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# Extraction and Estimation of Nerostimulous from Different Tea Brands and Its Application

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## ABSTRACT

*Caffeine is an odourless, slightly bitter bioactive heterocyclic amine present in many plants. It is found in beverages such as coffee or tea and in chocolates, coca cola. Caffeine is a drug, which stimulates the central nervous system (CNS). A number of over the counter pain relievers, headache remedies and antihistamines also contain caffeine. Among alkaloids – Caffeine is probably the most widely used drug.*

*Three brands of tea were purchased from market namely Red Label, Parivar, and Vikram. At first, we extracted caffeine from tea by solvent extraction. Furthermore, extract was analyzed for its caffeine content by thin layer chromatography, and other chemical tests. The results show that coffee contains higher amount of caffeine as compared to tea leaves. Caffeine content of tea sample was 20-26.5mg/g of the product. As per the US food and Drug administration these levels are considered safe in healthy adults. Furthermore, antibacterial activity and effect of extract concentration on plant growth, germination was evaluated.*

***Keywords**: Alkaloid, Central Nervous System (CNS), Caffeine, Chromatography, Extraction.*

## I. INTRODUCTION

Caffeine (1,3,7-trimethylxanthine), the molecular formula of which is C8H10N4O2, is the most widely consumed stimulant in the world can be consider to be constructed from the purine ring system, which is important biologically, being found in nucleic acids and nucleotides and in few organisms as alkaloids. Caffeine was first discovered in tea in 1827, and was named theine, and later it was found in mate, coffee and various other plants and the term theine was then dropped [1].Tea (Camellia sinensis) and coffee are the most popular beverages for centuries, primarily due to their pleasant taste and stimulant effects [2]. Millions of people throughout the world drink a cup of tea or coffee in the morning to induce wakefulness and/or at some other time of day to overcome the physical and mental exertion.Tea and coffee contains biologically active secondary plant metabolites such as alkaloids, polyphenols, flavonoids and tannins etc. that are considered to be beneficial to human health [3]. Many scientific studies have shown that regular consumption of tea or coffee can prevent or reduce the risk of development of many chronic diseases [4].

Caffeine was extracted by liquid chromatography by liquid-liquid extraction method and its identity and purity can be confirmed by thin layer chromatography and by recording lambda max in water. The results show that coffee contains higher amount of caffeine as compared to tea leaves. Coffee content of Caffeine were range from 39-41mg/g, while tea sample contain 20-26.5mg/g [5]. As per the US food and drug administration these levels are considered safe in healthy adults [6]. Many scientific studies have shown that regular consumption of

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tea or coffee can prevent or reduced the risk of development of many chronic diseases. However, there are some conflicting reports also, but the overall balance is towards the benefit sides [7].

**Table 1 Caffeine Content of Common foods and Drugs [7].**

| | |
|---|---|
| Coffee | 103 mg per cup |
| Instant coffee | 57 mg per cup |
| Coffee, decaffeinated | 2 to 4 mg per cup |
| Tea | 30 to 75 mg per cup |
| Cocoa | 5 to 40 mg per cup |
| Anacin, Bromo-Seltzer, Midol | 32 mg per pill |
| Excedrin, Extra Strength | 65 mg per pill |
| Dexatrim, Dietac, Vivarin | 100 mg per pill |

In present work we considered three brands of tea were purchased from market namely Red Label, Parivar, and Vikram. Anhydrous sodium carbonate acts as base. The base convert's tannins in to their sodium salts being ionic these salts are not soluble in solvents like methylene chloride so remain in the aqueous layer during extraction. These allow purer caffeine to be extracted. Anhydrous sodium sulphate used to dry the solvents and remove any water that may be present before you start evaporating the solvent off. Propan-1-ol is used to extract caffeine from an aqueous extract of tea because caffeine more soluble in propan-1-ol. Petroleum ether is used for precipitation formation in extraction solution, after extraction to form caffeine powder. We studied different application of extracts on plant growth and its antibacterial activity against gram negative bacteria.

## II. MATERIALS AND METHODS

### 2.1. Solid-Liquid Extraction

Solid-liquid extraction allows soluble components to be removed from solids using a solvent. Application of this unit operation includes obtaining oil from oil seed or leaching of metal salts from ores.

An everyday example is the preparation of tea or coffee. Here, water (solvent) is used to remove the tea or coffee flavours (transition component) from the tea or coffee powder (extraction material, consisting of solid carrier phase and transition component). This results is are drinkable coffee or tea (solvent with dissolved flavours) [8].

