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Short Communication

ESTIMATION OF CRUDE CAFFEINE IN DIFFERENT TYPES OF TEA

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ABSTRACT

Caffeine is a xanthine alkaloid found in various sources such as cocoa seeds, coffee beans, tea leaves, etc. Its content in tea is estimated by methods like spectroscopy, thin layer chromatography, liquid- liquid extraction, etc. In this study, the content of crude caffeine was estimated using techniques like liquid- liquid extraction and thin layer chromatography (TLC). It is the presence of caffeine in tea that keeps us alert and active and enables us to work efficiently when consumed in proper amounts. This aromatic compound on one hand can help fight against diseases like cancer while on the other hand can harm ones health by increasing heart rate and blood pressure and causing mental ailments like depression and anxiety when consumed in small amounts.

Keywords: Tea, Caffeine, Properties, Metabolism, Thin Layer Chromatography (TLC), Aromatic Compound

1. INTRODUCTION

Tea plant is native to east and south Asia and is originally found in China and Burma. The Chinese invented tea as medicine, but in the 17th century the British began consuming tea. Later, it was introduced to various countries like India. Nowadays, tea is consumed in almost every continent. It is known by various names such as 'chai' in India, 'teh' in various regions of southeast Asia, 'cha' in China, etc . The different types of tea include green, white, black, oolong, post fermented tea. It can be also flavoured using various ingredients like mint, jasmine, ginger, lemon and cinnamon. Tea plant is mainly grown in acidic soils on hill slopes at about 1500 ft. It requires about 127 cm of rainfall [1].

According to Mathew Harbowy et al [2], the chemical components of tea include theobromine; theophylline; caffeine; amino acids like theanine; carbohydrates like glucose, fructose, etc.; vitamins A, B1, B6, B12, E, C, K minerals like zinc, potassium, magnesium, etc.

1.1. Benefits of tea

Mauro Serafini et al [3] emphasized that tea acts as an antioxidant by boosting the blood plasma antioxidant defenses. This prevents the production of free radicals and oxidative stress. According to Sharangi et al [4], green tea consists of anticancer components like polyphenols and catechins which help prevent metastasis and angiogenesis. The antioxidant property of tea prevents DNA damage and reduces the risk of various cancers like colorectal, stomach, urinary bladder, skin and lung cancer caused due to excessive smoking as stated by Sharangi et al [4]. Tea provides an anti-inflammatory effect [4]. This property of tea is used in the treatment of swelling, burns and other skin ailments.

Tea helps in improving digestion by treating diarrhoea, indigestion and diseases like dysentery [3-5]. It also provides an antiviral effect and helps in the treatment of diseases like genital warts. It reduces the risk of cardiovascular disease [5]. Tea can be used to treat various lung diseases like emphysema, bronchitis, asthma by relaxing and opening the air passages [4]. It has an antimicrobial and anticariogenic effect which helps prevent tooth decay as stated by Hamilton-Miller [6].

1.2. PROPERTIES OF CAFFEINE

Caffeine which is also called 1, 3, 5-trimethylxanthine mainly occurs as white crystals in its pure form according to Changquan Calvin Sun et al [7].

According to Hiroshi Ashihara et al [8], xanthine is the precursor for the synthesis of caffeine. Various intermediates like 7-methylxanthosine, 7-methylxanthine, theobromine are formed. Biodegradation of caffeine gives rise to theophylline, xanthine, uric acid, allantoin and finally carbon dioxide and ammonia is produced .

According to Fredholm et al [10], caffeine when consumed in low quantities keeps us alert and active and helps us work efficiently. It inhibits adenosine which is an inhibitor of various neurotransmitters . It reduces the risk of depression and provides relief from headache [10, 9]. It also increases the speed of perception and enables people to make proper decisions as stated by Janet Bryan et al [11]. According to Kristel Diepvens et al [12], caffeine helps in weight control by preventing the degradation of intracellular cAMP and reduces the risk of various cancers like lung, endometrial, hepatocellular cancer, etc. According to

a research by Didier Laurent et al [13], the intake of caffeine before exercise, causes increase in beta-endorphin release which increases exercise performance.

Caffeine when consumed in higher doses causes effects like reduction of time of sleep [10], increase in blood pressure caused due to the vasoconstriction, which is caused due to the stimulation of norepinephrine which is a hormone produced by the adrenal glands as stated by Morten Bottcher et al [14]. Excess caffeine also causes caffeine addiction which includes symptoms like anxiety . It also stiffens arteries and increases intraocular pressure which causes conditions like glaucoma [6,9]. There is an increase in the risk of diabetes due to increase in the secretion of insulin by the pancreatic β-cells [15].

The estimation of caffeine involves various methods like spectroscopy, Thin Layer Chromatography, liquid-liquid extraction, etc. Liquid- liquid extraction is a unit operation which involves the use of a third solvent to dissolve the desired component depending on its polarity. This enables separation of a desired component out of a mixture of two miscible substances.

X-ray crystallography [16] can be used to determine the crystal structure of caffeine. X-ray crystallography uses x-ray producing sources called synchrotrons. In this technique, the x-rays interact with the spatial arrangement of valence electrons. The diffracted x-rays are used to produce an image of the sample. This method can be used to determine the geometry of the molecule including the angles of crystal faces.

Spectroscopy [16] is a technique used to study the interaction between matter and the energy radiated. Spectroscopy can be of various types such as ultraviolet, infrared, NMR (Nuclear Magnetic Resonance), etc. Depending on the source used (UV rays, IR rays, magnetic field, etc.)

