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Comparative Study of Caffeine Estimation and Measurement of Acid Value from Different Commercially Available Brands of *Camellia sinensis* L. An Affordable Beverage

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ABSTRACT

Caffeine is a well-known psychoactive drug obtained from the leaves of *Camellia sinensis* L. (family Theaceae). The estimation of caffeine content is of critical importance since its intake in large doses can cause physiological dependence and addiction. The study accounts for the variability of caffeine content and acid value (a measure of bitterness level and erosive potential) for the first time in three commercially available tea brands like Hi-tea (India), Al Kbous tea (Jordan), and Tetley tea (England). For the study, a simple, reliable and cost-effective method of polar, non-polar extraction was followed. Caffeine aqueous extract was subjected to fractionation using chloroform, and then concentrated using a rotary evaporator. The measurement of acid value involved the titration of diluted tea samples with sodium hydroxide. Caffeine was isolated as a needle-shaped crystalline substance, tested positive for Murexide (purple coloration). The melting point (237°C) was determined using the Buchi melting point apparatus. After the quantitative measurement, caffeine content was found to be highest in Al Kbous tea (0.52g/20gm), followed by Hi-tea (0.45g/20gm) and Tetley tea (0.39g/20gm).The acid value and pH were found to be as follows- Al Kbous acid value 0.0204 (pH 4.5±0.003), Hi-Tea acid value 0.0188 (pH 4.9±0.001) and Tetley tea acid value 0.0124 (pH 5.1±0.001). Quantitative estimation showed an inverse relationship between acid values and the pH. Al Kbous had the highest caffeine content with possibly higher addiction rate and highest acid value (lowest pH), which makes it very bitter in taste whereas, Tetley showed the lowest caffeine content with possibly least addiction rate and least acid value (highest pH) which contributes to its mildly bitter taste and Hi-Tea quantitatively lies somewhere in the middle.

1. INTRODUCTION

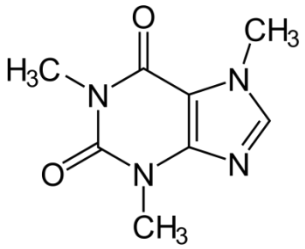

Figure No. 1: Chemical Structure of caffeine

C. sinensis L. (tea) belonging to family Theaceae, a plant native to China and Southeast Asia, is globally a very important plant for human beings. The tea made from the leaves has been consumed by humans for thousands of years as a stimulant and is the second most widely consumed beverage after the water[5]. The use of tea socially and habitually by people all over the world is known since 3000 BC. The refreshing feeling and the pleasant taste that it provides are so deeply felt that the potential benefits can be often studied. Tea is a sign of a welcome drink, which depicts the hospitable culture of Arabs. With the long tradition of drinking tea, Saudi Arabia boasts the second largest retail tea market in the Middle East.

Caffeine as an active ingredient is the world's most widely consumed psychoactive drug present in leaves of *C. sinensis* L. and chemically is known as 1,3,5 trimethyl xanthine (figure no. 1). Its chemical formula is $C_8H_{10}N_4O_2$. Caffeine is a white crystalline powder that tastes very bitter and it occurs naturally in many plants, including coffee beans, cola, and cocoa nuts[2]. Other natural sources of caffeine are guarana berries and yerba mate.

Caffeine is a central nervous system stimulant that reduces fatigue, improves alertness, wakefulness, motor coordination and the muscle strength[10]. The consumption of 1-1.5gm/day is associated with a condition called caffenism, in which there are a wide range of unpleasant symptoms like nervousness, irritability, insomnia, headache, and palpitations.

The tea brands do not reveal information like caffeine content or acid value on their package labels. This hinders a consumer from making well-informed choice. The tea brands should mention the information about the percentage of secondary metabolite (caffeine) and also its pharmacological effect on consumers on the label so that consumer can make a right choice.

