PROJECT REPORT

On

"CAFFEINE EXTRACTION AND COMPARATIVE QUANTIFICATION IN CHOSEN BRANDS OF TEA, COFFEE AND GREEN TEA"

Submitted by

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In partial fulfillment for the award of the

Bachelor's Degree in Chemistry

DEPARTMENT OF CHEMISTRY AND CENTRE FOR RESEARCH

ST. TERESA'S COLLEGE (AUTONOMOUS)
ERNAKULAM

2023-2024

ST. TERESA'S COLLEGE (AUTONOMOUS)

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B.Sc. CHEMISTRY PROJECT REPORT

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This is to certify that the project **"CAFFEINE EXTRACTION AND COMPARATIVE QUANTIFICATION IN CHOSEN BRANDS OF TEA, COFFEE AND GREEN TEA"**is the work done by **SARA BABU, HRIDHYA C J, NANDINI P AND VANDANA A.**

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CERTIFICATE

This is to certify that the project work entitled "**CAFFEINE EXTRACTION AND COMPARATIVE QUANTIFICATION IN CHOSEN BRANDS OF TEA, COFFEE AND GREEN TEA**" is the work done by **SARA BABU, HRIDHYA C J, NANDINI P AND VANDANA A** under my guidance in the partial fulfilment of the award of the Degree of Bachelor of Science in Chemistry at St. Teresa's College (Autonomous), Ernakulam affiliated to Mahatma Gandhi University, Kottayam.

DR. NISHA T P

Project Guide

DECLARATION

I hereby declare that the project work entitled "**CAFFEINE EXTRACTION AND COMPARATIVE QUANTIFICATION IN CHOSEN BRANDS OF TEA, COFFEE AND GREEN TEA**" submitted to Department of Chemistry and Centre for Research, St. Teresa's College (Autonomous) affiliated to Mahatma Gandhi University, Kottayam, is a record of an original work done by me under the guidance of **DR. NISHA T P, ASSISTANT PROFESSOR,** Department of Chemistry and Centre for Research, St. Teresa's College (Autonomous), Ernakulam and this project work is submitted in the partial fulfilment of the requirements for the award of the Degree of Bachelor of Science in Chemistry.

SARA BABU
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Contents

Chapter 1

Introduction

1. 1 BEVERAGE

A beverage is broadly defined as any liquid that can alleviate thirst. Water stands as a fundamental example of a beverage, and the category encompasses an array of options such as tea, coffee, milk, juice, and even alcoholic drinks like beer. Whether served hot or cold, non-alcoholic or alcoholic, beverages cover a spectrum of liquids meant for consumption. The term "beverage" extends beyond a mere functional description, often embracing a diverse range of drinks that cater to various tastes and occasions. From the soothing warmth of tea or coffee to the refreshing qualities of water or juice, beverages span a spectrum of flavours and textures, offering a multitude of choices for consumers. In the realm of dining establishments, bars, and retail settings, the term "beverage" is commonly employed as a versatile replacement for the more general term "drink." This broad classification encompasses everything potable, and in some contexts, it is used to differentiate between styles of drinks, such as soft drinks or alcoholic beverages. Thus, "beverage" becomes a comprehensive term that encapsulates the vast array of liquid refreshments available for consumption. In essence, the term "beverage" encapsulates a wide spectrum of liquid options, emphasizing not only their function in quenching thirst but also their varied nature, making them suitable for different tastes, occasions, and preferences.

Beverages have become a very big part of our diets and are necessary to stay hydrated. Beverages have undeniably become integral to our daily

routines, serving as more than just a means of hydration. We indulge in a variety of drinks for diverse reasons, ranging from celebratory moments and socializing with friends and family to combating boredom or simply quenching our thirst. While water stands out as one of the most essential liquids, playing a crucial role in maintaining the efficient functioning of our bodies, our beverage choices extend far beyond mere survival needs. The significance of water cannot be overstated, as it contributes to the well-being of our skin, aids in digestion, promotes healthy nails and hair, and ensures overall bodily hydration. Despite not being essential for survival, other beverages have seamlessly woven themselves into the fabric of our daily lives, providing a source of refreshment and tranquillity. Beyond the physiological benefits, beverages have become intertwined with cultural, social, and personal rituals. From toasting during celebrations to sipping coffee during moments of reflection, these drinks have a remarkable ability to enhance our experiences. Moreover, the sheer variety of beverages available allows individuals to tailor their choices to personal preferences and moods. The ubiquity of beverages also reflects a cultural and societal shift, where the act of consuming drinks has transcended mere sustenance. Whether enjoying a cup of tea to unwind after a long day or sharing a round of drinks with friends, beverages have become a means of creating and enhancing connections. In essence, while not indispensable for survival, beverages have secured a lasting place in our routines, providing not only physical nourishment but also contributing to our emotional and social well-being.

1.1.1 TEA

Tea is the most popular beverage in the world, drank after water. The dried leaf of the Camellia sinensis plant is used to make tea. The fragrant

beverage known as tea is made by steeping fresh or over-cured Camellia sinensis leaves in hot or boiling water. This evergreen shrub is native to East Asia and is said to have started its journey in the borderlands of northern Myanmar and southwest China. There are three different types of tea: oolong (semi-fermented), green (non-fermented), and black (fermented), depending on how the leaves are picked and processed. The production and processing methods used to generate these main types of tea vary depending on the many drying and fermenting processes that affect the tea's chemical makeup. Green tea is made from young tea leaves that are steamed, pan-fired, dried, graded, and then sold for consumption without going through any fermentation process. In order to stop the natural enzyme activity from fermenting the tea leaves, pan firing is necessary. To prepare black tea, tea leaves are fermented for a few hours and then either steamed, flame-fired, or smoke-fired. Partial oxidation of the leaf, which occurs halfway through the process for producing black and green tea, is how oolong tea is made. The process of making black tea involves first oxidizing the tea leaves by exposing them to air. The flavor of the leaves is enhanced during this oxidation process, which also gives them a deep brown hue. Following that, the leaves are either left unaltered or roasted, dried, and crushed. The health benefits of green tea, such as its chemo preventive and chemotherapeutic properties against cancer, have received the most research; nevertheless, new findings suggest that black tea may possess similar health-promoting attributes and effects.

1.1.2. CHEMICAL COMPOSITION OF TEA

Tea shoot consists of enzymes, biochemical intermediates, carbohydrates, proteins, and lipids. Furthermore, tea shoots are unique due to their high concentration of methyl xanthine and polyphenols, which include caffeine

and other purines like theophylline and theobromine. These two classes of chemicals, together with other compounds linked to the aroma of tea, are primarily responsible for the distinct flavour of tea, which may account for its popularity as a beverage. The chemical composition of tea shoots changes with agro climatic state, season, cultural activity, and the type of material.

