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Extraction and Analysis of Caffeine from Various Sources: A Review

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Abstract
Caffeine is a naturally occurring alkaloid found in tea, coffee shrubs, cola, guarana, mate, and various seeds, leaves and fruits of some plants. It is a central nervous system (CNS) stimulant that is believed to act by serving as an antagonist of adenosine receptors on neurons. It is a bitter, shiny white alkaloid and stimulant drug of the methylxanthine class. It acts as a natural pesticide because it can paralyse or kill insects feeding on the plant. This is why caffeine is classified by the Food and Drug Administration as GRAS (generally recognised as safe that the toxic amount covers 10 grams in the average adult) are much higher than commonly used doses less than 500 milligrams. This work will focus on the procedure for caffeine extraction and their working principles and various analytical methods for its separation and detection. Some known mechanisms of action explain the effects of caffeine. Using caffeine as a raw material, we can prepare an Anacin drug with several medicinal uses. Caffeine keeps us attentive and energetic and facilitates us to work powerfully when consumed properly. This aromatic complex can help fight diseases like cancer. On the other hand, it can trouble one's health by increasing heart rate and blood pressure and causing depression and anxiety when addicted in a small amount.
Keywords: *Caffeine, Extraction, Methylxanthine class.*

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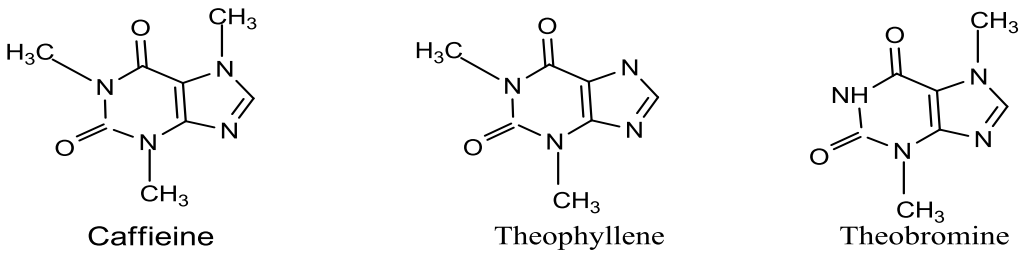
I. Introduction

Caffeine is a naturally occurring chemical stimulant found in numerous plants' leaves, seeds, and fruits. Caffeine is the world's most widely consumed psychoactive drug; however, it is legal and unregulated in nearly all parts of the world. It stimulates the central nervous system, reducing fatigue and drowsiness. It can improve athletic performance, muscular strength, and power. Caffeine riches chemically known as 3, 7 – dihydro – 1, 3, 7 – trimethyl – 1H – purine – 2, 6 – dione or 1, 3, 7 – trimethylxanthine having chemical formula $C_8H_{10}N_4O_2$[1] was first discovered in 1827 belonging to the alkaloid family containing nitrogen in their heterocyclic ring structure [2]. Coffee originated in China and was the sole producer until the British Empire colonised India and began large coffee plantations.

Coffee drinking is still an important ritual in Japan and England, from the choreographed Japanese coffee ceremony to the daily coffee time held in homes and offices across Brazil. Currently, Brazil is the highest consumer of coffee in the world. Caffeine is a naturally occurring substance found in the leaves, seeds, or fruits of more than 63 plant species worldwide. The most common sources of caffeine are coffee, cocoa beans, cola nuts, coffee leaves, yerba mate, guarana berries and the yaupon holly. The amount of caffeine in coffee varies by brand, but the average in common brands is typically about 30 – 40 mg caffeine/coffee bag. Caffeine is the purine-based Pseudo alkaloids because they were not directly derived from amino acid [3]. Pharmacologically, caffeine acts as a CNS stimulant, mild diuretic, and a natural pesticide, increasing blood pressure, increasing heart rate, stimulating gastric motility, algicides, and bactericides [4, 5].

Caffeine was first isolated from coffee in 1819 by a German chemist, Friedlieb Ferdinand Runge. Its pure form is an odourless white solid with a melting point of 235° - 238° C. Caffeine is classified as a heterocyclic purine base with two or more nitrogen elements in its rings. Caffeine is weakly basic, and its solubility in water at room temperature is quite low (2g/100ml) but increases dramatically in boiling water (66g/100ml).



Chemical structure of methylxanthines

The main methylxanthine in tea is the stimulant caffeine. Other methylxanthines found in tea are two chemically similar compounds, theobromine and theophylline, which play a major role in the long-term popularity of non-alcoholic beverages and foods such as coffee, tea, cocoa, chocolate and a variety of soft drinks. It is combined with painkillers (such as aspirin or acetaminophen) and a chemical called ergotamine for treating migraine headaches[6].

