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Estimation of Caffeine from energy drinks and Tea sample available in the Regional Market using UV-Visible Spectrophotometer

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ABSTRACT: Background: Caffeine which is widely present in commonly used non-alcoholic beverages and teas are widely consumed for many purposes. Various concentrations of caffeine are present in different drinks. Their pharmacological action includes CNS stimulant, diuretic, antioxidant, and anti-inflammatory. Caffeine also has different side-effects which may lead to other health disorders. **Aim:** The study was performed to determine the caffeine content in tea, soft drinks and energy drinks that are available in regional markets. **Method:** It was carried out by UV-visible Spectrophotometer method using chloroform as the extracting solvent and concentration of caffeine was determined at 274 nm wavelength. Eight different samples were taken for the study. **Results:** The maximum caffeine concentration of energy drinks was observed in monster (10.58 µg/ml) and lowest in sting (7.8 µg/ml). In soft drinks, thumbs up shows (3.3 µg/ml) & coca cola (4.5 µg/ml) of caffeine content. While tea samples show lower concentration of caffeine in Assam tea (2.960 µg/ml) and denzong tea (1.21 µg/ml) of caffeine. The present study gave preliminary information about the concentration of caffeine in tea, soft drinks and energy drinks. **Conclusion:** The concentrations of caffeine in all the drink samples and tea were found below the maximum allowable limits set by the food regulatory bodies.

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INTRODUCTION:

Caffeine is an alkaloid of the methyl xanthine family found in many foods and beverages including coffee, tea, soft drinks, and energy drinks. They are a class of organic compounds that are basic in nature. Pure Caffeine is a white crystalline xanthine alkaloid which is bitter in taste, odourless fleecy masses, that act as a psychoactive stimulant drug and a mild diuretic. Caffeine is also known as 1,3,7-trimethyl xanthine or 3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione.

Caffeine is a purine base present along with other bases like theophylline & theobromine present in coffee, tea etc. Caffeine serves as an ingredient in many carbonated soft & energy drinks. It is used to reduce physical

fatigue and restore mental alertness when weakness or drowsiness occurs. Excess amounts of caffeine can result in physiological disorders like anxiety, irritability, muscle twitching, nervousness, and respiratory alkalosis [1-3]. The Food and Drug Administration (FDA) defines caffeine as a Generally Recognised as Safe (GRAS) substance. However, the maximum amount of caffeine in carbonated beverages as mentioned by the FDA is limited to 0.002 % (FDA 2006) [4,5]. The addictive nature of caffeinated drinks is the reason for the rise in the consumption of soft and energy drinks. There are certain standards set by the regulating bodies in the country like FSSAI (Food Safety and Standards Authority of India), which has 300 mg/L as the maximum limit for caffeine in non-alcoholic beverages. The beverages containing caffeine more than 145 mg/l should be labelled as "Contains Caffeine".

Caffeine acts as a stimulant to the central nervous system in humans and therefore it is used as a recreational drug and as medical substances to effectively reduce exhaustion and to restore awareness when unexpected weakness or dizziness occurs [6]. In the liver, caffeine is broken down into the following three primary metabolites namely, paraxanthines (84 %), theobromine (12 %) and theophylline (4 %) [7].

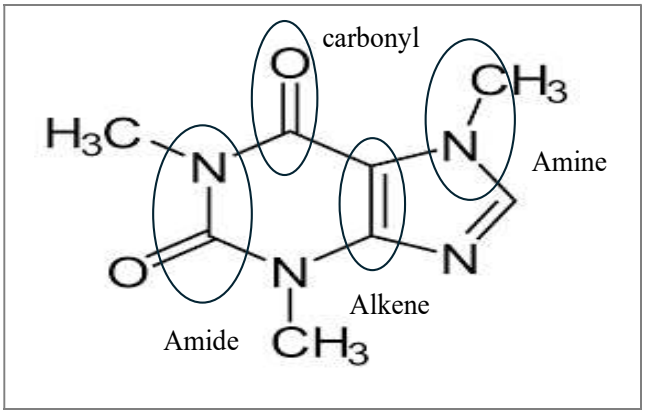

Fig 1. Chemical structure and functional group of caffeine.

Caffeine is also called 1,3,7-trimethylpurine-2-6-dione which belongs to the family of methylxanthines (alkaloids). The physical properties of caffeine include molecular formula $C_8H_{10}N_4O_2$, molar mass as 194.19g/mol, boiling point 178 °C, melting point 238 °C, and density is 1.23 g/cm^3. The compound has a carbon and nitrogen based molecule composed of a purine ring (a pyrimidine ring and an imidazole ring), both of which have two nitrogen rings. Their chemical structure has functional groups such as amine, carbonyl group, alkene and amide [8,9] .