. Total polyphenols, which consist of flavanols, flavanols, flavanol glycosides, polyphenolic acids, and depsides, account for roughly 30% of the dry weight in a tea shoot. The primary oxidizable chemicals in tea leaves are flavanols or catechins. The two main catechins found in tea leaves are (-) epigallocatechin (EGC) and (-) epigallocatechin gallate (EGCG). The cytoplasmic vacuoles contain the catechins, which are important during fermentation.

1.1.4 COFFEE

A beverage made from roasted coffee beans is called coffee. Coffee is dark in colour, bitter, and slightly acidic. Its caffeine content is what gives it its energizing properties to people. When it comes to hot drinks, it has the largest sales worldwide.

To make unroasted green coffee beans, the seeds from the fruits of the coffee plant are separated. To make a cup of coffee, the beans are roasted, crushed into tiny particles, and usually soaked in hot water before being filtered out. There are many different ways to make and serve coffee (such as espresso, French press, coffee latte, or canned coffee that has already been brewed).

Coffea arabica Linn, a member of the Rubiaceae family, is what makes coffee. Just three of the 70 species of coffee are grown commercially.25%

of production comes from Coffea arabica, while 75% comes from Coffea canephora, and less than 1% by Coffia liberica.

1.1.5 CHEMICAL COMPONENTS OF COFFEE

Coffee's primary ingredients include caffeine, tannin, fixed oil, protein, and carbs. It has 2–3% caffeine, 3–5% tannin, 13% protein, and 10%–15% oil in it. There is caffeine in the form of a chlorogenic acid salt.

1.2 CAFFEINE

The primary ingredient in drinks like coffee and tea is caffeine. It can also be found in chocolate, soda, and energy beverages. It is the main alkaloid found in coffee, tea, and a few other drinks.

As an odourless, white powder with smooth, sparkling needles, caffeine is a purine alkaloid with an unpleasant taste. The formula for it is $C H_{10}N_4O_2$.

Purines are a class of heterocyclic chemicals that includes caffeine. It is chemically known as 1,3,7-trimethylxanthine and 1,3,7-trimethyl-2,6-dioxopurine. Its systematic name is 3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione. The molar mass of caffeine is 194.19 grams. It is a methyl xanthine alkaloid purine with a white crystal structure that is chemically related to guanine and adenine in DNA and RNA. The caffeine molecule's nitrogen atoms are all essentially planar. Despite the fact that some are frequently depicted with three single bonds, these atoms' lone pairs engage in resonance with nearby double-bonded carbon atoms, causing them to adopt a sp2 orbital hybridization.

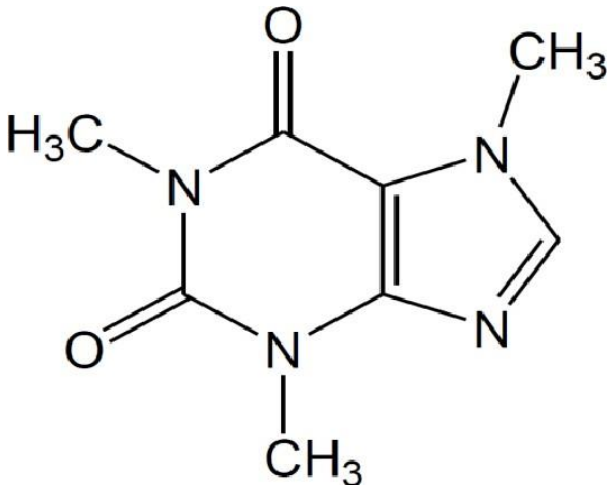

Figure 1.1: Structure of Caffeine

Caffeine has a melting point between 235 °C and 238 °C. At room temperature, caffeine is only slightly soluble in water (2 g/100 mL), but it dissolves completely in boiling water (66 g/100 mL). Additionally, it dissolves in ethanol somewhat

(1.5 g/100 mL). Due to its weak base (pK_a of approximately 0.6), it needs a strong acid to be protonated. Since caffeine lacks stereo genic centres, it is categorized as an achiral molecule.

Caffeine's xanthine core is made up of two fused rings: imidazole and pyrimidinedione. In turn, the pyrimidinedione has two amide functional groups that are primarily found in a zwitterionic resonance, which is when the nitrogen atoms are double linked to the amide carbon atoms next to them. As a result, every single atom in the pyrimidinedione ring system is sp^2 and planar. The imidazole ring also has a resonance. Therefore, the

fused 5,6 ring core of caffeine contains a total of ten π electrons and hence according to Huckle's rule is aromatic.

The amount of time needed for the body to excrete half of a dose of caffeine is known as its biological half-life, and it differs greatly between people based on a number of variables, including age, liver enzyme function, pregnancy, and other medication use. Caffeine's half-life in healthy humans is three to seven hours. The half-life is prolonged in the latter trimester of pregnancy, about doubled in women using oral contraceptives, and reduced by 30 to 50% in adult male smokers. Half-lives in infants can reach 80 hours or longer, but they can decrease quickly with maturity, possibly to less than the adult value by the time they are 6 months old. Caffeine's clearance is reduced by over 90% when taken with the antidepressant fluvoxamine (Luvox), and its elimination half-life is increased by over ten times, from 4.9 hours to 56 hours.

As soon as you ingest caffeine, your body absorbs it swiftly from the stomach into your bloodstream. After there, it goes to the liver where it is broken down into substances that have an impact on different organ functions. It works by inhibiting the effects of adenosine, a neurotransmitter that causes fatigue and mental relaxation. Adenosine levels often rise during the course of the day, making you feel more and more exhausted and wanting to sleep. By attaching itself to brain adenosine receptors without activating them, caffeine promotes sleep. As a result, there is less fatigue since the effects of adenosine are blocked. It might also raise blood levels of adrenaline and stimulate dopamine and norepinephrine in the brain. This combination increases arousal, alertness, and focus by stimulating the brain even more. Caffeine is frequently referred to as a psychoactive drug due to its effects on the brain. Moreover, caffeine usually takes affect really

quickly. For example, the quantity in a single cup of coffee can enter the bloodstream in as little as 20 minutes, and it takes approximately an hour to achieve full effect.

1.2.1 HEALTH BENEFITS OF CAFFEINE

It's well known that caffeine helps people feel less tired and have more energy. This is due to the fact that caffeine boosts the levels of other neurotransmitters, such as dopamine, in your brain that control your energy levels by blocking the receptors of the neurotransmitter adenosine. Over time, regular coffee consumption may be associated with a decreased risk of type 2 diabetes. Some study indicates that coffee may help protect against some neurodegenerative disorders, like Alzheimer's disease and Parkinson's disease, although studies have shown conflicting results. People who consistently drank coffee had a considerably lower risk of acquiring Parkinson's disease, per one evaluation of thirteen trials. Furthermore, over time, usage of coffee also delayed the advancement of Parkinson's disease. Coffee may contribute to weight loss and be associated with a reduction in body fat. Additionally, research indicated that coffee drinkers had higher rates of physical activity. Three to five cups of coffee a day were linked to a 15% lower risk of heart disease, according to one research.

