Effective Black Tea Caffeine Extraction and UV/VIS Spectrophotometer Analysis of a Local Tea Brand

Muhammad Shahab Alam[1,2,*], Rasheedullah[3], Zubair Hashmi[3], Nusrat Kiran[3], Muhammad Bilal[4]

[1]Department of Basic Science & Humanities, Dawood University of Engineering & Technology, Karachi,

[2]H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi

[3]Department of Chemical Engineering, Dawood University of Engineering & Technology, Karachi,

[4]Department of Industrial Engineering & Management, Dawood University of Engineering & Technology, Karachi,

*Corresponding Author: Muhammad Shahab Alam,

Keywords: Caffeine, Extraction, Black Tea, UV/VIS Spectrophotometer,

Abstract: Using various techniques, varied amounts of caffeine were extracted from tea leaves. However, it was discovered that approach-B is a revolutionary approach used to extract caffeine from black tea leaves. The UV-Spectrophotometer is used to evaluate the caffeine content in black tea leaves. Method-B offers several benefits over traditional extraction method-A, including a shorter extraction time, a greater yield, reduced impurity levels, and less energy use.

INTRODUCTION

Caffeine is an alkaloid found in tea, one of the most popular caffeinated beverages consumed worldwide [1]. As an organic substance with at least one nitrogen atom, caffeine is classified as an alkaloid. It is a member of the methylxanthines chemical class, along with theophylline and theobromine, which are closely related substances [2].

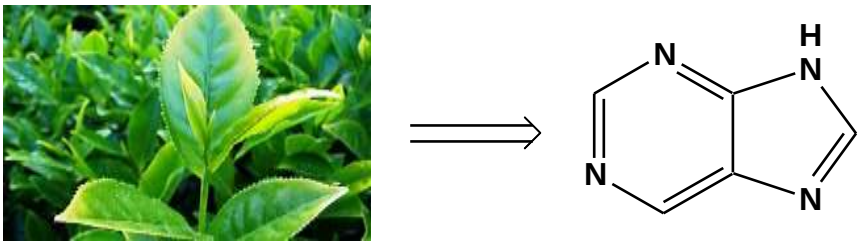

Figure-1: *Chemical structure of caffeine*

Tea shrubberies containing the Caffeine, also have acidic compound namely tannins, cellulosic materials, pigments and chlorophyll. To isolate the Caffeine from these tea shrubberies and leaves, it must be available in free basic forms Caffeine is invigorating due to cyclic backbone arrangement analogous to structure of a base named purine of DNA [3].

Chemical Structure of Purine

Tea leaves also contain additional organic substances known as tannins [4], commonly known as gallic acid (GA), in addition to caffeine. Caffeine and GA may both be dissolved in water [5]. However, due to dipole-dipole interactions brought on by caffeine's greater polarity and the formation of H-bonds with both water and caffeine, caffeine has a significant attraction for water. The use of intermolecular attractions in GA may theoretically be used to strengthen the dipole-dipole interface [6]. Additionally, caffeine is employed as a food ingredient that is subject to FDA regulation. Caffeine's usage as a stimulant in various over-the-counter and prescription drugs is governed by the FDA. Caffeine is frequently indicated as an active component in stimulants, cold treatments, and a variety of painkillers. Depending on the amount, caffeine, a pharmacologically active chemical, may have a modest stimulant effect on the central nervous system. Caffeine is typically eliminated few hours after administration and does not accumulate in the body over time [7]. Cocoa beans, kola nuts, guarana berries, yerba mate, and yaupan holly are other sources of caffeine. In fact, it is naturally present in many plant species' leaves, seeds, or fruits [8]. Caffeine's scientific name is 1, 3, 7 trimethyl-1H-purine, although it is also known as 1, 3, 7 trimethyl xanthine.

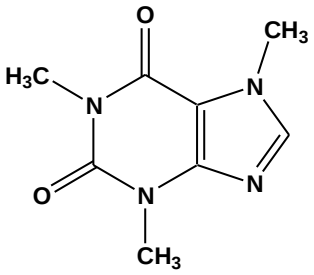

Figure-2: *Structure of Caffeine*

The molecular mass is 194.19 g/mole and the chemical formula is $C_8H_{10}N_4O_2$. Its boiling point is 178 °C, and it has a 138 °C melting point. It has a white colour, is mildly soluble in water, tastes bitter, and has no odor.

Caffeine may provide greater protection for human brain cells, lowering the risk of developing a number of diseases, including Parkinson's. Regular coffee drinking might stimulate the gallbladder and reduce the risk of gallstone formation. Because caffeine causes the blood vessels to constrict, some headache discomfort may be lessened. Coffee reduces inflammation and may offer protection against a number of heart-related illnesses. Enhances the effectiveness of analgesics and treats migraines and asthma attacks.

