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Extraction and Estimation of Caffeine Content in Tea, Soft Drinks, and Energy Drinks Marketed in the Al-Jabal El-Khder Region, Libya

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Abstract

Tea, soft drinks, and energy drinks are widely consumed by young people and are especially popular at social gatherings, despite containing caffeine, a stimulant with potentially addictive and adverse health effects. Caffeine occurs naturally in tea and is commonly added to soft and energy drinks to enhance alertness and temporarily boost energy levels, making these beverages appealing in social contexts. However, frequent intake of caffeine has been associated with several negative health outcomes. This study aimed to determine the caffeine content in popular beverages using UV/VIS spectrophotometry. Samples were collected in 2024 from supermarkets across various locations in the Al-Jabal El-Akhdar region. Dichloromethane was employed as the extraction solvent, and a quartz cuvette was used to hold samples in the spectrophotometer to measure absorbance at 272 nm. Results revealed that caffeine concentrations were highest in energy drinks, with Red Bull and Bison containing 82 ± 0.173 ppm and 69 ± 1.082 ppm, respectively. Among soft drinks, Pepsi (Tripoli) had 41 ± 0.374 ppm, while Coca-Cola had the lowest at 35 ± 1.212 ppm. Tea samples showed a range from 39 ± 0.954 ppm to 61 ± 0.361 ppm, with the highest in Ahmed's black tea and the lowest in Al Najea's green tea. All samples were below the FDA caffeine limit of 200 ppm.

Keywords: Caffeine, tea, Drinks, UV/VIS spectrometry, Extraction

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استخلاص وتقدير محتوى الكافيين في الشاي والمشروبات الغازية ومشروبات الطاقة المسوقة في منطقة الجبل الأخضر، ليبيا

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الملخص

تَحظى المَشروبات مِثل الشَّايِ و المشروبات الغازية و مشروبات الطاقة بشعبية واسعة بين الشباب، وتُعد خيارًا رائجًا في المناسبات الاجتماعية. ومع ذلك، تحتوي هذه المشروبات على مادة الكافيين ذات الخصائص المنبهة التي قد تؤدي إلى الإدمان وتسبب آثارًا صحية سلبية. يوجد الكافيين بشكل طبيعي في الشاي، ويُضاف إلى معظم المشروبات الغازية ومشروبات الطاقة لتعزيز اليقظة وزيادة النشاط، مما يفسر انتشارها في الأوساط الاجتماعية. إلا أن الاستهلاك المفرط للكافيين ارتبط بعدة تأثيرات صحية سلبية. هدفت هذه الدراسة إلى تحديد محتوى الكافيين في عينات من هذه المشروبات باستخدام تقنية مطيافية الأشعة فوق البنفسجية والمرئية (UV/VIS). جُمعت العينات في عام 2024 من محلات تجارية في مناطق مختلفة من الجبل الأخضر. استُخدم ثنائي كلورو الميثان كمذيب للاستخلاص، واستُخدمت كوفيتات من الكوارتز لقياس الامتصاص عند الطول الموجي 272 نانومتر. كشفت النتائج أن تركيزات الكافيين كانت أعلى في مشروبات الطاقة، حيث احتوى ريد بول وبايسون على (69± 1.082 ppm و 82±0.173 ppm) على التوالي. من بين المشروبات الغازية، احتوى بيبسي (طرابلس) على (0.374 ppm ± 41)، بينما سجّل كوكاكولا أقل نسبة تركيز (35 ± 1.212 ppm). وتراوحت نسب الكافيين في عينات الشاي بين بين (61 ± 0.361 – 39 ± 0.954 ppm)، ، وكان أعلى تركيز في شاي أحمد الأسود وأقل تركيز في شاي النجع الأخضر. وكانت جميع العينات أقل من الحد الأقصى المسموح به من قِبل وكالة الغذاء والدواء الأمريكية (FDA) والبالغ 200 ppm.

الكلمات المفتاحية: الكافيين، الشاي، المشروبات، مطيافية الأشعة فوق البنفسجية/المرئية، الاستخلاص.