1.2.2 CAFFEINE METABOLISM

Caffeine is rapidly and completely absorbed in humans within an hour of ingestion. When it is consumed in beverages caffeine is absorbed rapidly in the gastrointestinal tract and is distributed throughout the body water. The primary mechanism for the stimulatory activity is to block adenosine

receptors and inhibit phosphodiesterase. This ability of caffeine results from the competitive binding of caffeine and paraxanthine to adenosine receptors and is of importance in contributing CNS effect. Due to this blocking of adenosine inhibitory effect through receptors, caffeine indirectly affects the release of norepinephrine, dopamine, serotonin, glutamate, acetylcholine, neuropeptides and gamma-aminobutyric acid.

Caffeine increases intracellular concentrations of cyclic adenosine monophosphate by inhibiting phosphodiesterase in skeletal muscles and adipose tissues. This promotes lipolysis via activation of hormone-sensitive lipases with the release of fatty acids and glycerol. Increased cyclo adenosine monophosphate led to increase in blood catecholamines.

Caffeine in high concentration, interfere with the uptake and storage of calcium in the sarcoplasmic reticulum of striated muscles and increase the translocation of calcium ions through the plasma membrane. Caffeine may also increase myofilamental sensitivity of calcium ion through its binding to ryanodine receptors in calcium channels of muscle and brain. Caffeine is metabolised and excreted in humans as paraxanthine and with repeated dosing can lead to development of tolerance and withdrawal symptoms.

1.3 CAFFEINE CONSUMPTION AND ITS ADVERSE EFFECTS

1.3.1. PHYSICAL EFFECTS

Coffee and other caffeinated beverages include caffeine, which can alter stomach acid output and gastrointestinal motility. High levels of caffeine use can hasten the loss of bone in postmenopausal women.

When people who have been devoid of caffeine for days or weeks suddenly consume substantial levels of caffeine (at least 250–300 mg, which is the same amount found in 2-3 cups of coffee or 5-8 cups of tea), their urine production is temporarily stimulated. Proximal tubular adenosine receptor blockage is the mechanism underlying this rise, which is caused by both a diuresis (an increase in water excretion) and a natriuresis (an increase in saline excretion). Dehydration risk may rise due to the sudden increase in urine production. Chronic caffeine users, however, get inured to this impact and have no further effects.

1.3.2. PSYCHOLOGICAL EFFECTS

The usual side effects of caffeine use include moderate anxiety, jitteriness, sleeplessness, increased sleep latency, and decreased coordination. These symptoms are not severe enough to need a mental diagnosis. Anxiety disorders may suffer from the detrimental effects of caffeine. A 2011 literature analysis found that coffee consumption may exacerbate anxiety and panic symptoms in Parkinson's disease patients. Caffeine can both produce and exacerbate anxiety at large amounts, usually more than 300 mg. Stopping caffeine usage can dramatically lower anxiety in some people.

1.3.3 ADDICTION

Depending on the definition of addiction, caffeine may or may not cause an addictive disorder. Since there has never been any evidence of compulsive coffee drinking, caffeine is not usually regarded as addictive. But some diagnostic models—like the ICD-10 and ICDM-9—classify caffeine addiction under a more general diagnostic category. Some claim that even if users are aware of the harmful repercussions on their health, they might

develop an addiction and become unable to cut back.

1.3.4 OVERDOSE

A condition known as caffeinism is linked to daily caffeine consumption of 1-3 grams (1,500 mg). Caffeine dependency and a variety of unpleasant symptoms, such as anxiety, agitation, restlessness, sleeplessness, headaches, and palpitations following caffeine usage, are typically present in this disease.

Caffeine intoxication, a clinically important transient syndrome that manifests during or soon after caffeine ingestion, is a state of central nervous system overstimulation that can be brought on by an excessive caffeine intake. Usually, this condition only manifests itself after consuming significantly more caffeine—usually, over 400–500 mg at a time—than what is commonly present in caffeinated beverages and pills. The DSM-5 states that if five (or more) of the following symptoms appear after a recent caffeine consumption, it may be necessary to diagnose caffeine intoxication: flushed face, diuresis, gastrointestinal disturbance, twitching of the muscles, rambling speech and thought processes, tachycardia or cardiac arrhythmia, periods of inexhaustibility, and psychomotor agitation.

1. 3.5 EFFECT OF CAFFEINE IN ENERGY DRINKS

Prolonged QT interval, palpitations, and hypertension are only a few of the short-term cardiovascular adverse effects linked to high caffeine consumption in energy drinks (at least 1 liter or 320 mg). Smaller caffeine intake in energy drinks did not cause these cardiovascular adverse effects.

1.4 AIM AND SCOPE OF THIS STUDY

We chose a total of eight tea, coffee, green tea brands for our comparative study. The tea brands include Kannan Devan, AVT, 3 Roses, Red label; coffee brands: Bru, Nescafe and green tea brands: Ripple and Lipton. These are the most commonly consumed brands by the Indians. To spread awareness among the consumers regarding the daily intake of caffeine from beverages.

Chapter 2

Materials and Methods

2.1 MATERIALS USED

- Tea/coffee/green tea samples
- 250 ml beaker
- 250 ml separating funnel

2.2 CHEMICALS REQUIRED

- Chloroform
- Lead acetate

2.3 PROCEDURE

In the experimentation process, 25 g samples of tea, coffee, and green tea were combined with 75 ml water and subjected to intense boiling. The resulting solution underwent filtration, followed by the addition of lead acetate, leading to the formation of a curdy brown precipitate. Further additions of lead acetate continued until no additional precipitate formed. The obtained solution underwent a secondary filtration, and the filtrate was reduced to 25 ml through heating. After allowing the solution to cool, 10 ml of chloroform was introduced, and the resultant mixture was transferred to a separating funnel, resulting in the formation of two distinct layers. The lower layer was carefully separated, and upon exposure to the atmosphere, chloroform evaporation ensued. Crystal-like structures that formed on the beaker walls were gently scraped off, weighed, and additional observations were meticulously recorded [1].