Additionally, type-2 diabetes and weight reduction are treated with caffeine For a healthy adult without any medical conditions, the U.S. Department of Agriculture (USDA) and the European Food Safety Authority (EFSA) define a safe caffeine consumption as up to 300–400 mg per day [8]. Drinking caffeinated coffee is significantly linked to osteoporosis, which is caused by a decline in bone mineral density. Caffeinated beverages can raise blood sugar levels and pose issues for diabetics if they are consumed every day.

Dehydration from caffeine's diuretic effects is possible. Some people may have trouble falling asleep, and caffeine interferes with deep sleep, which can make them tired during the day [9]. It is one of the most often misused medications and is quite addictive. The list of withdrawal effects from caffeine includes fatigue, headache, nausea, and illness [10]. Caffeine dissolves differently in various solvents. Chloroform, dichloromethane, acetone, ethyl acetate, water, methanol, ethanol, and carbon tetrachloride are the solvents in which caffeine is least soluble [2].

Table 1. *Caffeine Content of Common Food and Drugs.*

Cocoa	5-40mg per cup
Milk Chocolate	6mg per Oz
Dexatrim Dietac Vivarin	200mg per pill
Dristan	16mg per pill
No-Doz	100mg per pill
Tea	30-75 mg per cup
Espresso	120 mg per 2 Oz
Baking Chocolate	35mg per Oz

Excedrin Extra Strength	65mg per pill
Coffee, Decaffeinated	2 to 4 mg per cup
Coca-Cola Classis	46mg per 12 Oz
Jolt Cola	72mg per 12 Oz
Coffee, Regular, Brewed	103 mg per cup
Anacin Bromo Seltzer Midol	32mg per pill
Instant Coffee	57mg per cup

Table 2. *Caffeine Content in Tea/Coffee Sample (Extraction with water).*

TEA/COFFEE SAMPLES	AMOUNT OF CAFFEINE (gm)
Kannan Devan	0.01
Palat	0.04
Brook Bond Red Label	0.01
3 Roses	0.02
AVT Coffee	0.62
AVT	0.03
Bru gold Coffee	0.68
Eastern Eastea	0.02

EXPERIMENTAL DETAILS

Materials

Erlenmeyer flask, 5g of Black tea (Tapal), Dichloromethane (SIGMA-ALDRICH), Separating funnel, dropper, Beakers, Charcoal, Methanol (AnalaR), Sodium carbonate (Xilong Chemical Co.Ltd), Distill water, vials, Balance, Electric heater, Silica Bed, Caffeine standard(Sigma-Aldrich) and Whatman no.1 filter papers.

Extraction Method-A

5g of tea leaves (Tapal) were weighted and add in 100ml of water along with 2g of sodium carbonate (Na_2CO_3). The purpose of Na_2CO_3 addition is to remove the tannins. Now heated up to boiling point and keep boiling for 10 minutes. After 10 minutes allow the mixture to cool down to separate the mixture of tea leaves and tannins vacuum filtration is used. Shift the filtrate to the funnel fitted in iron stand. Add 50ml DCM to funnel and extract the DCM fraction. Repeat this for three times (50ml x 3=150ml). the next step is to evaporate the DCM using Rotary Evaporator to collect the caffeine containing DCM fraction 0.14g [9].

Extraction Method-B

5g of tea leaves (Tapal) were weighted and add in 100ml of water along with 2g of sodium carbonate (Na_2CO_3). Now heated up to boiling point and keep boiling for 10 minutes. After 10 minutes allow the mixture to cool down and shifted to the funnel fitted in Iron stand. Add 50ml DCM and extract the DCM fraction. Repeat this for three times (50ml x 3 =150ml).

The next step is to evaporate the DCM using Rotary Evaporator to collect the caffeine containing DCM fraction 0.16g.

Figure-3: *Liquid-Liquid Extraction of Caffeine.*

Extraction Method-C

In this method 5g of tea was added in DCM directly and left for three days. After three days the DCM was change to greenish type and the whole DCM was divided into two parts. The first half part (C-1) was treated with charcoal to absorb the coloring materials. After one day the DCM was white and the whole color was absorbed by charcoal and we get the caffeine 0.01g.The second half part (C-2) was treated reversely which was shift to funnel and add solution of 6g of sodium carbonate in 100ml water and left for one day and after extraction of DCM we get 0.05g.

Extraction Method-D

In this method 5g of tea leaves were add in a 50ml DCM along with charcoal at the same time. After one day the charcoal and DCM was filter through filter paper and we get a white DCM which can be evaporate using Rotary Evaporator and we get 0.04g.