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1. Introduction

Caffeine, also known as 1,3,7-trimethylxanthine, 1,3,7-trimethyl-2,6-dioxopurine, 3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione, or methyltheobromine, is a purine alkaloid compound. It is an odorless compound with a slightly bitter taste, naturally occurring in many plant species. Globally, over 63 plant species contain this chemical in their leaves, seeds, or fruits. The most significant natural sources of caffeine include coffee (*Coffea spp*.), tea (*Camellia sinensis*), chocolate (*Theobroma cacao*), maté (*Ilex paraguariensis*), guarana (*Paullinia cupana*), guayusa (*Ilex guayusa*), wild guarana seeds (*Paullinia yoco*), cascara (*Croton eluteria*), and cola nuts (*Cola Vera*) (Heckman et al., 2010; Komes *et al.*, 2009).

Chemically, caffeine ($C_8H_{10}N_4O_2$) is a chemical compound composed of carbon, nitrogen, and oxygen, structured with a purine ring system that integrates both pyrimidine and imidazole rings, each containing two nitrogen atoms. This molecular structure incorporates various functional groups, including amine, carbonyl, alkene, and amide groups (Sharma et al., 2023). Caffeine appears as a white substance, either in the form of needles or powder, with a density of 1.2 g/cm^3. It has a melting point of 235 °C and a boiling point of 178 °C, which makes it stable at room temperature. Its solubility in water is limited to 2.17 grams per 100 liters, and its molar mass is 194.19 g/mol (Reddy et al., 2024). Caffeine is a derivative of xanthine, formed by the addition of three methyl groups. These methyl groups contribute to its solubility and pharmacological properties, classifying caffeine as a stimulant for the central nervous system. The molecule exhibits planarity, and its conjugated π-electron systems enhance its chemical stability. Additionally, weak dipole-dipole interactions and van der Waals forces are involved in caffeine's interactions with various enzymes and receptors, including adenosine receptors, explaining its stimulating effects (McLellan et al., 2016). Caffeine acts as a weak base with a pKa value of approximately 10.4, remaining mostly un-ionized under physiological conditions. This characteristic boosts its lipophilicity, allowing it to cross the blood-brain barrier quickly, further explaining its effects. The log P (partition coefficient) of caffeine is -0.07, signifying a balanced nature between hydrophilicity and lipophilicity (Ferré, 2008).

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Pharmacologically, caffeine acts as a central nervous system stimulant by antagonizing adenosine receptors, mainly A1 and A2A, promoting wakefulness and reducing fatigue. Its half-life ranges from 3 to 7 hours and is influenced by metabolic factors such as liver enzyme activity and genetic polymorphisms (Kolahdouzan and Hamadeh, 2017). In the liver, caffeine is primarily metabolized by cytochrome P450 enzymes, especially CYP1A2, producing three main metabolites: paraxanthine (84%), theobromine (12%), and theophylline (7%), each with distinct physiological roles (Cappelletti et al., 2015). Smoking accelerates caffeine metabolism, resulting in a reduced half-life, whereas liver dysfunction can impair its breakdown, extending its systemic presence. Additionally, several drugs, including antidepressants and oral contraceptives, inhibit CYP1A2, slowing caffeine clearance (Grzegorzewski et al., 2022). Following oral ingestion, caffeine is rapidly and completely absorbed, with effects initiating within 15 to 30 minutes and lasting for hours. In adults, caffeine's half-life—defined as the time required to eliminate 50% of the compound—varies with factors like age, weight, pregnancy, medication, and liver function. Among healthy adults, the average half-life is approximately four hours, with observed ranges from two to eight hours (Babu et al., 2008).
Many over-the-counter and prescription medications contain caffeine because of its vasoconstricting and anti-inflammatory properties, which complement analgesics and can sometimes increase their efficacy by up to 40%. The dosages of drugs like Midol™ and Vanquis™, which contain caffeine, range from 33 to 60 mg and are used to treat general pain. In combination with non-steroidal anti-inflammatory drugs and ergotamine, it is used therapeutically to alleviate migraine headaches. Prescription headache drugs including Fiorinal, Orphenadrine, and Synalgos include 30–60 mg of caffeine, while Anacin™, Excedrin™, Goody's™ headache powder, and Pain Reliever Plus contain 32–65 mg (Renner et al., 2007; Sawynok, 1995).
Due to its stimulant properties, caffeine is widely used in beverages, medications, and supplements. However, excessive consumption can lead to side effects such as increased heart rate, insomnia, anxiety, and, in extreme cases, caffeine toxicity. The primary objective of this study was to extract and determine the concentration of caffeine in well-known tea brands, soft drinks, and energy drinks.