Caffeine is most used to improve mental alertness but has many other uses. Diet pills and cosmetic companies are the largest consumers of caffeine. It can also be used to manufacture body wash, soap, lip balm, facial scrub, and other products such as caffeine lipstick. Caffeine is incorporated in different pharmaceutical preparations along with acetylsalicylic acid, ascorbic acid, codeine, paracetamol and other analgesics and antipyretic preparations [7]. The amount of caffeine in food and beverage products varies depending on the serving size, product type, and preparation method. Tea, which we generally drink, is made from the leaves of an Asian Evergreen known as Camelia Sinesis. The caffeine content of tea leaves depends on the variety and where they were grown; most tea has 3 - 5 % by weight [8]. The optical transition properties of caffeine were measured in different solvents (dichloromethane, water, chloroform, and ethyl acetate). Caffeine has the highest optical transitions in dichloromethane than the other solvents. Caffeine can be extracted more at the boiling temperature than at 30° C. Caffeine can be extracted from cocoa by various methods, such as water extraction, supercritical carbon dioxide extractions and organic solvent extraction [9]. Solvents such as chloroform, methyl chloride, ethanol, acetone, and ethyl acetate are commonly used to extract caffeine [10]. Several methods can be used for this extraction, such as Soxhlet extraction, Ultrasonic extraction, and Heat Reflux extraction [11,12]. Heat Reflux extraction is one of the common methods used to extract caffeine from Cocoa seeds on a laboratory scale.

The level of caffeine can vary depending on what is consumed. A piece of chocolate may have as little as 5 mg, while energy drinks contain as much as 160 mg[13,14]. Make sure to read the labels of pain medications and diet pills, as products can have levels of caffeine as high as 200 mg. Michigan State University Extension recommends moderate doses of caffeine 200 - 300 mg daily, equivalent to 2 – 4 cups of brewed coffee and is considered safe for most adults. Consume more than 500 - 600 mg of caffeine daily, which equals 4 – 7 cups of coffee. You may be prone to health problems, including insomnia, nervousness, nausea or gastrointestinal problems, elevated heartbeat, headaches, etc. Caffeine is the most widely consumed psychoactive substance and can be a mild central nervous system stimulant. Caffeine is found in several things ingested by people. It is physically addictive. The caffeine extracted can be further converted into items we enjoy daily – sodas, cosmetics, and pharmaceutical products [15].

Properties of Caffeine

- Systematic name – 1, 3, 7 – trimethyl – 1H – purine – 2, 6 (3H, 7H) - Dione
- Other names – 1, 3, 7 – trimethylxanthine and 1, 3, 7 – trimethyl – 2, 6 – dioxopurine
- Molecular formula – $C_8H_{10}N_4O_2$
- Molecular mass – 194.19 g/mole
- Melting point – 238° C
- Solubility in water – slightly soluble
- Caffeine stimulates the central nervous system and the action of the heart and lungs.
- It increases blood pressure and promotes urine formation.

Advantages of Caffeine[16,17,18]

- Caffeine may help protect human brain cells, which lowers the risk of developing some diseases, such as Parkinson's.
- Caffeine reduces the risk of mortality and chronic diseases. Coffee reduces inflammation and may help prevent certain heart-related illnesses.
- Caffeine causes the blood vessels to constrict, which may help relieve some headaches, pain, and asthma attacks.

- Regular cups of coffee may stimulate the gallbladder and reduce the risk of gallstones. Caffeine is also used for weight loss and type 2 diabetes.
- Caffeine increases the potency of analgesics, and very high doses are used, often in combination with ephedrine, as an alternative to illegal stimulants.
- Caffeine creams are applied to the skin to reduce redness and itching in dermatitis.

Caffeine content in common Food and Drugs [1]

Up to 400 mg of caffeine daily is safe for most healthy adults. That's roughly the amount of caffeine in four cups of brewed coffee, 8 - 10 cans of cola or two energy short drinks. The caffeine content in beverages may vary widely, especially in energy drinks [(19)]. Caffeine in powder or in liquid form can provide toxic levels of caffeine, The U.S. Food and Drug Administration has cautioned. Just a single teaspoon of powdered caffeine equals around 28 cups of coffee. Such high levels of caffeine can cause serious major health problems and possibly death.

