika et al., 2010).
Caffeine (1,3,7-trimethyxanthine), theophylline (3,7- dimethylxanthine), and theobromine (1,3-dimethylxanthine) are in the family of alkaloid methylxanthines. Paraxanthine (84\%) Increase free fatty acid levels in the blood plasma, theobromine ( $12 \%$ ) increases urine volume while, theophylline (4\%) relaxes smooth muscles of the bronchi and is used to treat asthma. Its formula is $\mathrm{C}_{8} \mathrm{H}_{10} \mathrm{~N}_{4} \mathrm{O}_{2}$, IUPAC ID: 1,3,7-Trimethylpurine-2,6-dione, molecular weight: 194.19 g , melting point is $236^{\circ} \mathrm{C}$, point at which caffeine sublimes is $178^{\circ} \mathrm{C}$, at atmospheric pressure, pH is $6.9(1 \%$ solution), specific gravity is 1.2 , volatility is $0.5 \%$, vapor pressure is 760 mmHg at $178^{\circ} \mathrm{C}$, solubility in water is $2.17 \%$, density 1.23 g/c. (Komes, Horzic, Belscak, Kova, Ganic, \& Baljak, 2009; Nour Viol et al., 2008; Ashihara \& Crozier, 1996; Abdul Mumin et al., 2006).
Caffeine can be synthesized through a variety of methods. However, a direct and more common approach is that of Methylation of theobromine as shown below.


Figure 3: Synthesis of caffeine. (Zuo, Chen, \& Deng, 2002).

## Justification and Significance of Research

Caffeine is widely used as stimulant in drinks and beverages. However, it was reported to show some adverse effects which affect the body systems such as: the nervous system, renal and skeletal system, cardiovascular system, respiratory system etc. This makes it necessary to assess the level of caffeine in some notable tea marketed in Nigeria.

## Problem of Research

The use of caffeine as a psychoactive stimulant in tea has been observed to have serious negative effects in humans' systems such as respiratory, nervous, cardiovascular, renal and skeletal systems. As such there is need to regulate and control the amount of caffeine in our food products for consumers' safety and health protection.

## Limitations of Research

This research is limited to only ten (10) samples. Sampling population is restricted to those tea brands marketed in Nigeria.

## Aims and Objectives

This project is aimed at assessing the level of caffeine in some tea samples marketed in Dutse, Nigeria and to find out whether they comply with the established regulations. The objectives include:
i. Sample treatment
ii. Extraction of caffeine from tea solution using different solvents.
iii. Quantification of caffeine using UV-Vis spectrophotometer.

## 2. Methodology

A set of ten (10) different tea brands were randomly selected from Dutse metropolis shops and markets. Five of which are Chinese green teas, four (4) were black teas; whereas, one was a red tea. All glass wares were washed with distilled water, and were dried in oven at 105 degrees Celsius for 2Hrs. A 100ppm stock standard of caffeine was prepared by dissolving 20 mg caffeine in 250 ml chloroform in a volumetric flask. Working standards were prepared by pipetting $0.1,0.2,0.3,0.4,0.5 \mathrm{ml}$ respectively aliquots of stock standard solution into separate volumetric flasks of 100 ml and dilute it with water and forms $10,20,30,40,50 \mathrm{mg} / \mathrm{L}$ standards solution. Two (2) grams of tea leaves was measured using a calibrated weighing balance. The weighed tea leaves were transferred into a 100 mL beaker and 40 ml of deionized water was added to it which was placed on a heating mantle and was left to boil. After boiling, it was left to cool to room temperature. Upon cooling, the solution was then filtered using a funnel and filter paper. To the filtered tea solution, 40 mL of solvent was added. This forms an immiscible layer which was separated using a separating funnel. The solvent layer was transferred into a cuvette and the absorbance was taken using UV/Visible spectrometer. This same process was repeated for the ten (10) samples mentioned above using five (5) distinct solvents. The cuvette was rinsed four times and dried before taking the next measurement. To make the result more reliable, three independent measurements were taken for each sample and the average values were taken.