Soft drinks are a type of non-alcoholic beverage that is usually but not always carbonated and consists of natural or artificial sweeteners, edible acids, synthetic flavours, and sometimes juice. The caffeine content of soft drinks varies by brand ranging from 10 to 50 mg per servings [10,11]. The US Food and Drug Administration, on the other hand limits the maximum amount in carbonated beverages to 6 mg/oz. As a result, the caffeine content of soft drinks range between 30 and 72 mg/355 ml (12oz) or 8.45 to 20.28 mg/100ml [12,13].

Tea is considered as one of the important sources of caffeine. Various kinds of tea have different concentrations of caffeine. Green tea comes with less amount of caffeine as compared to black tea and oolong tea. Consumption of tea originated from east Asian countries such as China, India, tropical and sub-tropical areas. Tea has various medicinal importance such as anti-inflammatory, CNS stimulant and other neuroprotective properties.

Energy drinks like red bull, bullet and monster contain caffeine, herbal extracts like guarana, ginseng, *Ginkgo biloba*, vitamin-B, amino acids derivatives like carnitine, and sugar derivatives like glucuronolactone and ribose . Energy drinks are said to have 80 to 300 mg of caffeine and 35 g of processed sugar per 8 oz servings [14-16]. The objective of the study is to determine whether the content of caffeine in soft drinks, energy drinks and tea leaves which are commercially available in the local market of Majhitar (East Sikkim) is within the label claimed or not with the help of a UV-visible Spectrophotometer.

MATERIALS AND METHOD:

Sodium carbonate (Na_2CO_3), Chloroform ($CHCl_3$), Iodine (I_2), are of analytical grade were procured from SRL, Maharashtra. Caffeine standard ($C_8H_{10}N_4O_2$) was obtained from SRL (Maharashtra).

Sample collection:

Different samples of black tea (Denzong Tea, Assam Tea), Energy Drinks (Red Bull, Monster, hell, sting) & soft drinks (thumbs up, coca cola) were purchased from the local markets of Majhitar, East Sikkim.

Instrument:

UV/Visible spectrophotometer (Shimadzu UV-1800) was used for the analysis of caffeine in samples such as (tea, soft drinks, energy drinks).

Wavelength selection:
The wavelength for maximum absorbance of caffeine was determined by scanning at 100 to 400 nm. The wavelength was found to be 274nm and used for further analysis [17].

Preparation of caffeine standard solution:
Caffeine stock solution (100 mg/ml) in chloroform was prepared in a 100 ml volumetric flask. From this, different dilutions were prepared in mg/ml such as (1, 5, 10, 15, 20, 25). Absorbances were measured at 274 nm [17].

Extraction of caffeine from soft and energy drinks and preparation of the solution:
About 20 ml of sample was heated for 20 min to remove carbon dioxide. It was kept at room temperature for cooling. 10ml of the sample was placed in a separating funnel, 1ml of (20% w/v) sodium carbonate & 5ml of chloroform were added to the sample. the content was swirled, and the lower layer was removed. An aliquot 0.1 ml of extract was mixed with chloroform (5 ml) and absorbance was measured at 274 nm under UV-Visible spectrophotometer [17].

Extraction of caffeine from black tea and preparation of the solution:
About 2 g of sample was added in 20 ml distilled water which is heated for 10 min. About 2 g of sodium carbonate was added to each sample for precipitating tannins. The sample was filtered and filtrates were concentrated to 5 ml by heating. From the given solution caffeine was extracted by adding 5ml of chloroform in a separating funnel and swirl for a few min. The lower caffeine-containing layer was separated and analyzed for caffeine content with UV-Visible spectrophotometer [17].

Table 1. Standard curve of Caffeine.

Sl. No.	Concentration (µg/ml)	Absorbance
1	1	0.044
2	5	0.212
3	10	0.394
4	15	0.549
5	20	0.772
6	25	0.936

RESULTS AND DISCUSSION:
A good linear relationship is observed between the absorbance and the concentration of the standard caffeine solution in the standard calibration curve shown in Fig 2.