Table-3: *Different methods of Extractions and Absorbance of resulting extracts.*

Method Name	Caffeine Extracted	Absorbance (A)/5ml	Concentration Of Caffeine (mg)
A	**0.14g**	**2.701**	**3.778**
B	**0.16g**	**3.553**	**4.977**
C-1	**0.01g**	**0.752**	**1.034**
C-2	**0.05g**	**0.7708**	**1.061**
D	**0.04g**	**1.2356**	**1.715**

Preparation of Standards

Caffeine stock standard solution was prepared by dissolving 0.0144 gram of caffeine standard in 50 ml solution of Acetonitrile and water by ratio of 1:9. Working standard solutions of 0.01, 0.1, 0.05 and 0.2 mg/mL were prepared by suitable dilution of stock solution. Calibration curve was obtained by plotting absorbance versus standard concentration of caffeine.

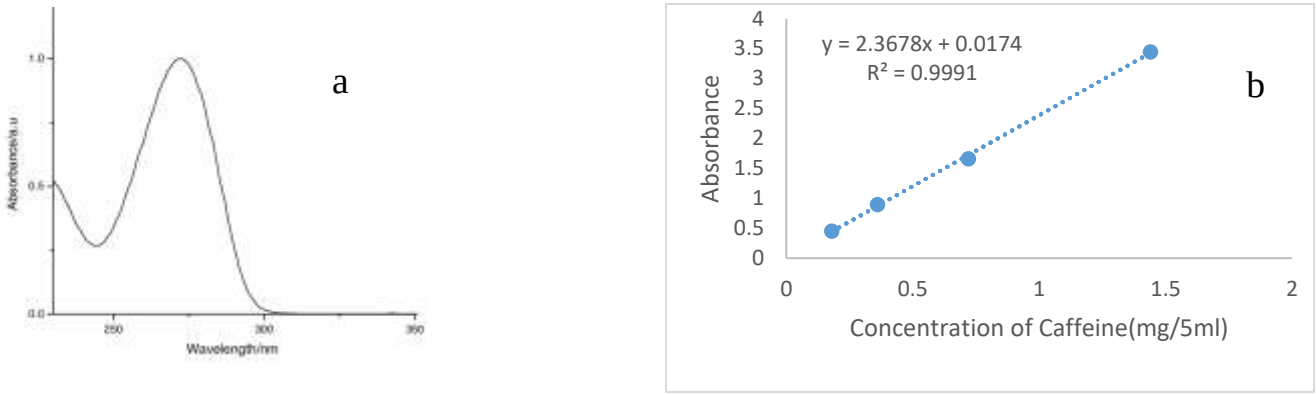

Figure-4: a.*UV of Caffeine and* ***b****.Calibration Curve of Caffeine.*

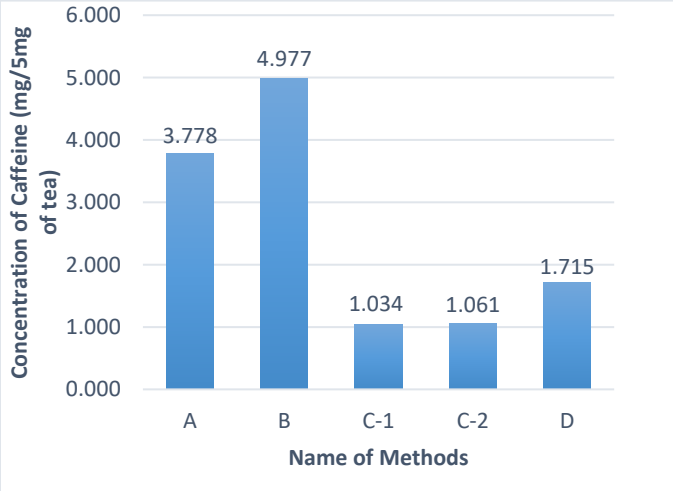

Figure-5. *Concentration of Caffeine in different samples.*

Result and Discussion:

Dichloromethane (DCM) will be used as a solvent to decaffeinate tea (Tapal) in this investigation. This investigation will be conducted to determine the caffeine content of tea leaves. It's okay that the caffeine content dropped with each use. Liquid-liquid extraction with vacuum filtration is used to extract the caffeine from tea [9], as is liquid-liquid extraction without vacuum filtration and adding tea leaves to DCM one at a time. The caffeine level of tea leaves was examined in the

current study, and it was discovered that the caffeine amount varied depending on the extraction process.