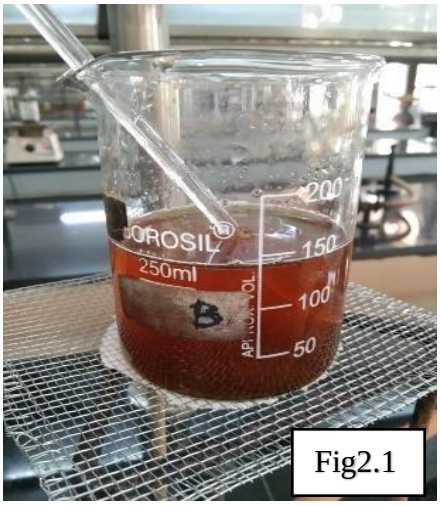

Fig2.1

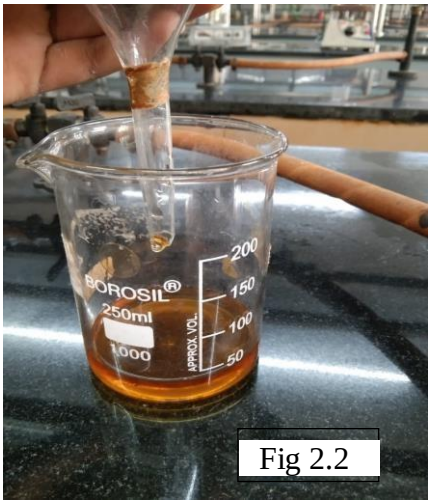

Fig 2.2

Fig 2.3


Fig 2.4

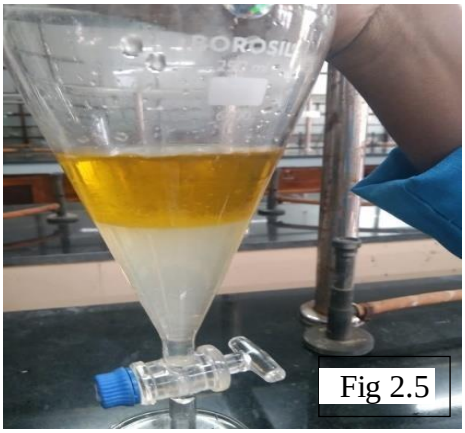
Fig 2.5

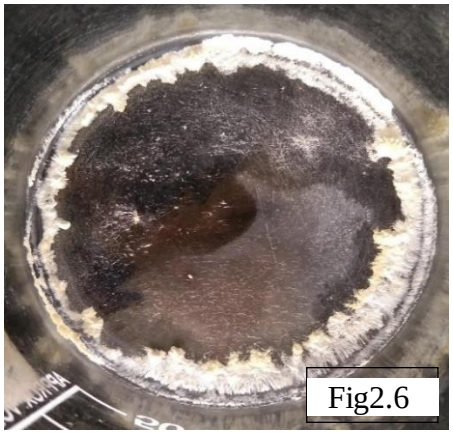
Fig2.6

Fig 2.1: The sample is heated

Fig 2.2: Filtration
Fig 2.3:Formation of precipitate after the addition of lead acetate
Fig 2.4: filtration of the precipitate
Fig 2.5: Separating the lower layer using separating funnel

2.4. CHARACTERISATION TECHNIQUES

For characterisation of the samples, Thin layer chromatography was used to determine the purity of the sample and FTIR was used to confirm that the substance obtained was caffeine.

2.4.1. THIN LAYER CHROMATOGRAPHY

Thin-layer chromatography was adopted for the confirmation of caffeine crystal. Purity of isolated caffeine compound was checked using Thin Layer Chromatography and compared with standard caffeine. The test sample was dissolved in chloroform and spotted on a precoated TLC plates with Silica Gel G (Merck) using solvent system chloroform: acetone: methanol (4:3:3 V/V). The plates were observed under UV light at 254 nm. Their Rf values were calculated and compared.

This solvent then moves up the plate via capillary action. As with all chromatography, some compounds are more attracted to the mobile phase, while others are more attracted to the stationary phase. Therefore, different compounds move up the TLC plate at different speeds and become separated. To visualize colourless compounds, the plate is viewed under UV light or is stained.

Retention factor (R_f)is a number that gives the degree of separation between compound and mixture. They range from 0 to 1. Rf value zero indicates that there is no separation between compound and the mixture. The substance with R_f value 1 indicates there is complete separation between compound and the mixture.

The Rf value of a component in Chromatography can be affected by several factors, including the type of stationary phase, the polarity of the solvent, the temperature, and the concentration of the components in the mixture. If the solvent only moves a short distance, then the Rf value will be small.

$$R_f \text{ value} = \frac{\text{Distance travelled by the substance}}{\text{Distance travelled by the solvent}}$$

2.4.2. FOURIER TRANSFORM- INFRARED SPECTROSCOPY

The extracted caffeine powdered samples were mixed with KBr and a pellet was prepared. FTIR spectra were then recorded on FT-IR spectrophotometer in the wave number range of 4000-400 cm^{-1} and compared with standard FTIR spectra.

After the literature reviews, the FTIR peaks of each of the samples were compared with that of the standard caffeine.

Chapter 3

Results and discussion

3.1 THIN LAYER CHROMATOGRAPHY

Table 2.1.1 : Amount of caffeine content in each of the sample

SAMPLE	CAFFEINE CONTENT
Kannan devan	0.328 g
AVT	0.418 g
3 roses	0.217 g
Red label	0.238 g
Lipton	0.131 g
Ripple	0.101 g
Nescafe	0.254 g
Bru	0.203 g

Following the procedure as detailed in Chapter 3, the amount caffeine obtained from each of the tea, coffee, green tea brands are shown in the table 2.1.1. From this table it is clear that, in tea brands, AVT has the high

highest caffeine content of 0.418g. In coffee samples, Nescafe has 0.254g, and in green tea sample, Lipton has 0.131g of caffeine content.

Table 3.1.1: Calculated R_f values of samples

SAMPLE	**Isolated caffeine R_f VALUE**
Kannan Devan	0.57
AVT	0.64
3 Roses	0.83
Red label	0.71
Lipton	0.72
Ripple	0.77
Nescafe	0.88
Bru	0.68

The R_f values of pure isolated caffeine was 0.63 and those of extracted caffeine ranges from 0.57-0.88 indicating that all samples contain caffeine. The extracted caffeine was identified by thin layer chromatography (TLC) by comparing the R_f values of sample and the standard which is mentioned in table 3.1.1 it was confirmed that the extracted material is caffeine.

Fig3.1Kannan

Fig3.2 AVT

Fig3.3 3 Roses

Fig 3.4 Red Label

Fig3.5 Lipton

Fig3.6 Ripple

Fig3.7 Nescafe Fig3.8 Bru

3.2 FOURIER TRANSFORM INFRARED SPECROSCOPY

The extracted caffeine powdered samples were mixed with KBr and a pellet was prepared. FTIR spectra were then recorded on FT-IR spectrophotometer in the wave number range of 4000-400 cm^{-1} and compared with standard FTIR spectra. The resultant peaks obtained showed the presence of various functional groups like N-H, C=O, C=C, -O-H, C-C in comparing with the standard caffeine showed similar peaks.