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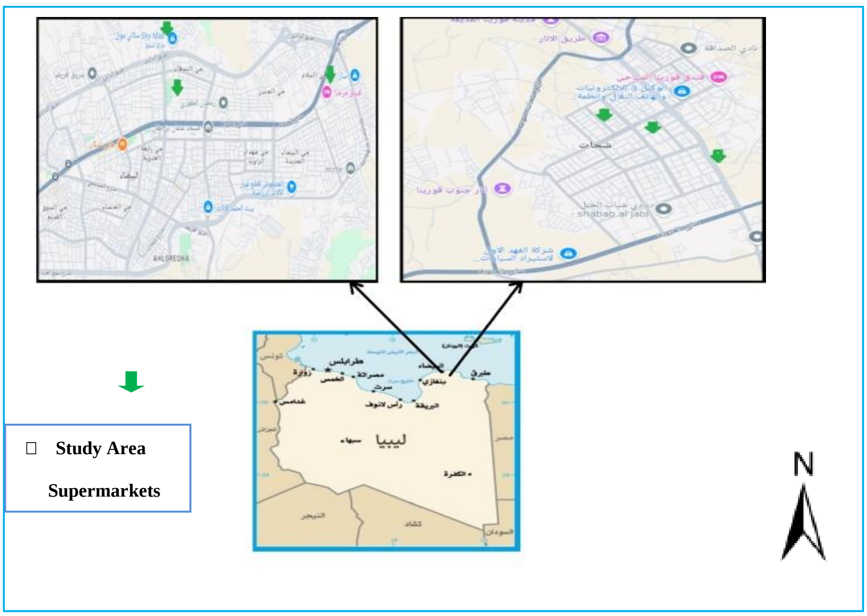
2. Material and Methods

2.1. Description of the Studied Area:

The study area is situated in northeastern Libya, occupying a strategic position along the country's eastern coast, which also forms part of the southern Mediterranean Basin. Markets in this region are dispersed across a wide area within the city. In recent years, the Libyan market has experienced increased openness, leading to the importation of food products from diverse and often unverified sources, with insufficient regulatory oversight. Furthermore, there has been a notable lack of adequate health supervision by the relevant authorities over both imported and locally produced food items. For this study, six local markets were selected in the cities of Al-Shahat and Al-Bayda, two of the most prominent cities in the Al-Jabal Al-Akhdar region (Figure 1).



2.2. Collection and Preparation of Samples

Overall, nine beverage samples were collected, with three replicates for each type (each replicate representing one bag or one bottle), from various commercial brands and flavors that were among the most popular and in-demand products in commercial stores in Shahat and Al-Bayda during the spring of 2024.The studied samples are detailed in Table (1). Following collection, the samples were placed in plastic bags or transfer containers, labeled with identification numbers, and stored in the laboratory until caffeine

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extraction and analysis were performed. All analyses were conducted in triplicate to ensure accuracy and reliability.

TABLE 1. Samples of selected beverages to estimate their caffeine content

Sample Type	Sample Code	Trade Name	Origin Country
Soft Drinks	S1	Pepsi	Tripoli
	S2	Coca Cola	Tripoli
Energy Drinks	S3	Red Bull	Austria
	S4	Bison	Turkey
Tea	S5	Ahmed black tea	Ceylon
	S6	Al-Taj black tea	Ceylon
	S7	Lipton black tea	China
	S8	Chinese's green tea	China
	S9	Al Najea green tea	China

2.3. Chemicals and Solvents:

Pure caffeine ($C_8H_{10}N_4O_2$), dichloromethane (CH_2Cl_2), Chloroform ($CHCl_3$) and sodium carbonate (Na_2CO_3), all of analytical grade, along with distilled water, were utilized to prepare the standard solutions necessary for constructing the calibration curve and extracting caffeine from various samples.