## 3. Results and Discussion

### 3.1. Results

## Results of absorption

The tables below are for the absorbance of caffeine scanned in the UV/Visible range of $200 \mathrm{~nm}, 250 \mathrm{~nm}, 300 \mathrm{~nm}$, $350 \mathrm{~nm}, 400 \mathrm{~nm}, 450 \mathrm{~nm}$ and 500 nm .

Table 1: Absorbance in Cyclohexanol

| Sample | 200 nm | 250 nm | 300 nm | 350 nm | 400 nm | 450 nm | 500 nm | Mean | $\pm$ STD |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| A | 0.999 | 0.998 | 0.997 | 0.992 | 0.924 | 0.876 | 0.764 | 0.936 | 0.089 |
| B | 0.997 | 0.997 | 0.997 | 0.998 | 0.998 | 0.996 | 0.994 | 0.997 | 0.001 |
| C | 0.996 | 0.996 | 0.996 | 0.997 | 0.995 | 0.994 | 0.992 | 0.995 | 0.002 |
| D | 0.99 | 0.988 | 0.998 | 0.987 | 0.985 | 0.969 | 0.951 | 0.981 | 0.016 |
| E | 0.984 | 0.985 | 0.983 | 0.982 | 0.977 | 0.87 | 0.68 | 0.923 | 0.115 |

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| F | 0.981 | 0.981 | 0.98 | 0.967 | 0.741 | 0.37 | 0.07 | 0.727 | 0.367 |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| G | 0.976 | 0.978 | 0.977 | 0.828 | 0.412 | 0.378 | 0.169 | 0.674 | 0.344 |
| H | 0.768 | 0.764 | 0.678 | 0.655 | 0.647 | 0.578 | 0.502 | 0.656 | 0.095 |
| I | 0.964 | 0.963 | 0.964 | 0.853 | 0.413 | 0.316 | 0.001 | 0.639 | 0.393 |
| J | 0.972 | 0.971 | 0.971 | 0.971 | 0.955 | 0.925 | 0.85 | 0.945 | 0.045 |

Table 2: Absorbance in Cyclohexane

| Sample | 200 nm | 250 nm | 300 nm | 350 nm | 400 nm | 450 nm | 500 nm | Mean | $\pm$ STD |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| A | 0.961 | 0.961 | 0.961 | 0.948 | 0.821 | 0.779 | 0.632 | 0.866 | 0.128 |
| B | 0.96 | 0.989 | 0.96 | 0.942 | 0.832 | 0.78 | 0.631 | 0.871 | 0.131 |
| C | 0.958 | 0.958 | 0.958 | 0.945 | 0.874 | 0.844 | 0.741 | 0.897 | 0.083 |
| D | 0.928 | 0.98 | 0.979 | 0.977 | 0.977 | 0.976 | 0.975 | 0.970 | 0.019 |
| E | 0.693 | 0.691 | 0.67 | 0.566 | 0.187 | 0.181 | 0.02 | 0.430 | 0.289 |
| F | 0.956 | 0.955 | 0.955 | 0.931 | 0.13 | 0.11 | 0.13 | 0.595 | 0.442 |
| G | 0.963 | 0.96 | 0.96 | 0.959 | 0.958 | 0.956 | 0.954 | 0.959 | 0.003 |
| H | 0.684 | 0.682 | 0.626 | 0.414 | 0.402 | 0.389 | 0.365 | 0.509 | 0.147 |
| I | 0.678 | 0.676 | 0.469 | 0.432 | 0.38 | 0.362 | 0.331 | 0.475 | 0.145 |
| J | 0.69 | 0.69 | 0.69 | 0.689 | 0.688 | 0.687 | 0.685 | 0.688 | 0.002 |