Table - 1 [19,20]

Food	**Average Content**
Coffee, Regular, Brewed	100 – 200 mg per cup
Coffee, Decaffeinated	2 – 4 mg per cup
Espresso	120 mg per 2 Oz
Instant coffee	57 mg per cup
Tea	30 – 75 mg per cup
Milk chocolate	6 - 10 mg per Oz
Baking chocolate	30 – 35 mg per Oz
Cocoa	5 – 40 mg per cup
Coca–Cola classis	46 mg per 12 Oz
Jolt cola	72 mg per 12 Oz
Dexatrim Dietic Vivarin	200 mg per pill
NO - Doz	100 mg per pill
Excedrin Extra Strength	65 mg per pill
Anacin Bromo Seltzer Midol	32 mg per pill
Dristan	16 mg per pill

Adverse Effects of Caffeine [21, 22]

- Daily caffeinated drinks can increase blood sugar levels and cause problems for people with diabetes.
- Caffeine is a diuretic that can cause dehydration, prevent some from falling asleep and interfere with deep sleep, leading to fatigue during the day.
- There is a significant association between drinking caffeinated coffee and the degrees of bone mineral density, which leads to Osteoporosis.

Table – 2: Caffeine content extracted with water in Tea/Coffee samples [15]

Coffee/Tea Samples	**Amount of Caffeine (gm)**
Bru Gold Coffee	0.68
AVT Coffee	0.62
Palat	0.04
Eastern Eastea	0.02
3 Roses	0.02
Kannan Devan	0.01
Brook Bond Red Label	0.01

Industrial applications of Caffeine: The caffeine so extracted can be further converted into items that we enjoy in our everyday life, e.g., sodas, cosmetics, and pharmaceutical products [23, 24.]

- Diet pills and cosmetic companies are the largest consumers of caffeine.
- It is also used with painkillers to prevent and treat headaches after epidural anaesthesia.
- Caffeine creams are applied to the skin to reduce redness and itching in dermatitis.
- It can also be used in manufacturing body wash, soap, lip – balm, facial scrub and several other products, such as caffeine lipstick.
- Caffeine is also used for weight loss and type two diabetes.

II. Materials and Methods

Various decaffeination methods, such as the Swiss water, Chemical solvent, and Supercritical carbon dioxide processes, are commercially used. The Swiss water process was very popular as it did not require chemicals and retained more flavour components. This has been replaced by the chemical solvent process requiring methylene chloride/ethyl acetate. These solvents are considered toxic and hazardous; their presence in the product and their effect on the environment and operators must be monitored carefully.

Table 3: Different methods adopted to extract caffeine [25, 26].

Name	Different methods
Muthanna J. Mohammed, Firas A. Al – Bayati, 2008	(1) Liquid - Liquid Extraction (2) Solid – Liquid Extraction
Khalida Khan, M Naeem, M Arshad and M Asif, 2012	Column Extraction
Satarupa Banerjee, Jyotirmoy Chatterjee, 2015	(1) Microwave Assisted Extraction (2) High Pressure Processing (3) Supercritical Fluid Extraction (4) Subcritical Water Extraction
Gonul Serdar, Ezgi Demir, Munevver Sokmen, 2015	(1) Citric Acid Water Extraction (2) Ethanol Extraction (3) Two-Step Water Extraction (4) High Temperature Pretreatment Water Extraction (5) Water Extraction (6) Solid - Liquid Extraction
Gonul Serdar, Ezgi Demir, Serhat Bayrak, Munevver Sokmen, 2017	Microwave Assisted Extraction

Extraction is a method used to separate organic compounds from a mixture of compounds. This technique selectively dissolves one or more compounds into an appropriate solvent. The solution of these dissolved compounds is referred to as the extract. Liquid - Liquid extraction (LLE) is a method to separate compounds or metal complexes based on their relative solubilities in two different immersible liquids, usually water (polar) and an organic solvent (non–polar). LLE is an essential technique in chemical laboratories, where it is performed using a variety of apparatus, from separatory funnels to counter current distribution equipment called mixture settlers. This type of process is commonly performed after a chemical reaction.

Solvent selection: Although water is almost always one of the liquids in the Liquid - Liquid extraction process, The choice of organic solvent is quite wide. A suitable extraction solvent needs five essential features [27]:

(i) The immiscible with the other solvent (usually water).
(ii) It has a relatively low boiling point to be easily removed from the compound after extraction.
(iii) Extract little or none of the impurities and other compounds present in the mixture.
(iv) Be non-toxic, non-reactive, readily available, and inexpensive.
(v) Has high solubility for the organic compound.