2.4. Instruments and Equipment:

UV/VIS spectrophotometer (JENWAY, Model 6405) was utilized for the analysis of caffeine in various samples. The equipment setup included a quartz cuvette with a 1 cm path length, a precision balance, a heating plate, a laboratory grinder, separating funnels with lids, volumetric flasks, beakers, funnels, pipettes, graduated cylinders, glass rods, and filter paper, all of which were essential for the analytical process.

2.5. Wavelength Selection:

The wavelength at which caffeine exhibits maximum absorbance was determined by scanning the range from 190 to 400 nm. It was found that caffeine absorbs most strongly at 272 nm, (AL-hitti and

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Ibrahim, 2009) used wavelength of 272 nm for analysis of caffeine, which is described.

2.6. Calibration Standard Preparation:

Caffeine stock solution (100 ppm) in chloroform was prepared in a 100 ml volumetric flask. From this, different dilutions were prepared in mg/ml, such as (2, 4, 6, 8, 10, and 12 ppm). Absorbances were measured at 272 nm

2.7. Caffeine Extraction Procedure

2.7.1. Soft and Energy Drinks:

To eliminate carbon dioxide, approximately 20 ml of the sample underwent heating for 20 minutes, followed by cooling to room temperature. Subsequently, 10 ml of the pre-treated sample was transferred to a separating funnel. To this, 1 ml of a 20% w/v sodium carbonate solution and 5 ml of chloroform were added. The mixture was then swirled, and the lower chloroform layer was collected. A 0.1 ml portion of this extract was diluted with 5 ml of dichloromethane, and its absorbance was measured at 272 nm using a UV-Visible spectrophotometer (Rehman and Ashraf, 2017).

2.7.2. Black and Green Tea:

Approximately (2 g) of sample was processed by adding 20 ml of distilled water and heating for 10 minutes. Around 2 g of sodium carbonate was added to each sample to precipitate tannins. The samples were then filtered, and the filtrates were concentrated to 5 ml by heating. Caffeine was extracted from the resulting solution by adding 5 ml of dichloromethane into a separating funnel, and the mixture was swirled for a few minutes. The lower layer containing caffeine was separated, and its caffeine content was analyzed using a UV-Visible spectrophotometer. Figure.2 illustrates the steps involved in extracting caffeine from tea samples (Wanyika et al., 2010).