Table 3: Absorbance in petroleum ether

| Sample | 200 nm | 250 nm | 300 nm | 350 nm | 400 nm | 450 nm | 500 nm | Mean | $\pm$ STD |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| A | 0.645 | 0.636 | 0.608 | 0.601 | 0.601 | 0.584 | 0.574 | 0.607 | 0.026 |
| B | 0.68 | 0.677 | 0.678 | 0.554 | 0.22 | 0.085 | 0.054 | 0.421 | 0.290 |
| C | 0.647 | 0.637 | 0.625 | 0.622 | 0.618 | 0.611 | 0.604 | 0.623 | 0.015 |
| D | 0.655 | 0.644 | 0.623 | 0.617 | 0.612 | 0.607 | 0.604 | 0.623 | 0.019 |
| E | 0.681 | 0.68 | 0.68 | 0.679 | 0.678 | 0.678 | 0.675 | 0.679 | 0.002 |
| F | 0.687 | 0.685 | 0.681 | 0.68 | 0.643 | 0.621 | 0.618 | 0.659 | 0.031 |
| G | 0.679 | 0.668 | 0.662 | 0.657 | 0.643 | 0.631 | 0.614 | 0.651 | 0.023 |
| H | 0.683 | 0.683 | 0.68 | 0.664 | 0.632 | 0.621 | 0.62 | 0.655 | 0.029 |
| I | 0.678 | 0.677 | 0.677 | 0.674 | 0.671 | 0.669 | 0.668 | 0.673 | 0.004 |
| J | 0.687 | 0.685 | 0.682 | 0.677 | 0.642 | 0.639 | 0.628 | 0.663 | 0.025 |

Table 4: Absorbance in chloroform

| Sample | 200 nm | 250 nm | 300 nm | 350 nm | 400 nm | 450 nm | 500 nm | Mean | $\pm$ STD |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| A | 0.683 | 0.682 | 0.684 | 0.395 | 0.395 | 0.395 | 0.395 | 0.518 | 0.154 |
| B | 0.677 | 0.676 | 0.673 | 0.669 | 0.664 | 0.66 | 0.651 | 0.667 | 0.009 |
| C | 0.699 | 0.698 | 0.695 | 0.693 | 0.692 | 0.69 | 0.688 | 0.694 | 0.004 |
| D | 0.687 | 0.687 | 0.686 | 0.084 | 0.683 | 0.681 | 0.678 | 0.598 | 0.227 |
| E | 0.701 | 0.7 | 0.7 | 0.697 | 0.694 | 0.691 | 0.684 | 0.695 | 0.006 |
| F | 0.684 | 0.682 | 0.681 | 0.679 | 0.674 | 0.671 | 0.668 | 0.677 | 0.006 |
| G | 0.684 | 0.683 | 0.677 | 0.674 | 0.673 | 0.671 | 0.662 | 0.675 | 0.008 |
| H | 0.679 | 0.677 | 0.673 | 0.661 | 0.654 | 0.653 | 0.649 | 0.664 | 0.012 |
| I | 0.689 | 0.688 | 0.686 | 0.686 | 0.632 | 0.621 | 0.615 | 0.660 | 0.035 |
| J | 0.693 | 0.693 | 0.692 | 0.691 | 0.69 | 0.688 | 0.685 | 0.690 | 0.003 |

Table 5: Absorbance in diethyl ether

| Sample | 200 nm | 250 nm | 300 nm | 350 nm | 400 nm | 450 nm | 500 nm | Mean | $\pm$ STD |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| A | 0.691 | 0.689 | 0.688 | 0.686 | 0.683 | 0.683 | 0.68 | 0.686 | 0.004 |
| B | 0.688 | 0.685 | 0.681 | 0.674 | 0.671 | 0.67 | 0.663 | 0.676 | 0.009 |
| C | 0.692 | 0.692 | 0.69 | 0.687 | 0.684 | 0.681 | 0.68 | 0.687 | 0.005 |
| D | 0.647 | 0.644 | 0.636 | 0.627 | 0.621 | 0.621 | 0.619 | 0.631 | 0.012 |
| E | 0.655 | 0.649 | 0.644 | 0.641 | 0.639 | 0.639 | 0.636 | 0.643 | 0.007 |
| F | 0.641 | 0.641 | 0.638 | 0.631 | 0.628 | 0.626 | 0.623 | 0.633 | 0.007 |
| G | 0.67 | 0.667 | 0.66 | 0.639 | $0 . .632$ | 0.627 | 0.619 | 0.647 | 0.022 |
| H | 0.079 | 0.677 | 0.671 | 0.659 | 0.652 | 0.649 | 0.647 | 0.576 | 0.220 |
| I | 0.691 | 0.689 | 0.688 | 0.687 | 0.685 | 0.685 | 0.682 | 0.687 | 0.003 |
| J | 0.689 | 0.681 | 0.668 | 0.655 | 0.653 | 0.65 | 0.637 | 0.662 | 0.018 |