The comparative study of caffeine estimation and measurement of acid value from different commercially available brands of *C. sinensis* L. are the main objectives of this study. The estimation of caffeine content is of critical importance since its intake in large doses can cause adverse physiological and psychiatric dependence (addiction).

The study accounts for the variability of caffeine content and acid value (a measure of the level of bitterness, i.e, taste and erosive potential) in three commercially available brands in Abha, KSA for the first time.

Similar studies in the past proved the role of tea in causing gastric irritation, peptic ulcers, and aerodigestive tract cancers[6,7,9]. The work from previous coworkers has also shown that the consumer should not drink caffeine more than 400 mg per person for health, 200 mg for pregnant, and 100 mg for children and adolescents to be safe[3,8]. FDA approves caffeine levels in the beverages to be safe for consumption and with a caution to avoid dependence. The intake of caffeine in pregnancy should be monitored to avoid complications[4]. More clinical studies on the effect of caffeine in pregnant females would lead to lesser miscarriages. Nowadays the addiction to caffeinated drinks are very common in the children too which is assumed to be the probable cause of hyperactivity[1]. Further the role of caffeine in people who consume these products in very large quantities can also be studied.

2. MATERIALS AND METHODS

2.1. Materials

The tea samples of three commercially available brands were procured from the local supermarket in Abha, KSA in the year 2021. These samples belong to three different regions of the world Tetley from England, Hi-Tea from India and Al Kbous from Jordan. The major solvent like chloroform was collected from the local chemical store. All the solvents and chemicals like sodium hydroxide, nitric acid and sodium carbonate used in study were of analytical grade. All the glassware including rotary evaporator used were thoroughly washed with water and detergent before usage.

2.2. Methods

2.2.1. Isolation of Caffeine

20g of tea sample was mixed with sodium carbonate and boiled with water for ten minutes followed by filtration. The resultant solution was then transferred to a pear-shaped separating

funnel in which 10 ml of chloroform was added. The aqueous phase got partitioned with the chloroform layer. The chloroform layer was collected and the process was repeated by adding 10 ml of chloroform until no separate layers were formed. The collected underneath chloroform sample was concentrated using a rotary evaporator to get a needle-shaped crystals (figure no. 2) of caffeine. For identification of caffeine, a murexide test (figure no. 3) was performed by adding two drops of nitric acid to the sample and then evaporating it to dryness, which turned it into yellow-colored residue, this was followed by the addition of two drops of sodium hydroxide to obtain a purple color. A similar procedure was performed for other samples also.

Figure No. 2: Isolated sample of caffeine:

Figure No. 3: Murexide test

2.2.2. Estimation of Acid Value

20g of tea sample was boiled with water for 15 minutes, allowing it to cool. The solution was then filtered and diluted by taking 5 ml of filtered sample with 20 ml of water. Titration of

this diluted solution was performed with N/50 sodium hydroxide using phenolphthalein as an indicator until the color changed from pink to colorless. Readings were noted and the process was repeated 3 more times.

3. RESULTS AND DISCUSSION

The amount of caffeine isolated from Al Kbous was 0.52g, followed by Hi-tea 0.45g and Tetley tea was 0.39g. The percentage yield of caffeine calculated from Al Kbous was 2.6 %, Hi-Tea 2.25 %, Tetley Tea 1.95 % (table no. 1). The Al Kbous Tea recorded pH of 4.5, acid value 0.0204 followed by Hi-Tea with pH of 4.9, acid value 0.0188 and Tetley Tea with pH of 5.1, acid value 0.0124 (table no. 2).

Calculation of percentage yield:

$$\% \text{ weight of caffeine in sample} = \frac{\text{weight of caffeine in sample}}{\text{total weight}} \times 100$$

Table No. 1: % of caffeine per 20gm sample each.