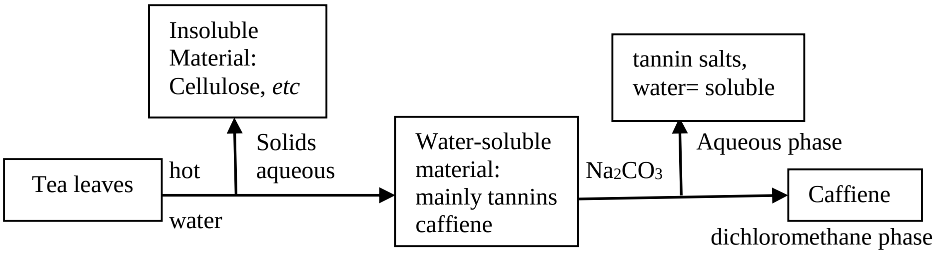

Figure 2. Schematic protocol for isolating and extracting caffeine from tea

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3. Results and Discussion

3.1. The maximum wavelength (λ max) for caffeine absorption and the standard curve:

Spectral scanning of caffeine absorption in water revealed the peak absorbance in the ultraviolet region at a wavelength of 272 nm, aligning with the findings reported by (Mufakkar et al., 2014), (Ogunney et al., 2017), and (Pradhan et al., 2020). Although most spectrophotometric methods for caffeine quantification utilize wavelengths between 270 and 274 nm, employing a quartz cuvette is crucial due to its exceptional transparency and non-absorption of ultraviolet radiation, unlike plastic or glass cuvettes. Notably, some Libyan studies have overlooked this critical detail. For instance, (Khalifa et al., 2023) used a glass cuvette, potentially compromising accuracy. Furthermore, (Al-Sadawi et al., 2016) utilized an inappropriate wavelength of 260 nm for caffeine absorption and misinterpreted the standard curve equation, potentially yielding inaccurate results. Figure (3) depicts the standard curve for caffeine solutions in water at the optimal absorption wavelength. The figure also presents the linear equation relating absorbance to concentration, demonstrating a high linearity (R^2) between concentrations and their corresponding absorbance values.

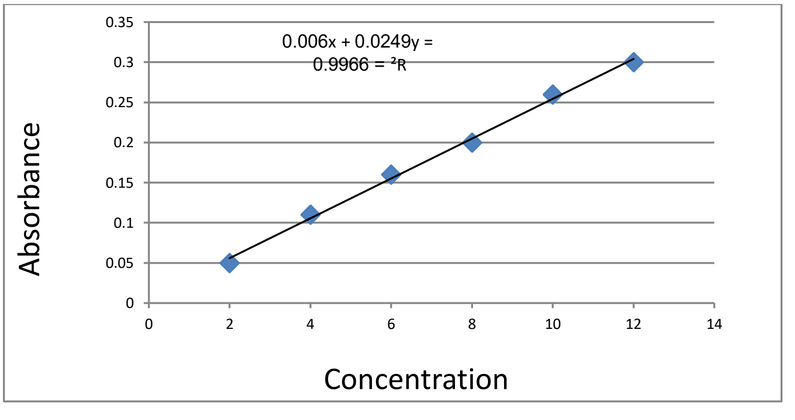

Figure 3. Calibration curve of standard caffeine solutions at 272 nm

3.2. Caffeine Contents in Beverage Samples:

Figure (4) highlights some of the effects of caffeine on human health, as summarized by Jabbar and Hanly (2013).

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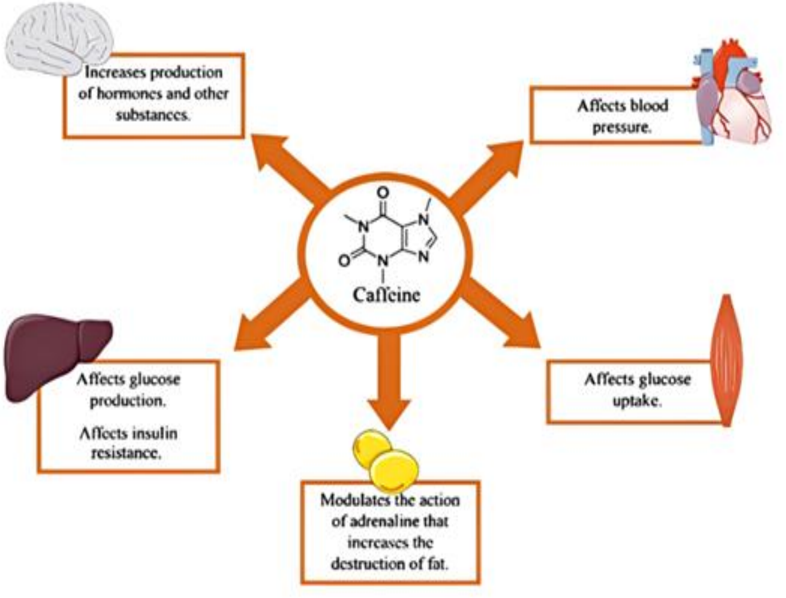

Figure 4. Caffeine acts on multiple systems in the body, including the heart, muscles, fat, liver, and brain