Table 6: Result of Concentration of Samples in Cyclohexanol

| Sample | Concentration (mg/L) |
| :--- | :--- |
| A | 49.051 |
| B | 49.906 |
| C | 49.901 |
| D | 48.821 |
| E | 48.201 |
| F | 35.561 |
| G | 34.211 |
| H | 33.721 |
| I | 32.561 |
| J | 48.501 |

Table 7: Result of Concentration of Samples in Cyclohexane

| Sample | Concentration (mg/L) |
| :--- | :--- |
| A | 46.532 |
| B | 46.721 |
| C | 47.011 |
| D | 48.621 |
| E | 22.311 |
| F | 29.065 |
| G | 49.132 |
| H | 24.561 |
| I | 23.111 |
| J | 34.501 |

Table 8: Result of Concentration of Samples in Petroleum Ether

| Sample | Concentration (mg/L) |
| :--- | :--- |
| A | 29.123 |
| B | 22.290 |
| C | 32.500 |
| D | 32.500 |
| E | 32.214 |
| F | 33.725 |
| G | 33.711 |
| H | 33.720 |
| I | 34.210 |

J 32.555

Table 9: Result of Concentration of Samples in Chloroform

| Sample | Concentration (mg/L) |
| :--- | :--- |
| A | 24.572 |
| B | 32.210 |
| C | 35.032 |
| D | 29.070 |
| E | 35.033 |
| F | 34.215 |
| G | 34.212 |
| H | 32.207 |
| I | 32.200 |
| J | 35.028 |

Table 10: Result of Concentration of Samples in Diethyl Ether

| Sample | Concentration (mg/L) |
| :--- | :--- |
| A | 34.492 |
| B | 34.215 |
| C | 34.548 |
| D | 32.550 |
| E | 32.663 |
| F | 32.555 |
| G | 48.505 |
| H | 27.321 |
| I | 34.548 |
| J | 32.206 |



Figure 4: absorbance for caffeine standard solution

### 3.2. Discussion

From the results obtained; it is apparent that cyclohexanol extracts caffeine more than the other solvents due to the relative high values of absorbance compared to those of other solvents, and the same holds for concentration according to Beer-Lambert’s law (concentration is proportional to the number of absorbing molecules). Hence, beer lambert's law is valid. The electronic transition responsible for caffeine absorbance is due to a $\pi \rightarrow \pi^{*}$ transition. It could be seen that sample B has the highest absorbance in cyclohexanol.

The results obtained showed that caffeine concentrations ranged from $22.290 \mathrm{mg} / \mathrm{L}-49.906 \mathrm{mg} / \mathrm{L}$. These were underneath the range of $170 \mathrm{ppm}-324 \mathrm{ppm}$ for caffeine concentrations in beverages reported by Mei, Mawahib, Mohammed, Badawi, and Abdalla (2012), and lower than the ranged of $440 \mathrm{ppm}-473 \mathrm{ppm}$ for caffeine concentration in tea samples reported by Mei et al. (2012).
The safety of caffeine intake has been assessed by several national regulatory scientific committees for use at the levels of consumption estimated by their respective populations.