In recently, the solid carrier phase will still contain some transition component after completion of the extraction. In addition, some of the solvent will be adoptively bonded to the solid carrier phase.To achieve the fastest and most complete solid extraction possible, the solvent must be provided with large exchange surfaces and short diffusion paths. This can be done by pulverising the solid to be extracted. However, an excessively small grain size can cause agglutination and make it more difficult for solvent to permeate.

In the simplest form of this unit operation, the extraction material and the solvent are mixed well. The solvent and the dissolved transition component are then removed and regenerated. The extraction material can also take the form of a fixed bed with the solvent flowing through the solvent. The solvent is normally regenerated using evaporation or distillation. The solvent is evaporated and a concentrated extract solution is left behind as the product. The solvent is condensed and can then be reused [9].

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## 2.2. Liquid-Liquid Extraction

### 2.2.1 Extraction using chloroform

Make aqueous solution by adding 4.58g of coffee powder to the 100ml of distilled water and 3g of sodium carbonate to increase the solubility of caffeine and boil it for few minutes.Cool down the solution to the room temperature. Transfer the raw coffee solution to the separating funnel. Add 10ml of chloroform and shake gently. Allow the mixture to stand and separate out the bottom chloroform layer to a beaker. Repeat this step five times. Add calcium sulphate to the separated chloroform to remove water. Shake well and stop until fluffy, cloudy effect. Weight the beaker which is being to hold the filtrate. Filter out the excess calcium sulphate, put the beaker with filtrate into hot water bath to evaporate chloroform (Boiling point 61.2°C) Weight the powder and calculate the amount of powder extracted [10].

### 2.2.2 Extraction using propanol

Add 2g of tea powder to 30 ml of distilled water and add 2g sodium carbonate. Boil the solution for 10 min on hot plate, decent the liquid into flask, add 20 ml of distilled water to the beaker and again boil it for 10 min. and decent it. Cool extract at room temperature. Transfer extract to the separating funnel and add 15 ml of propanol to the separating funnel. Shake the mixture gently; remove the glass stopper to vent any vapour that may have built up. Allow two layers to fully separate. Transfer organic layer to beaker and repeat the process with fresh propanol for 3-6 times. Add a small amount of sodium sulphate to dry organic layer and stir till it swirls like a snow globe. Place the beaker on a hot plate and when the volume of material in the beaker is between 3-5 ml start adding petroleum ether by pipet. When beaker begins to get cloudy remove the beaker from heat and allow it to cool. Filter the extracts allow it to air dry and then apply the sublimation process [11].

3.2.3 Extraction using other method

200 ml of coffee extract was taken in 500 ml of conical flask and 20 ml diluted $H_2SO_4$ (2 ml conc. $H_2SO_4$ +18 ml distilled water) was added. This mixture of 220 ml volume was then heated, at temperature 90°C ± 2°C maintained in a water bath and reduced the volume of the mixture to about 50 ml. The concentrated mixture was again filtered through whatman-42 and collected in the separating funnel. Then 20 ml chloroform was added with the filtrate in separating funnel and shaken well for 20 times.The washed chloroform from the bottom of the separating funnel was collected in 50 ml conical flask. The same filtrate was then washed thoroughly with different volumes (viz. 20, 15, 10 and 5 ml) and the total volume of the collected chloroform was washed with 5 ml 1% KOH in a clean separating funnel and was collected in a 50 ml oven dried conical flask which was previously weighed. The layer was dried using water bath and sublimation was done [12].

## 2.3 Analysis of caffeine

2.3.1 Qualitative analysis of caffeine by thin layer chromatography (TLC)

TLC is a simple, quick, and inexpensive procedure that gives a quick answer as to how many components are in a mixture. TLC is also used to support the identity of a compound in a mixture when the Rf of a compound is compared with the Rf of a known compound (preferably both run on the same TLC plate). A TLC plate is a sheet of glass, metal, or plastic which is coated with a thin layer of a solid adsorbent (usually silica or alumina). A small amount of the mixture to be analysed is spotted near the bottom of this plate. The TLC plate is then placed in a shallow pool of a solvent in a developing chamber so that only the very bottom of the plate is in the liquid. This liquid is the mobile phase, and it slowly rises up the TLC plate by capillary action.