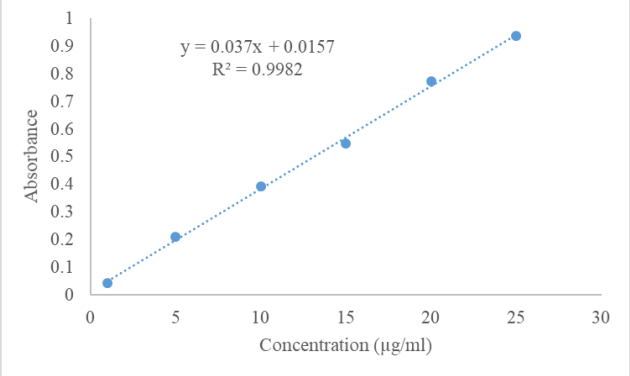

Fig 2. Standard calibration curve for caffeine.

Table 2. Table for Caffeine content in samples taken (Energy drinks, soft drinks, and Tea leaves).

Sample	Conc. of caffeine detected (µg/ml)
Energy drinks	
a. Red bull	8.2
b. Monster	10.58
c. Sting	7.8
d. Hell	10
Soft drinks	
a. Thumbs up	3.3
b. Coca cola	4.5
Tea leaves	
a. Denzong tea	1.21
b. Assam tea	2.960

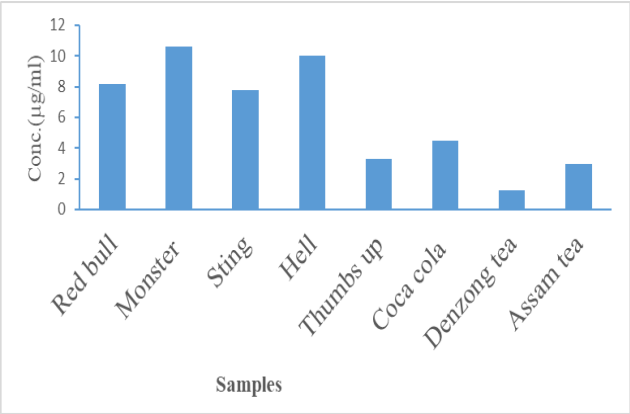

Fig 3. Concentration of caffeine in different samples.

In this study, caffeine content in the energy drinks, soft drinks, and tea leaves were determined using UV-Visible spectroscopy. For this determination, popular samples were collected from the local market. The sample included energy drinks like Red Bull, Monster, Hell, sting, soft drinks like thumbs up & coca cola & tea

samples like Assam tea, Denzong tea. From energy drinks it was found that the caffeine content is higher in the monster (10.58 µg/ml), hell (10 µg/ml) followed by red bull (8.2 µg/ml), and for soft drinks, the caffeine concentration was quite low as compared to energy drinks where thumbs up (3.3 µg/ml), coca cola shows (4.5 µg/ml) concentration of caffeine. From the tea sample it was found that the caffeine content is higher in Assam tea (2.960 µg/ml) as compared to denzong tea (1.21 µg/ml). From the result obtained, the concentration of caffeine was given in highest order as monster > Hell > red bull > sting > coca cola > thumbs up > assam tea > denzong tea. Determination of caffeine content in non-alcoholic beverages and tea is considered important due to high consumption among the college students, young generations for refreshing during entertainment and travelling hours [18]. High intake of caffeine leads to various adverse effects, determination of caffeine is necessary. From the study the caffeine content in the samples taken for study was found to be less than the specified limit that is 0.02 % (FDA limit).

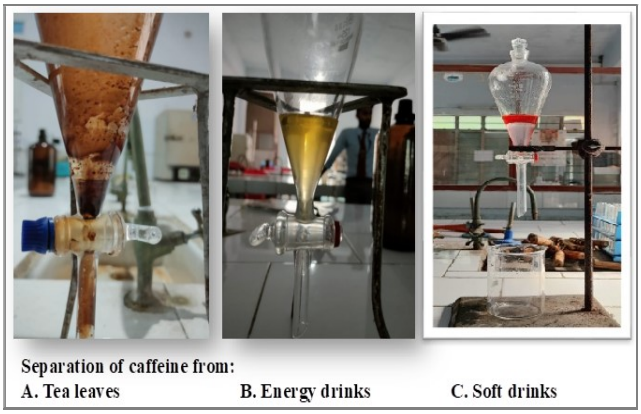

Fig 4. Separating images of caffeine from the samples.

CONCLUSION:

UV-Visible spectrophotometer is regarded as the most suitable method for the quantitative analysis of caffeine concentration in soft drinks, energy drinks and tea. This method is sensitive, accurate and economical. Despite the small number of samples analyzed, the present study gave preliminary information about the concentration of caffeine in tea, soft and energy drinks. The study led to the conclusion that all samples (energy drinks, soft drinks and tea samples) were found to contain caffeine concentration within the limit specified by FDA.

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