3.2.1 IR spectrum of caffeine extracted from Kannan Devan (tea)

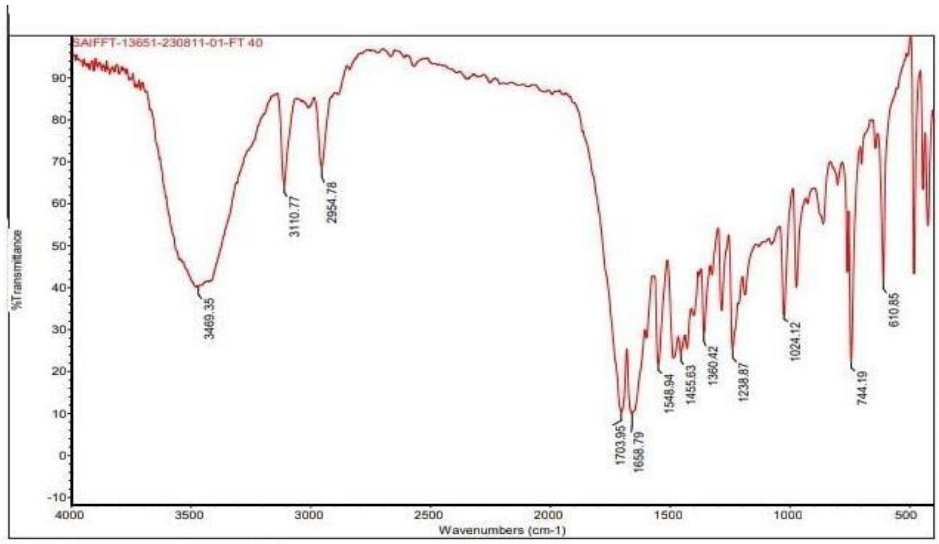

Figure 3.2.1: IR spectrum of coffee brand Kannan Devan

The caffeine extracted from this brand shows characteristic absorption giving rise to several peaks. The peak at 3469.35 cm^{-1} corresponds to O-H stretching vibration, and 3110.77 cm^{-1} corresponds to N-H stretching vibration. The peaks indicate the presence of Aromatic C-H stretching at 2954.7 cm^{-1}, −C=N ring stretching at 1703.95 cm^{-1}, C=O of C6-ring stretching at 1658, C=C stretching at 1548.94 cm^{-1}, C-N stretching vibration at 1455.63 cm^{-1}, C-N stretching at 136.42 cm^{-1}, C-O bond stretching at 1238.87 cm^{-1}, C-C stretching at 1024.12 cm^{-1}, Aromatic C-H bending at 744.19 cm^{-1}.

Functional groups	**Characteristic absorption** (in cm^{-1})
O-H stretching vibration	3469.35
N-H stretching vibration	3110.77
Aromatic C-H stretching	2954.7
−C=N ring stretching	1703.95
C=O of C6-ring stretching	1658
C=C stretching	1548.94
O-H angular deformation	1455.63
C-N stretching	1360.42
C-O bond stretching	1238.87
C-C stretching	1024.12
Aromatic C-H bending	744.19

3.2.2 IR spectrum of caffeine extracted from AVT (tea)

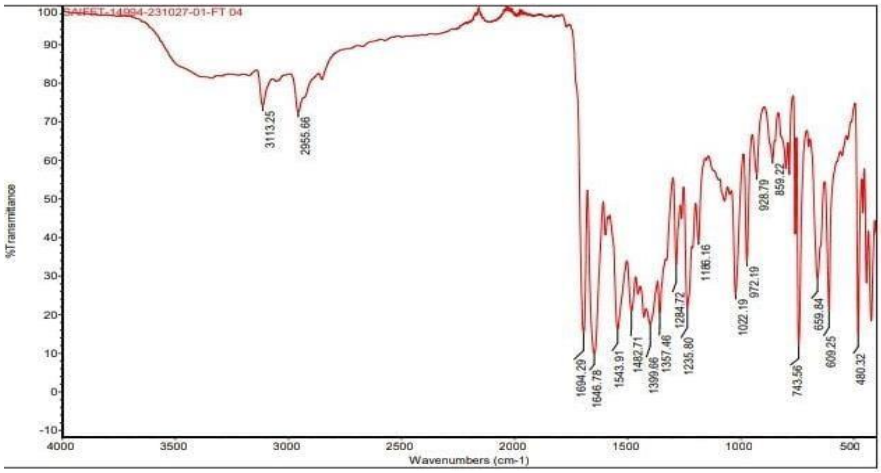

Figure 3.2.2 IR spectrum of tea brand AVT

Functional groups	**Characteristic absorption** (in cm^{-1})
N-H stretching vibration	3113.25
Aromatic C-H stretching	2955.66
−C=N ring stretching	1694.29
C=O of C6-ring stretching	1646.78
C=C stretching	1543.91
O-H angular deformation	1482.71
C-N stretching	1399.66
C-O bond stretching	1235.80
C-C stretching	1022.19
Aromatic C-H bending	743.56

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3.2.3 IR spectrum of caffeine extracted from 3 Roses (tea)

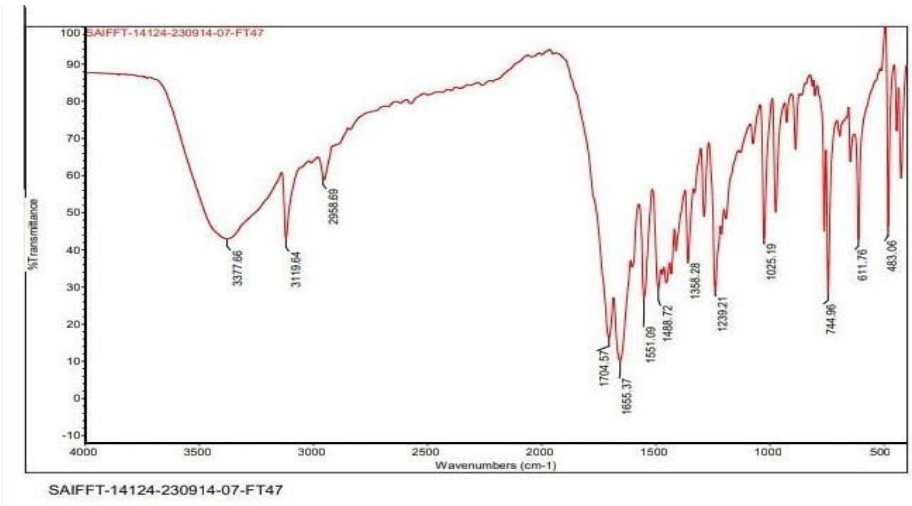

Figure 3.2.3 IR spectrum of tea brand 3 Roses

Functional groups	**Characteristic absorption** (in cm^{-1})
O-H stretching vibration	3377.66
N-H stretching vibration	3119.64
Aromatic C-H stretching	2958.69
−C=N ring stretching	1704.57
C=O of C6-ring stretching	1655.37
C=C stretching	1551.09
O-H angular deformation	1488.72
C-N stretching	1358.28
C-O bond stretching	1239.21
C-C stretching	1025.19
Aromatic C-H bending	744.96