From table (2), Figure (5) illustrates the caffeine concentration (ppm = µg/ml) in the samples selected for this study. The data clearly indicate that the Red Bull energy drink exhibited the highest concentration among all the selected beverages (82 ± 0.173 ppm), while the Coca-Cola drinks showed the lowest concentration (35 ± 1.212 ppm). The Bison energy drink also presented a relatively high concentration compared to the other beverages (69 ± 1.082 ppm), confirming that energy drinks generally possess the highest caffeine content. The caffeine concentration in the Pepsi drink (Tripoli) was found to be 41 ± 0.374 ppm. These findings align with observations by (Ahmed et al., 2016), who reported high caffeine concentrations in energy drinks (ranging from 32.05 to 101.705 ppm) and lower concentrations in other beverages (ranging from 10.69 to 42.17 ppm). Furthermore, the caffeine concentration analysis results are largely consistent with Al-(Sadawi et al., 2016), who documented a caffeine concentration of 45.7 ± 0.5 ppm in Pepsi and 41.59 ± 6.1 ppm in Coca-Cola. They also reported elevated caffeine levels in energy drinks, such as 79.7 ± 3.47 ppm in Red Bull. Notably, all cola drink samples in this study remained within the maximum permissible limit for such beverages.

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Global caffeine levels in carbonated soft drinks and energy drinks fluctuate depending on the brand. The US Food and Drug Administration (FDA) has established a caffeine limit of (200 ppm) for soft and energy drinks. If the permissible caffeine limit in soft drinks is exceeded, it can lead to health risks such as insomnia, high blood pressure, and stomach problems. The FDA may take legal action against violators. Individual sensitivity to caffeine varies, necessitating monitoring of daily consumption. In a study by (Ahmed et al., 2005), caffeine concentrations in cola products (Regular Cola, Diet Cola, and Lemon Cola) in Riyadh, Saudi Arabia, ranged from 8.84 ± 0.02 ppm to 18.58 ± 0.04 ppm. Furthermore, (Gerald et al., 2014) reported that daily caffeine consumption from cola products in Riyadh falls between 1.07 and 4.79 mg, suggesting that this consumption does not exceed the acceptable daily intake (ADI). The European Food Safety Authority (EFSA) indicates that a daily caffeine intake of up to 400 mg is considered safe for healthy adults. For pregnant and breastfeeding women, EFSA recommends limiting daily caffeine consumption to a maximum of 200 mg (EFSA, 2015). Caffeine is added to soft drinks and energy drinks primarily for its stimulating effects on the central nervous system, which help increase alertness, reduce fatigue, and improve concentration. In energy drinks, caffeine is often combined with other ingredients like sugars, vitamins, and amino acids to enhance physical and mental performance, particularly during activities requiring prolonged focus or exertion. Additionally, caffeine acts as a flavor enhancer in some beverages, providing a slightly bitter taste that complements the overall profile of the drink. Finally, the inclusion of caffeine in these products caters to consumer demand for beverages that boost energy levels and combat tiredness, especially among younger and active individuals (Alsunni, 2015).

Regarding tea samples, from table (2), Figure (6) shows caffeine concentrations in black and green tea samples, and indicates that the black tea has higher caffeine content than green tea. The black tea brands Ahmed, Al-Taj, and Lipton had concentrations of 61 ± 0.361 ppm, 58 ± 0.490 ppm, and 55 ± 1.004 ppm, respectively. Chinese green tea contained 41 ± 0.374 ppm of caffeine, while Al-Naja green tea had 39 ± 0.954 ppm. The findings of (Shar et al., 2017) align with these results, as their study also noted that black tea contains higher caffeine levels than green tea. They found caffeine concentrations in various tea types and brands ranging from 52 ppm

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to 111 ppm, with green tea having the lowest caffeine content (52 ppm), and Lipton tea and Danedar Tapal tea reaching the highest concentrations of 110 ppm and 111 ppm, respectively. No global or national organizations set mandatory limits for caffeine levels in tea (including black and green tea), unlike some other substances or beverages such as carbonated drinks with added caffeine. However, some organizations provide guidelines and recommendations regarding general caffeine intake, which can apply to tea consumption. (FDA, 2016) recommends that most healthy adults can consume up to 400 milligrams of caffeine per day without negative effects. Notably, many previous studies on caffeine concentration in tea reported higher values than those found in this study. These studies often based their findings on dry weight and expressed the concentration as a percentage, where 1% equals 10,000 ppm.