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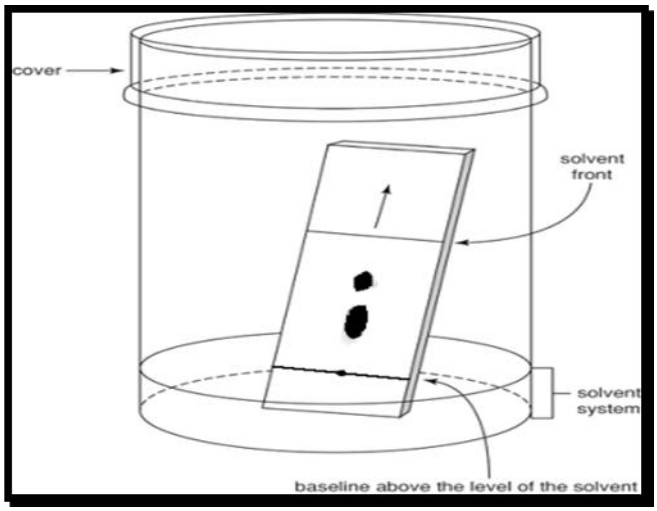


**Fig.3. Thin layer chromatography**

As the solvent moves past the spot that was applied, equilibrium is established for each component of the mixture between the molecules of that component which are adsorbed on the solid and the molecules which are in solution. In principle, the components will differ in solubility and in the strength of their adsorption to the adsorbent and some components will be carried farther up the plate than others. When the solvent has reached the top of the plate, the plate is removed from the developing chamber, dried, and the separated components of the mixture are visualized. If the compounds are colored, visualized is straightforward. Usually the compounds are not colored, so a UV lamp is used to visualize the plates [12].

In this experiment we have used iodine vapours for the spot observation in thin layer chromatography method. The staining of a TLC plants with iodine vapour is among the oldest methods for the visualization of organic compounds. It is based upon the observation that iodine has a high affinity for both unsaturated and aromatic compounds. To 100ml of beaker few crystals of iodine is added. Iodine has a high vapour pressure for a solid and the chamber will become rapidly become saturated with the iodine vapour. Then insert TLC plate and allow it to remain within the chamber until it develops a light brown colour over the entire plate. Commonly, if desired compound has an affinity for iodine, it will appear as dark brown spot on a lighter brown background. Carefully remove the TLC plate at this point and gently circle the spots with a dull pencil. The iodine will not remain on the TLC plate for long periods of time so circling these spots is necessary if one wishes to refer to these TLC's at a later date [14].

### 2.3.2 Quantitative analysis of caffeine by iodometric back titration

Sulphuric acid added to standard caffeine solution and extract solution separately.Iodine were added to solution, Brown-red precipitate formed. After filtration, filtrates were titrating against sodium thiosulphate solution by adding few drops of starch solution as indicator. The brown-red precipitate solution converts to colourless. Reading was taken, till get 2-3 consistent reading [15].

### 2.4 Antibacterial effect of caffeine

*E. coli* DH5α bacterial strain was used for the whole antibacterial process. To start the growth, 2 ml of overnight cultured *E .coli* stock was added to 100 ml of NB medium containing 0.12% of glucose with 0.01%, 0.1%, 0.5%, 1% and without caffeine (control) respectively. And incubated on rotary shaker at 30°C for 24 hours. Optical density measurements at 600 nm were used to monitor the concentration of bacteria [14]. 12 Petri plates were prepared with NB agar medium, 6 containing caffeine (1%) and 6 without caffeine (control).Different

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dilutions of bacteria used that is 10, 100, 1000, 10000, 100000, 1000000.20 µl of each bacterial solution was poured in Petri plates. Incubated at 30°C for 48 hours. The plates were visually estimated and bacteria colony is counted [16].

### 2.5 Effect on plant growth

We planted mung beans in three different pots labelled as standard caffeine, 2%extract with water.For first 5 days only water was given to the plants and after 5 days height measured and standard caffeine solution and extract solution was given to the plants.For next 5 days plants were provided with respective solutions and height was measuredAverage height from each pot were calculated [17].

## III. RESULT AND DISCUSSION

### 3.1 Extraction

For time of the solvent extraction, we use the different tea powder brand like Red label, Vikram and Parivar. Extraction was done with this tea powder using propanol, petroleum ether for the powder formation of extract. We obtained powder but we did not get caffeine in it. We observed the $\lambda_{max}$ of the same extract solution, $\lambda_{max}$ of caffeine is at 274 nm. We did not get correct maximum wavelength, because it may not contain caffeine or may be contain impurities.