Thin layer chromatography (TLC) [17] is used to separate a mixture of two non volatile substances. A sheet of glass, plastic, or aluminium foil, coated with a thin layer of adsorbent material, like silica gel, aluminium oxide, or cellulose which acts as the stationary phase.
A solvent acts as the mobile phase which moves up the plate by capillary action. Separation occurs as the rate at which different analyses ascend the TLC plate is different. The objective of the research is to compare the caffeine content of different tea brands.

2. MATERIALS AND METHODS

Three tea samples (green tea, local tea, black tea) each weighing 6.45 g is added to boiling distilled water to prepare an infusion. Reagents used include sodium chloride, sodium carbonate, propanol, sodium sulphate, ethanol and 10% sodium hydroxide. Solvent for TLC is prepared using butanol, acetic acid, distilled water.

2.1. Procedure

Three tea samples of 6.45 g are weighed using an electronic weighing balance. The weighed tea samples are emptied into three separate beakers and boiling distilled water is added to each of these beakers. An infusion of the tea in hot water is prepared by constant stirring using a glass rod as shown in fig. 1.

Fig. 1: Preparation of Tea Infusion

After the tea infusion was cooled to room temperature, 1-2 g of sodium carbonate and 24-26 g of sodium chloride is added to each of the beakers and mixed properly with a glass rod. The content of each beaker is subjected to filtration using a filter paper inserted into a funnel. Fig, 2 shows the filtrates are collected in conical flasks.

Fig. 2: Preparation of Sample for Liquid-Liquid extraction

The filtrates from the conical flasks are emptied into separating funnels. About 15 ml of propanol is added to each separating funnel. The separating funnel is inverted many times. After the separation into aqueous and organic phases has occurred, the organic phases of each separating funnel are collected in beakers. To the aqueous phase, about 15 ml of propanol is added; the separating funnel is inverted many times. The previously obtained organic phase is added back into the separating funnels. About 25 ml of 10% NaOH solution is added to each separating funnel. The separating funnels are inverted for a number of times as shown in fig. 3.

Fig. 3: Liquid Liquid Extraction

After the separation into aqueous and organic phases has occurred, the organic phase from each separating funnel is collected in separate beakers. The beakers are placed in a water bath and are heated to evaporate the solvent . After the solvent has evaporated, the crude caffeine obtained is weighed as shown in fig. 4. Qualitative analysis of the obtained crude caffeine is carried out.

Fig. 4: Separation of Crude Caffeine

This is followed by Thin Layer Chromatography. Before TLC is carried out, the crystals obtained are treated with ethanol. Butanol, acetic acid and distilled water in the ratio 4:1:5 is used as a solvent. After various time intervals, the TLC plates are analysed under UV light (fig. 5). The distance moved by solvent and caffeine are measured and the R_f values are calculated.The obtained R_f values of different samples are compared graphically.

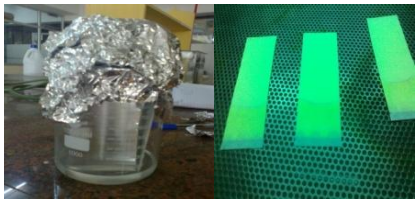

Fig. 5: Thin Layer Chromatography

3. RESULTS AND DISCUSSION

1. The qualitative analysis of caffeine showed the following results:

To the sample of crude caffeine, small amount of chloroform was added. After the mixture is evaporated to dryness, few drops of hydrogen peroxide and 1M HCl was added. After evaporating, small amount of ammonia solution was added. No colour change was observed after addition which suggests the presence of caffeine.

3.1. Analysis by TLC

Table 1: Observations for trials 1 and 2

	Distance travelled (cm) after 20 min			
Trial	Green tea	Local tea	Black tea	Solvent
1	4.5	4.7	4.4	6
2	4.9	5	5.1	6.5

Table 2: R_f Values

	R_f values		
Trial	Green tea	Local tea	Black tea
1	0.75	0.78	0.73
2	0.75	0.77	0.78

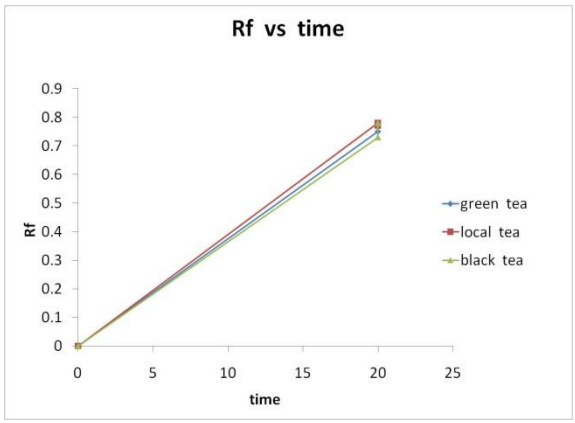

Fig. 6: Graph of R_f values vs. time

The slope for the graph of local tea is the greatest. Hence the amount of caffeine for the three tea brands can be given as:

Local tea > Green tea > Black tea

The intake of caffeine in the form of beverages like tea can enhance our health by improving digestion, respiration, oral health, etc. Recent researches have revealed that caffeine in particular concentration can be used as a treatment of cancer due to its antioxidant properties. But this same compound when taken in excess causes caffeine addiction and mental illness.

4. REFERENCES

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