Commercial brands (origin)	Weight of Caffeine in 20 gram of tea sample	
	Amount of Caffeine (g)	% of Caffeine
Al Kbous - Tea (Jordan)	0.52	2.6
Hi - Tea (India)	0.45	2.25
Tetley - Tea (England)	0.39	1.95

Table No. 2: Quantitative estimation of pH & acid value

Tea Sample	pH	Acid Value	Normality
Al Kbous - Tea (Jordan)	4.5	0.0204	5.1
Hi - Tea (India)	4.9	0.0188	4.7
Tetley - Tea (England)	5.1	0.0124	3.1

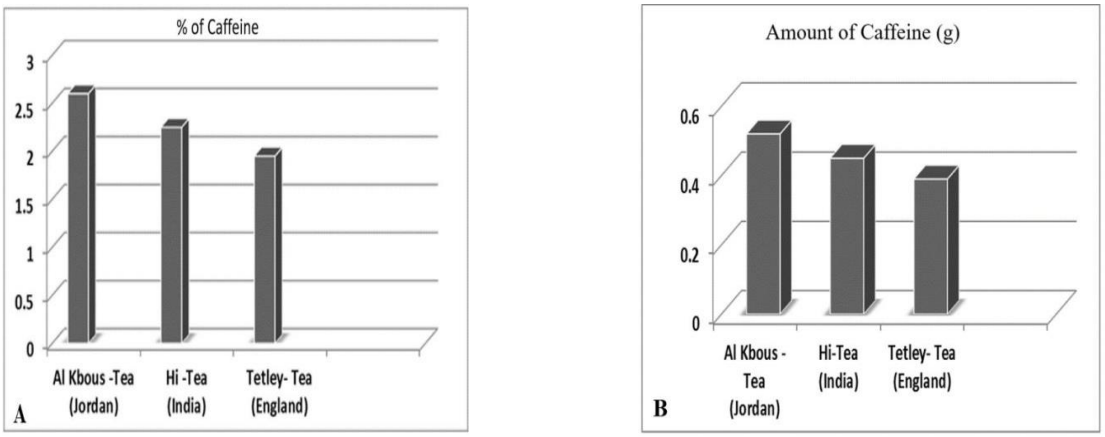

Figure No. 4: (A) % of caffeine graph; (B) Amount of caffeine (g) graph.

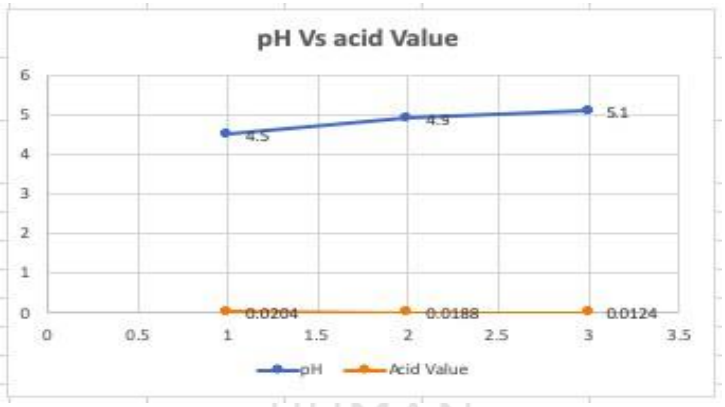

Figure No. 5: pH Vs acid value graph

4. CONCLUSION

We chose three commercially available brands of tea, Tetley from England, Hi-Tea from India, and Al Kbous from Jordan to calculate the amount of caffeine. It was found that percentage yield of caffeine is highest in Al-Kbous with a possibly higher degree of dependence in the frequent tea users followed by Hi-Tea and Tetley Tea respectively. It is safe to assume that caffeine content varies probably due to rainfall, soil nutrients, brewing conditions and age of the leaf. Acid value and pH shows inverse correlation i.e. Al Kbous with highest acid value and least pH is bitter in taste with higher erosive potential than Hi-Tea and Tetley respectively.

Today's computer-driven world is the root cause of many lifestyle-related diseases and moderate consumption of the natural product 'tea' would lead to a safer world with a happier life.

5. ACKNOWLEDGEMENTS

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