3.2.4 IR spectrum of caffeine extracted from Red Label (tea)

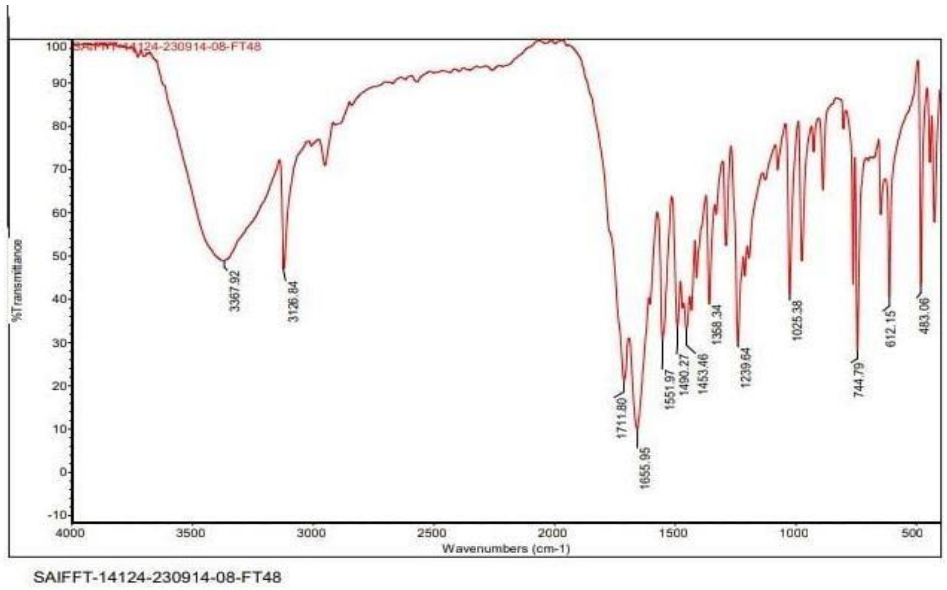

Fig 3.2.4 IR spectrum of tea brand AVT

Functional groups	**Characteristic absorption** (in cm^{-1})
O-H stretching vibration	3367.92
N-H stretching vibration	3126.84
−C=N ring stretching	1711.80
C=O of C6-ring stretching	1655.95
C=C stretching	1551.97
O-H angular deformation	1490.27
C-N stretching	1368.34
C-O bond stretching	1239.64
C-C stretching	1025.38
Aromatic C-H bending	744.79

3.2.5 IR spectrum of caffeine extracted from Lipton (green tea)

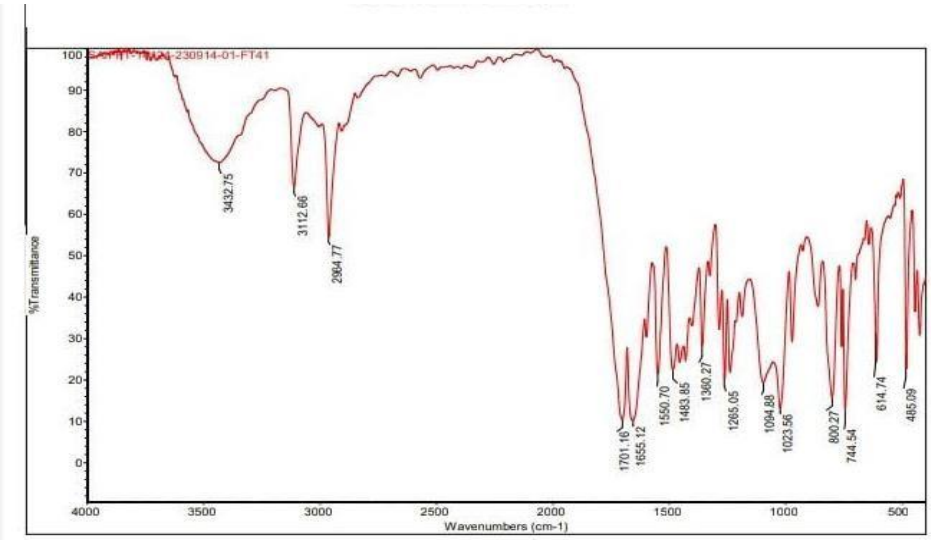

Fig3.2.5 IR spectrum of green tea brand Lipton

Functional groups	**Characteristic absorption** (in cm^{-1})
O-H stretching vibration	3432.75
N-H stretching vibration	3112.66
Aromatic C-H stretching	2964.77
−C=N ring stretching	1701.16
C=O of C6-ring stretching	1655.12
C=C stretching	1550.70
O-H angular deformation	1483.85
C-N stretching	1360.27
C-O bond stretching	1265.05
C-C stretching	1023.56
Aromatic C-H bending	744.54