Table – 4: Various solvents for caffeine extraction [15]

S. No.	Solvents	Description	% Extraction
1	Water	Non-toxic, Complex process that removes little flavouring components	94 – 96 %
2	Ethyl Acetate	Removed caffeine and little flavour compounds, Mildly toxic.	96 – 98 %
3	Dichloromethane	Removes caffeine and some flavouring compounds, Highly toxic	94 – 96 %
4	Supercritical CO_2	Selectively removes caffeine and very few flavour compounds, expensive	96 – 98 %
5	Acetone	Removes caffeine and little flavour compounds, mildly toxic	96 – 98 %

Dichloromethane is used as a solvent in liquid–liquid extraction because caffeine has higher solubility in dichloromethane than other solvents. After separating the organic layer from the separating funnel, it is then kept for evaporation to evaporate the dichloromethane present in it. Raw crude yellowish caffeine is further sent to recrystallisation to obtain pure white caffeine. Ethanol is used for recrystallisation as a solvent. The success of extraction involving a natural product is often expressed as percentage recovery.

$$\% \text{ Recovery} = \frac{\text{Gram of Caffeine Recovered}}{\text{Grams of Tea Leaves}}$$

The percentage recovery is called the purified percent recovery or crude present recovery. The extraction with the highest percent recovery is considered the most successful extraction.

Confirmative test for Caffeine detection

Murexide test can be carried out for caffeine detection as follows:

In a watch glass, a small amount of a sample with 2 – 3 drops of concentrated Hydrochloric Acid is mixed. Use a glass rod for mixing. Then, we add a few small crystals of potassium chlorate and mix well. Heat the watch glass over a boiling water bath until the sample is dry. Allow to cool. Moisten with a drop of bench (2 mol dm^{-3}) ammonia solution. The sample should turn purple.

Analysis Techniques for Caffeine [28, 29]

- **Thin Layer Chromatography (TLC):** There are different chromatographic methods, such as paper chromatography, gas chromatography, etc. They have the same principle: different solutes have different solubility in solvent/different solutes have different degrees of tendency to be dissolved in the same solvent. As the solution (containing the solvent with the dissolved solutes) moves along a stationary solid surface (a solid surface), different solutes adsorbed onto the solid surface to different extent as they have different degrees of adsorption characteristics (due to the different degrees of dissolve tendency). The less soluble solute will be retained first, and the more soluble solutes will be retained afterwards. (Note: No two substances have the same solubility and adsorption characteristics. Different solutes will then be separated on the different positions of the solid surface.) The retention Factor (R.F.) of each component is calculated as follows.

$$R\,F = \frac{\text{Grams of caffeine recovered distance travelled by the component substances from the base line}}{\text{Distance travelled by the solvent from the base line}}$$

Pure caffeine and the extract were analysed in the same TLC plate and compared for any difference in their R.F.

- **Gas Chromatography (G.C.):** Gas chromatography analysis of caffeine can be carried out with a Perkin – Elmer gas chromatograph equipped with flame ionisation detector (FID) G.P. – 100 – Printer plotter and an electronic integrator, employing a bounded phase fused silica capillary column B.P. – 1 (30 m X 0.32 m ID; 0.25 film thickness) coated with polydimethylsiloxane. Nitrogen gas can be used as carrier gas at 0.4 ml/min flow rate and 10 psi inlet pressure. Temperature is programmed from 60 to 160° C. The sample is injected with a split ratio.
- **Iodometric Back Titration:** Caffeine reacts with an excess accurately known amount of iodine in an acidic environment, forming an insoluble precipitate. Then, the insoluble precipitate is removed by filtration. Using titration by a standard sodium thiosulphate solution with the starch solution as an indicator, we can determine the amount of remaining iodine and, thus, the amount of caffeine that can be found. Here are the chemical equations:

$$C_8H_{10}N_4O_2 + 2I_2 + H_2SO_4 \longrightarrow C_8H_{10}N_4O_2.HI.I_4 + KHSO_4$$

$$I_2 + 2\,Na_2S_2O_3 \longrightarrow 2NaI + Na_2S_4O_6$$

- **Spike Test:** By adding a known amount of standard caffeine in distilled water and raw coffee solution, then carry out solvent extraction. By comparing the extraction results, we can analyse the spiked caffeine's recovery percentage and solvent extraction's efficiency.

III. Conclusion

The level of caffeine in all brands is found within the range. Caffeine in low quantity keeps us alert and active and enhances our performance. Increasing the speed of perception and decision-making also helps in weight loss and cancer prevention. However, high doses cause insomnia, rising blood pressure, and anxiety. Tea and coffee are rich in antioxidants and are the most widely used beverages worldwide. They also have medicinal properties. Caffeine is the most commonly used psychoactive drug in the world. It is a pharmacologically active substance and can be a mild central nervous system stimulant, depending on the dose. The purified caffeine is then analysed by using Thin Layer Chromatography, Gas Chromatography, Iodometric Back Titration, and Spike Test. The serious concern about the potential use of caffeine for pathogenic effects has made it one of the most broadly studied drugs. We can conclude that coffee's caffeine content is relatively high compared to other beverages. Therefore, we can also state that caffeine is highly soluble in dichloromethane compared to other solvents.

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