According to the Food Standards Agency U.K., drinks containing more than $150 \mathrm{mg} / \mathrm{L}$ of caffeine must be labelled with the term 'high caffeine content' in the same field of vision as the name of the food. This must be accompanied by an indication of the amount of caffeine per 100 ml in the product. No other labelling is currently required by law and this labelling does not apply to drinks such as tea and coffee.
The recommended upper daily intake levels of caffeine have been set by the Korean Food and Drug Administration. For adults less than 400 mg of caffeine per day, for pregnant women less than 300 mg and for children less than 2.5 $\mathrm{mg} / \mathrm{kg}$ of body weight. In Taiwan, Upper limit of caffeine is $320 \mathrm{mg} / \mathrm{L}$ for beverages other than tea and coffee (Heckman et al., 2010). Mexican regulations do not include any upper limit for the addition of caffeine to beverages. However, flavored non-alcoholic beverages containing more than $20 \mathrm{mg} / 100 \mathrm{ml}$ are considered beverages with added caffeine, which must be printed on the label (Heckman, et al., 2010). In Brazil, beverages containing 80mg of caffeine are considered liquid compounds ready for consumption and the regulations foresee an upper limit of 350 $\mathrm{mg} / \mathrm{L}$ (Heckman et al., 2010).
According to Health Canada, (2010), the maximum recommended daily intake of caffeine is $45-85 \mathrm{mg}$ per day for children (2-4 years), 125 mg per day for teenager (13 years) and 400 mg per day for adult.
USFDA has cited $400 \mathrm{mg} /$ day as an amount not generally associated with dangerous, negative effects. It has however not set a level for children, but the American Academy of Pediatrics discourages the consumption of caffeine and other stimulants by children and adolescents. In Nigeria, there has been no set limit by the regulatory bodies for caffeine in beverages as most of the products are being imported into the country. As such, they usually use the set standards by international bodies such as the FDA. The acceptable daily intake of caffeine is $400 \mathrm{mg} /$ day (USFDA, 2010). Caffeine concentration in all the tea samples were below the FDA set standards. This implies that a daily consumption of one cup of tea may have no adverse effect on the consumer. Chile regulation classified beverages containing 80 mg of caffeine ( $320 \mathrm{mg} / \mathrm{L}$ ) as sports drinks and the regulation does not include an upper limit. Rather, it states a daily consumption higher than 500 mg of caffeine should not be consumed (Heckman et al., 2010).

## 4. Conclusion

Debate regarding the overall risks and benefits of caffeine has gained momentum in recent times. Health researchers agree that caffeine consumption can have adverse health consequences, particularly at high doses. Among the most common negative effects are increased anxiety, panic attacks, increased blood pressure, increased gastric acid, bowel irritability, and insomnia.
This study analyzed the caffeine concentrations of tea brands in Nigerian markets. Results for caffeine concentration in the energy drinks ranged between $20 \mathrm{mg} / \mathrm{L}-49 \mathrm{mg} / \mathrm{L}$. Caffeine concentration in all the samples were below the FDA set standard of 400 mg per day.

## Recommendations

Tea have been associated with adverse health effects and the claims made by manufacturers about the benefits of tea do not highlight risks associated with excessive consumption of a combination of the ingredients contained in tea. Long term effects of tea consumption of children and young people have not been adequately studied.
Therefore, it is recommended that further research be carried out on the adverse effects of energy drinks on children. Research is also needed to be done on the effects of the combination of ingredients on health and excessive consumption of those ingredients to children and adolescents. People need to be educated and given proper awareness on the health risks associated with caffeine containing beverages
It is recommended that it should be made compulsory for manufacturers to fully disclose the exact concentrations of ingredients including stimulants such as caffeine and guarana used on the labels of their products so that people are aware of what they consume.
Manufacturers should also provide prominent health and safety warning labels on each container, alerting consumers to the risks associated with consuming caffeine and other stimulants contained in energy drinks.

Agencies such as NAFDAC should conduct further research on the health and safety of caffeine containing beverages, focusing particularly on the impact of underage drinking. They should also develop a national media and public awareness campaign about the risks associated with energy drinks.

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