3.2.6 IR spectrum of caffeine extracted from Ripple (green tea)

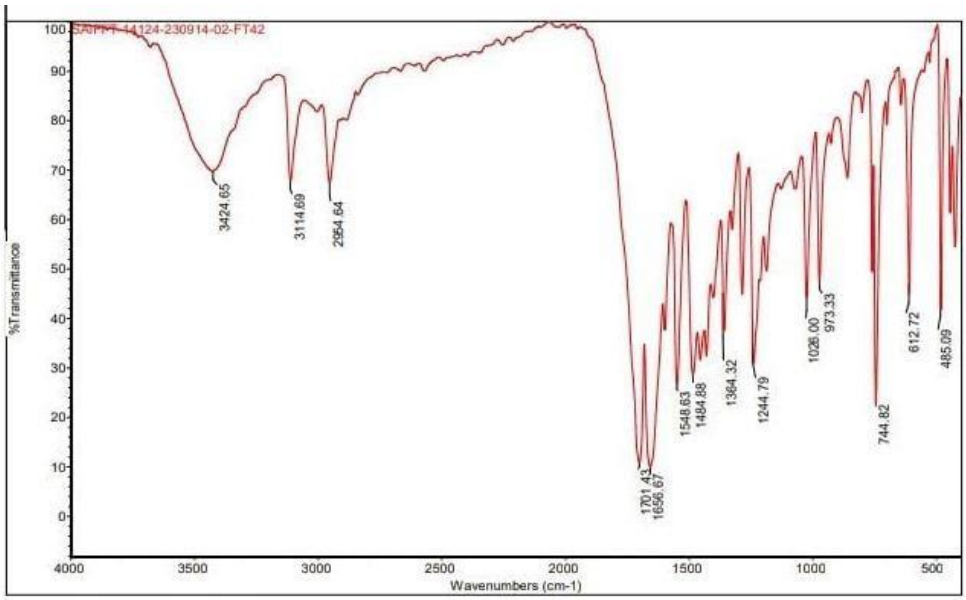

Fig3.2.6 IR spectrum of green tea brand Ripple

Functional groups	**Characteristic absorption** (in cm^{-1})
O-H stretching vibration	3424.65
N-H stretching vibration	3114.69
Aromatic C-H stretching	2964.64
−C=N ring stretching	1701.43
C=O of C6-ring stretching	1656.63
C=C stretching	1548.63
O-H angular deformation	1484.88
C-N stretching	1634.32
C-O bond stretching	1244.79
C-C stretching	973.33
Aromatic C-H bending	744.82

3.2.7 IR spectrum of caffeine extracted from Nescafe (coffee)

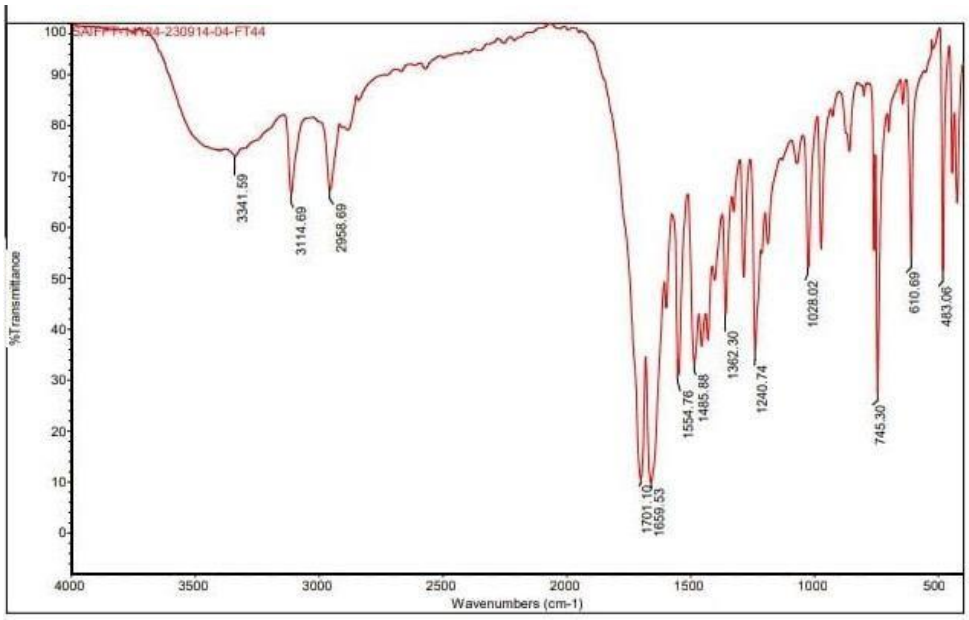

Fig 3.2.7 IR spectrum of coffee brand Nescafe

Functional groups	**Characteristic absorption** (in cm^{-1})
O-H stretching vibration	3341.59
N-H stretching vibration	33114.69
Aromatic C-H stretching	2958.69
−C=N ring stretching	1701.10
C=O of C6-ring stretching	1659.53
C=C stretching	1554.76
O-H angular deformation	1485.88
C-N stretching	1362.30
C-O bond stretching	1240.74
C-C stretching	1028.02
Aromatic C-H bending	745.30

3.2.8 IR spectrum of caffeine extracted from Bru (coffee)

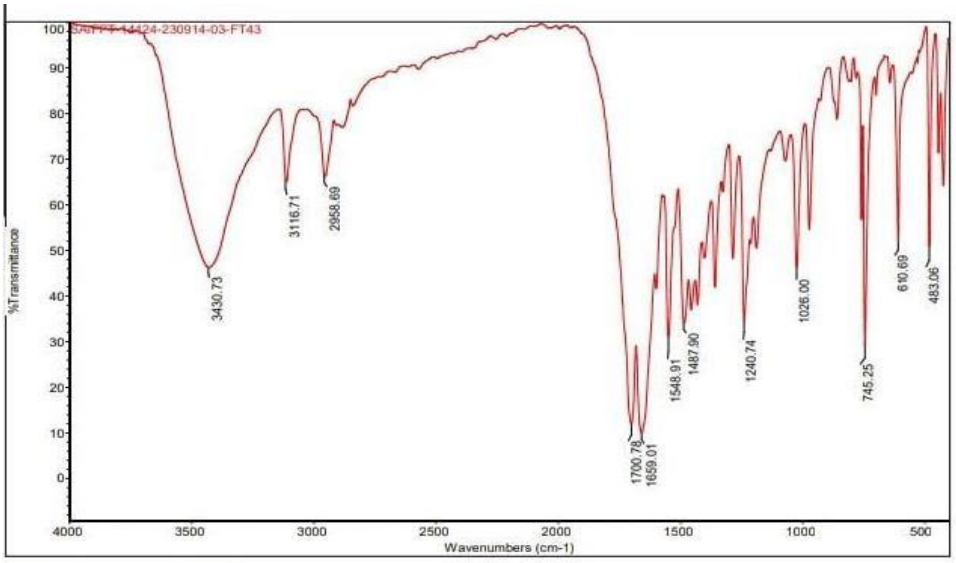

Fig 3.2.8 IR spectrum of coffee brand Bru

Functional groups	**Characteristic absorption** (in cm^{-1})
O-H stretching vibration	3430.73
N-H stretching vibration	3116.71
Aromatic C-H stretching	2958.69
−C=N ring stretching	1700.78
C=O of C6-ring stretching	1659.01
C=C stretching	1548.91
O-H angular deformation	1487.90
C-O bond stretching	1240.74
C-C stretching	1026.00
Aromatic C-H bending	745.25

Chapter 4

Conclusions

Based on the comprehensive analysis conducted in "CAFFEINE EXTRACTION AND COMPARATIVE QUANTIFICATION IN CHOSEN BRANDS OF TEA, COFFEE, AND GREEN TEA" it is evident that caffeine content varies significantly among different brands and types of beverages. Our findings reveal that AVT, Nescafe, and Lipton exhibit the highest caffeine content in tea, coffee, and green tea, respectively, as observed in Table 2.1.1. Moreover, through Thin Layer Chromatography (TLC), brands such as Three Roses, Nescafe, and Ripple demonstrated the highest Rf values, indicating lower caffeine content compared to others within their respective categories. Consequently, brands like Three Roses tea, Bru coffee, and Ripple green tea may be perceived as better options for individuals seeking beverages with lower caffeine levels. However, it is important to note that caffeine consumption habits vary among individuals, and while the shift towards green tea consumption appears advantageous due to its comparatively lower caffeine content, complete avoidance of tea and coffee may not be feasible for all. Future research could delve deeper into the health implications of consuming beverages with varying caffeine levels, considering factors such as cardiovascular health, cognitive function, and sleep patterns. Additionally, exploring methods to mitigate caffeine content while preserving flavor and aroma in traditional beverages could offer innovative solutions for consumers seeking moderation in caffeine intake without sacrificing taste preferences.