Table (2) also shows that the maximum consumption limits for all drinks are significantly high. Despite compliance with the 200 ppm threshold, the relatively high caffeine levels in Red Bull and Bison reduce the maximum safe daily volume that can be consumed, especially by pregnant and lactating women, who should not exceed 200 mg/day. For instance, the MPL for Red Bull is only 2.44 liters/day for this group, emphasizing the need for cautious intake, particularly among sensitive populations. Similarly, Bison's MPL for pregnant women is 2.90 liters/day. The FDA does not specifically regulate the caffeine content in tea as it does in certain beverages like sodas or energy drinks. However, caffeine levels in tea occur naturally and can vary widely depending on the type of tea and how it is brewed. The FDA considers up to 400 milligrams (mg) of caffeine per day (about 4-5 cups of coffee) generally safe for most adults (Zhang et al., 2014). Medically, caffeine serves as a central nervous system stimulant, as an adjunct in treating apnea of prematurity, in treating migraine headaches with some pain relievers, and as an adjunctive analgesic and diuretic (Ngwoke et al., 2015). Excessive caffeine intake can lead to various health issues, including cardiovascular problems, sleep disturbances, headaches, and negative effects on bone health. Caffeine also stimulates stomach acid, potentially causing reflux and increasing urine production, which could lead to dehydration. Notably, reports of caffeine poisoning are increasingly rising, particularly among children and adolescents who consume energy drinks (Deasi, 2020).

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TABLE 2 Caffeine concentrations (ppm) in tea, soft drink, and energy drink samples, and their maximum permissible daily intake

Sample name	Caffeine concentration (mean±SD)	Maximum Permissible Limit (MPL) for daily consumption in liters (L) according to EFSA	
		Children and adults	Pregnant and lactating
Pepsi	41±0.500	9.76	4.79
Coca Cola	35±1.212	11.43	5.71
Red Bull	82±0.173	4.88	2.44
Bison	69±1.082	5.80	2.90
Ahmed black tea	61±0.361	6.56	3.28
Al-Taj black tea	58±0.490	6.90	3.45
Lipton black tea	55±1.004	7.27	3.64
Chinese green tea	41±0.374	9.76	4.88
Al Najea green tea	39±0.459	10.26	5.13