The results of a number of studies using various extraction techniques lead us to the conclusion that method-B (4.977mg) yields a caffeine concentration, Table-3, that is considerably higher than that of described method-A [9] (3.778mg). The reported method-A requires more time, a vacuum filter, and has a lower yield than method-B. Due to this, we employed technique B, which provides the maximum yield. However, after the presentation of Method-B, there was a sizable amount of product loss during the process since the reaction was unable to proceed without complications, resulting in a lower than 100% yield. This implies that there was substantial product loss during the process, which was caused by emulsion formation.

Additionally, it's important to keep in mind that tea leaves and solvents cannot react entirely, making a yield of 100% impractical. Additionally, it was discovered that the amount of water added caused the caffeine content to drop. Due to the fact that caffeine dissolves differently in water depending on the temperature, ranging from 2.2 mg/ml at ambient temperature to 180 mg/ml at 80°C to 670 mg/ml at 100°C [11]. In method-C1 we have use the Charcoal which may absorb some amount of caffeine along with coloring materials due which the concentration of caffeine is (1.034mg) less than method-C2 (1.061mg) in which we doesn't use the charcoal and use the $NaCO_2$ which remove and precipitate the coloring materials along with tannins.In method-D the tea leaves and charcoal add to DCM at the same time and filter after one day, which give more caffeine (1.715mg) as compare to Method-C1 (1.034mg).

Conclusion:

Different methods were used to extract varying amounts of caffeine from tea leaves. The UV-Spectrophotometer method quantified caffeine in black tea leaves, while a novel approach called Method-B was developed for caffeine extraction. Compared to the conventional Method-A, Method-B offered several advantages. It had a shorter extraction time, higher yield, lower impurity levels, and reduced energy consumption. Method-B's accelerated extraction time made it efficient in time-sensitive situations. Its higher yield meant more caffeine could be extracted, increasing overall efficiency. Lower impurity levels ensured a purer caffeine product. Lastly, Method-B's lower energy consumption aligned with sustainability principles. Overall, the UV-Spectrophotometer method and Method-B improved caffeine extraction from tea leaves by providing accurate quantification and enhanced efficiency, yield, purity, and sustainability.

References

[1] M. Zahra, M. Rehman, and I. Qayyum, "Isolation of Natural Caffeine From Lipton™ Black Tea Through Acid-Base Liquid-Liquid Extraction Approach, Its Medical Significance and Its Characterization By Thin Layer Chromatography and IR Analysis," *Nat Prod Chem Res, an open access journal,* vol. 9, 2021.

[2] A. Shalmashi and F. Golmohammad, "Solubility of caffeine in water, ethyl acetate, ethanol, carbon tetrachloride, methanol, chloroform, dichloromethane, and acetone between 298 and 323 K," *Latin American applied research,* vol. 40, p. 283, 2010.

[3] Z. Ruan, D. Ghorai, G. Zanoni, and L. Ackermann, "Nickel-catalyzed C–H activation of purine bases with alkyl halides," *Chemical Communications,* vol. 53, pp. 9113-9116, 2017.

[4] M. Khajeh, S. Fard, M. Bohlooli, M. Ghaffari-Moghaddam, and A. Khatibi, "Extraction of caffeine and gallic acid from coffee by electrokinetic methods coupled with a hollow-fiber membrane," *Journal of Food Process Engineering,* vol. 40, p. e12565, 2017.

[5] C. Lin, G. Xia, and S. Liu, "Modeling and comparison of extraction kinetics of 8 catechins, gallic acid and caffeine from representative white teas," *LWT-Food Science and Technology,* vol. 83, pp. 1-9, 2017.

[6] W. E. Acree Jr, "Comments on "Thermodynamic modeling studies of aqueous solubility of caffeine, gallic acid and their cocrystal in the temperature range of 303 K–363 K"," *Fluid Phase Equilibria,* vol. 463, pp. 32-33, 2018.

[7] S. Parvathy, A. Luiz, and J. T. Varkey, "Chemical analysis of caffeine content in tea and coffee samples," *Asian Journal of Science and Applied Technology,* vol. 3, pp. 1-4, 2014.

[8] M. Yunus and B. Nulamuga, "Extraction and Characterization of Caffeine: A Biochemical Compound Contained In Some Locally Consumed Tea Leaves (Camellia Sinensis)," *International Journal of Research and Scientific Innovation,* vol. 7, pp. 1-7, 2020.

[9] A. Chaugule, H. Patil, S. Pagariya, and P. Ingle, "Extraction of caffeine," *Int J Adv Res Chem Sci,* vol. 6, pp. 11-19, 2019.

[10] D. Costill, G. P. Dalsky, and W. Fink, "Effects of caffeine ingestion on metabolism and exercise performance," *Medicine and science in sports,* vol. 10, pp. 155-158, 1978.

[11] K. L. Williamson and K. M. Masters, *Macroscale and microscale organic experiments*: Cengage Learning, 2016.