References

1. Parvathy, S., Luiz, A., & Varkey, J. T. (2014). Chemical Analysis of Caffeine Content in Tea and Coffee Samples. *Asian Journal of Science and Applied Technology*, *3*(1), 1–4. https://doi.org/10.51983/ajsat-2014.3.1.790
2. Adnadjevic, B., Koturevic, B. and Jovanovic, J. (2017) 'Comparative kinetic analysis of isothermal extraction of caffeine from guarana seed under conventional and microwave heating', *Chemical Engineering Research and Design*, 118, pp. 61–70. Available at: https://doi.org/https://doi.org/10.1016/j.cherd.2016.12.006.
3. Alka Gupta* and Jiya Lal Maurya (2023) 'Extraction and Analysis of Caffeine from Various Sources: A Review', *International Journal of Research in Engineering and Science (IJRES)*, 11(10), pp. 103–108.
4. Aniket Chaugule, Hitesh Patil, Shreyans Pagariya, P.I. (2019) 'Extraction of Caffeine', *International Journal of Advanced Research in Chemical Science (IJARCS)*, 6(9), pp. 11–19. Available at: https://doi.org/http://dx.doi.org/10.20431/2349-0403.0609002.
5. Chen, Z. *et al.* (2002) 'Determination of caffeine as a tracer of sewage effluent in natural waters by on-line solid-phase extraction and liquid chromatography with diode-array detection', *Water Research*, 36(19), pp. 4830–4838. Available at: https://doi.org/https://doi.org/10.1016/S0043-1354(02)00221-X.
6. ejene Getachew, Bedasa Gidisa, A.B. and Jemal Mohmmed, D.M. (2021) 'QUANTIFICATION OF CAFFEINE CONTENT IN COFFEE BEAN BY EXTRACTION METHOD AT JIMMA ZONES, ETHIOPIA', *International Journal of Research in Engineering and Science (IJRES)*, 3(5).
7. Fraga, S. *et al.* (2020) 'Sequential high-pressure extraction of caffeine and bioactive compounds from caferana seeds (Bunchosia

glandulifera)', *The Journal of Supercritical Fluids*, 165, p. 104958. Available at: https://doi.org/https://doi.org/10.1016/j.supflu.2020.104958.

8. Gloess, A.N. *et al.* (2013) 'Comparison of nine common coffee extraction methods: instrumental and sensory analysis', *European Food Research and Technology*, 236(4), pp. 607–627. Available at: https://doi.org/10.1007/s00217-013-1917-x.
9. H. N. Wanyika*, E. G. Gatebe, L. M. Gitu, E.K.N. and C.W.M. (2010) 'Determination of caffeine content of tea and instant coffee brands found in the Kenyan market', *African Journal of Food Science*, 4(6), pp. 353 – 358.
10. Jindal Diksha, Singh Jarnail, P.R.K. and P.H.C. (2019) 'ISOLATION OF CAFFEINE FROM TEA BAGS', *EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH*, 6(7), pp. 502–505.
11. Klebanov, G.S., Mednikova, L.N. and Ovcharova, A.D. (1967) 'Extraction of caffeine from aqueous solutions', *Pharmaceutical Chemistry Journal*, 1(4), pp. 221–223. Available at: https://doi.org/10.1007/BF00770195.
12. Lou, Z. *et al.* (2012) 'Removal of caffeine from green tea by microwave-enhanced vacuum ice water extraction', *Analytica Chimica Acta*, 716, pp. 49–53. Available at: https://doi.org/https://doi.org/10.1016/j.aca.2011.07.038.
13. M.M. Paradkar, J.I. (2006) 'A Rapid FTIR Spectroscopic Method for Estimation of Caffeine in Soft Drinks and Total Methylxanthines in Tea and Coffee', *Journal of Food Science*, 67(7), pp. 2507–2511. Available at: https://doi.org/10.1111/j.1365-2621.2002.tb08767.x.
14. Ms. R. R. Shinde, P.N.H.S. (2017) 'Extraction of Caffeine from Coffee and preparation of Anacin drug', *International Journal of Engineering Research and Technology.*, 10(1).
15. Murray, S.D. and Hansen, P.J. (1995) 'The Extraction of Caffeine from Tea: An Old Undergraduate Experiment Revisited', *Journal of Chemical Education*, 72(9), p. 851. Available at: https://doi.org/10.1021/ed072p851.
16. Park, H.S., Im, N.G. and Kim, K.H. (2012) 'Extraction behaviors

of caffeine and chlorophylls in supercritical decaffeination of green tea leaves', *LWT - Food Science and Technology*, 45(1), pp. 73–78. Available at: https://doi.org/https://doi.org/10.1016/j.lwt.2011.07.023.

17. Rahimi, A. *et al.* (2018) 'Selective determination of caffeine in foods with 3D-graphene based ultrasound-assisted magnetic solid phase extraction', *Food Chemistry*, 262, pp. 206–214. Available at: https://doi.org/https://doi.org/10.1016/j.foodchem.2018.04.035.
18. Sereshti, Hassan and Smadi, Sohelia, S. (2014) 'A rapid and simple determination of caffeine in teas, coffees and eight beverages', *Food Chemistry*, 158.
19. Theodoridis, G. and Manesiotis, P. (2002) 'Selective solid-phase extraction sorbent for caffeine made by molecular imprinting', *Journal of Chromatography A*, 948(1), pp. 163–169. Available at: https://doi.org/https://doi.org/10.1016/S0021-9673(01)01457-1.
20. Wang, L. *et al.* (2012) 'Column-chromatographic extraction and separation of polyphenols, caffeine and theanine from green tea', *Food Chemistry*, 131(4), pp. 1539–1545. Available at: https://doi.org/https://doi.org/10.1016/j.foodchem.2011.09.129.