We used another extraction method which is mentioned in material and methods 3.1, we failed with this method. Then we go with another method for the extraction and we also changed the solvent as chloroform. During extraction the chloroform become plane brown. After extraction we obtained powder of pale yellow with strong smell. Comparing with pure caffeine and observation, it's obvious that the extraction does contain contamination where pure caffeine is white powder.

### 3.2 Thin layer chromatography

We carry out TLC to prove the existence of caffeine in the extract. In the first part we carry out extraction from tea. We carried TLC for that, we used solvent system as ethylscetate:methanol:water(10:1.35:1) and we got retention factor as 0.294, 0.308 and 0.308 for the brands of tea that is red label, vikram and parivar respectively. The RF value of caffeine did not match with research paper. From this we can concluded that the extract do not contain caffeine.

In the next part we used coffee to extract the caffeine, and we carry TLC using the same solvent system as above. We run two spots one is extract and other is standard caffeine solution. We got RF value for standard caffeine spot as 0.73 and for extract solution as 0.698. From this we can concluded that the extract contain caffeine and we successfully extract the caffeine from the coffee. It may because coffee contain more amount of caffeine than tea and although some amount of caffeine loosed during extraction process it retains some amount of caffeine as it is not in the case of tea.

### 3.3 Iodometric back titration

Iodometric back titration used to find out the amount of caffeine in extract.Accuracy for Iodometric back titration is 97.1%Amount of caffeine we calculated by this method is 25.009 mg/100 m

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### 3.4 Antibacterial effect of caffeine

#### 3.4.1 Liquid medium

For each flask OD observed at 600 nm, we got maximum OD for control flask as 0.6408 and 0.3246 for 0.01% caffeine concentration flask. Lower OD was observed in 1% flask.At higher concentration of caffeine, it affects the growth of bacteria. At lower concentration bacterial growth is high.Hence caffeine decreases the growth of bacteria in liquid media at high concentration.

**Table2. Measurements of OD in liquid medium**

| Serial no. | Concentration of caffeine | 24 hours | 48 hours |
|---|---|---|---|
| 1 | Control | 0.6408 | 0.1155 |
| 2 | 0.01% | 0.3246 | 0.0471 |
| 3 | 0.1% | 0.3340 | 0.0441 |
| 4 | 0.5% | 0.3702 | 0.0418 |
| 5 | 1% | 0.0260 | 0.0061 |

#### 3.4.2 Solid medium

We observed higher number of colonies of bacteria in 10000 times dilution control plate.Bacterial colonies were not observed in any experimental plates. Hence caffeine suppresses the bacterial growth on the solid media.

**Fig.1. 48 hours incubated plates**

### 4.5 Effect of caffeine on plant growth

To study the effect of caffeine on plant growth we studied three different pots with planted mung beans for which first five days of plantation we provide only water to all three plants. After five days, we named the pots as standard caffeine, extracted caffeine, and water and to them we provide the standard caffeine solution, extract solution, and water respectively. The height is measured of plants in each pot up to five days and then the average height is measured, and the result shows that the standard caffeine containing pots as shown in table.

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Table 3. Growth of plants over 5 days

| Day | 1 | 2 | 3 | 4 | 5 | Avg. Height |
|---|---|---|---|---|---|---|
| Water | 10.5 | 15.16 | 15.86 | 15.88 | 16.73 | 14.82 |
| Std. caffeine | 13.48 | 18 | 18.33 | 22.41 | - | 18.05 |
| Extract | 14.66 | 17.42 | 18.31 | 18.5 | 18.66 | 17.51 |

## V.CONCLUSION

After above procedure of extraction of caffeine, we conclude that the solvent used for extraction that is chloroform is good for the caffeine extraction. Due to the nature of partition coefficients of solvent-solvent extraction, the amount of loss of caffeine could be reduced by increasing the number of portion of chloroform used in the process.

The TLC results proved that caffeine was extracted successfully from the raw coffee solution. The Iodometric back titration is a simple and an accurate method to determine the amount of caffeine in aqueous solution. It requires simple apparatus and common chemicals only. Our results showed that it is an accurate method. It has very high accuracy, about 97.1%.

Then we see the applications of caffeine, first is antibacterial effect of caffeine. Our results show a clear antibacterial effect of caffeine. The sensitivity of bacteria to caffeine can vary greatly depending on caffeine concentration. This effect could be taken into account in medical practice.

Another application of caffeine that we have seen is effect of caffeine on plant growth. Standard caffeine solution stimulates the growth of plants, but the survival of plants is good in the extract pot, because extract may contain other compounds which may favourable for the survival of plants.

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