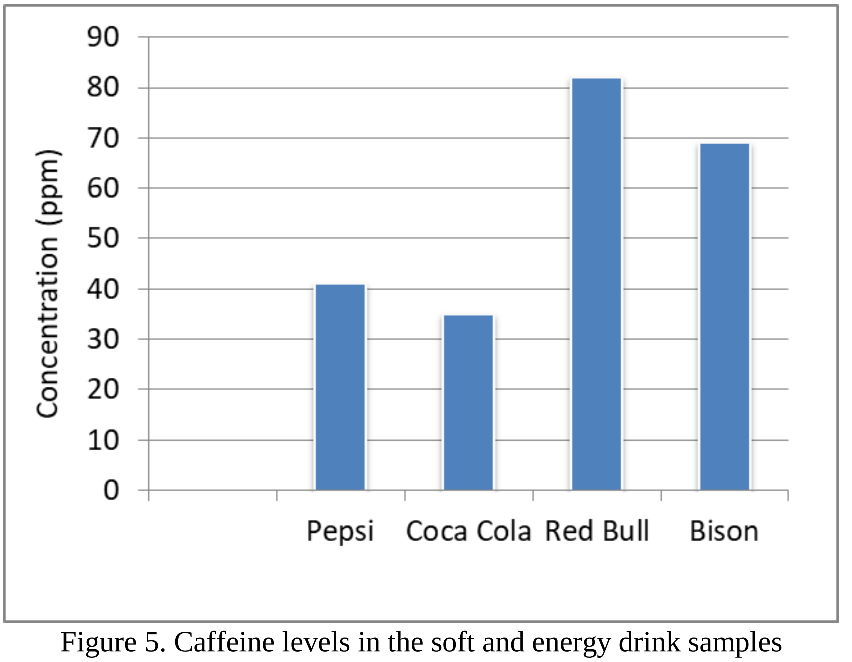

Figure 5. Caffeine levels in the soft and energy drink samples

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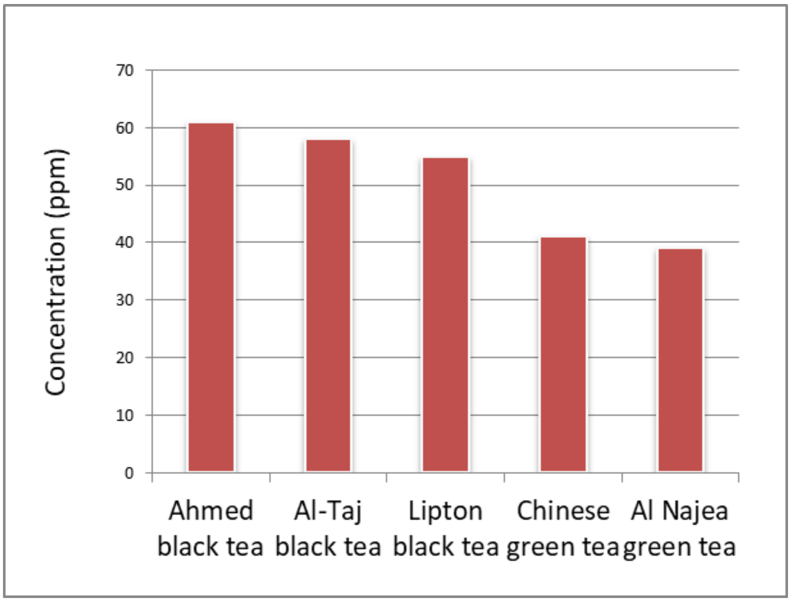

Figure 6. Caffeine levels in the black and green tea samples

4. Conclusion

This study aimed to estimate the caffeine concentration in four samples of soft drinks and energy drinks, in addition to five samples of black and green tea obtained from local and regional markets. Researchers employed the spectrophotometric method for caffeine determination, using dichloromethane as the extraction solvent. A quartz cuvette was used to hold the liquid samples during spectrophotometric analysis, and absorbance readings were taken at a wavelength of 272 nanometers. The results showed that the energy drinks Red Bull and Bison contained the highest caffeine concentrations among the soft drinks tested, measuring (82 ± 0.173 ppm) and (69 ± 1.082 ppm), respectively. Pepsi (Tripoli) had a caffeine concentration of (41 ± 0.500 ppm), while Coca-Cola recorded the lowest at (35 ± 1.212 ppm). Caffeine levels in tea samples ranged from (39 ± 0.954 ppm) to (61 ± 0.361 ppm). Ahmed black tea exhibited the highest concentration, whereas Al-Naja green tea showed the lowest. In general, black tea samples were found to have higher caffeine contents compared to green tea. These findings reflect notable variations in caffeine content across commonly consumed beverages in the region, highlighting the need for further investigation and consumer awareness regarding caffeine intake.

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Based on these findings, the study proposes the following recommendations:

- There is a need for consumer education on the potential health risks associated with excessive caffeine consumption. Public awareness campaigns can help inform the public, especially young people, about the caffeine content in popular beverages such as soft drinks and energy drinks, encouraging moderation in their consumption.
- Conduct further research to estimate caffeine concentrations in a broader range of beverages and food products where caffeine is used as a food additive, including dietary supplements, sports supplements, energy drinks, and soft drinks. Compare these concentrations against local and international standards, and raise awareness about the potential negative health effects of caffeine on patients and various age groups.
- Given the high caffeine content in energy drinks and the increasing consumption among young people, it is recommended to study caffeine exposure from these beverages and evaluate it against the acceptable daily intake (ADI) limit.
- Collaborate with relevant authorities to regulate caffeine consumption and develop a consumer guide for products containing caffeine, following the example of several Arab countries that have already implemented similar guidelines.

Conflict of interest

The authors declare that there are no conflicts of interest.

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