Determination of caffeine in tea brands using HPLC in Ethiopia.

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Abstract, Caffeine is an alkaloid occurring naturally in some 60 plant species, of which cocoa beans, kola nuts, tea leaves and coffee beans are the most well-known. Tea is the most commonly and widely used soft beverage in the household. It acts as a stimulant for central nervous system and skeletal muscles. That is why tea removes fatigue, tiredness and headache. It is also increases the capacity of thinking. It is also used for lowering body temperature. The principal constituent of tea, which is responsible for all these properties, is the alkaloid-caffeine. The objective of this study is to determine caffeine in tea sample is the major objectives of this study. Addis and wushwush Tea were the two brands of this study. It is found that the normality of extract is more in wushwush tea is more than in Addis tea.

Keywords: Caffeine, Tea, Structure of Caffeine, Properties of Caffeine and side effect of caffeine

Objective of the study; To determine the concentration of caffeine and acid contents in tea samples.

1. Introduction:

1.1 Background; Caffeine is found naturally in over 60 plants such as coffee and cocoa beans, tea leaves, kola nut, yerba mate, and guarana. It's also used in soft drinks, energy drinks and drugs such as some cold and pain remedies. Coffee and tea are the main sources of caffeine for adults, while soft drinks are the main sources for children.

Caffeine is a stimulant and the most commonly used drug in the world. Every day, millions consume it to increase

Wakefulness, alleviates fatigue, and improves concentration and focus.

Caffeine is a very common substance and is found in coffee, tea, soft drinks, chocolate and “stay awake” pills such as vitamin. It can be synthesized or isolated from natural sources. Caffeine is closely related to theophylline and theobromine.Pure caffeine takes the form of white, hexagonal crystals, which can be broken into a soft powder. It has a melting point of 235^{0C}-238^{0C} and a molecular weight 194.19g/mol. It is easily soluble in chloroform and partially soluble in water. Caffeine is a naturally occurring chemical stimulant called trimethylxanthine. Its chemical formula is C8H10N4O2. It is a drug and actually shares a number of traits with more notorious drugs such as amphetamines, cocaine and heroin. In its pure form, caffeine is a white crystalline powder that tastes very bitter. It is medically useful to stimulate the heart and also serves as a mild diuretic, increasing urine production to flush fluid out of the body. Caffeine can increase blood pressure and cause vasoconstriction. Caffeine is an alkaloid occurring naturally in some 60 plant species, of which cocoa beans, kola nuts, tea leaves and coffee beans are the most well-known. Other natural sources of caffeine include yerba mate, guarana berries, Guayas, and the yaupon holly. Caffeine is added to many popular soft drinks, and is also a component of a number of pharmacological preparations and over-the-counter medicines including

analgesics, diet-aids, and cold/flu remedies.

1.2. Structure and Physical and Chemical Properties

- Molar mass: 194.19 g/mol
- IUPAC ID: 1, 3,7-Trimethylpurine-2, 6-Dione
- Melting point: 238 °C
- Boiling point: 178 °C
- Density: 1.23 g/cm³
- Formula: C8H10N4O2

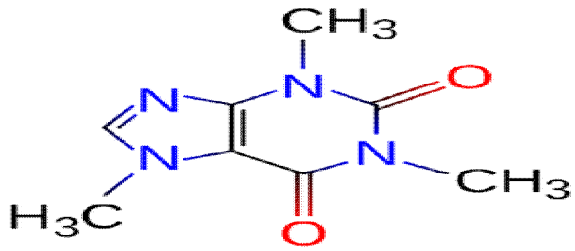


Fig -1; Structure of caffeine

2. CAFFEINE SIDE EFFECTS & SAFETY

Caffeine is likely safe for most adults when used appropriately.

Caffeine is possibly unsafe when taken by mouth for a long time or in fairly high doses. Caffeine can cause insomnia, nervousness and restlessness, stomach irritation, nausea and vomiting, increased heart rate and respiration, and other side effects. Caffeine can make sleep disorders in patients with acquired immunodeficiency syndrome (AIDS) worse. Larger doses might cause headache, anxiety, agitation, chest pain, and ringing in the ears.

Caffeine is likely unsafe when taken by mouth in very high doses as it can cause irregular heartbeats and even death.

2.1. Special Precautions & Warnings:

2.1.1 Children: Caffeine is possibly safe when taken appropriately by mouth or intravenously (by IV), as well as when used in amounts commonly found in foods and beverages.

2.1.2 Pregnancy and breast-feeding: Caffeine is possibly safe in pregnant or breast-feeding women when used daily amounts of less than 200 mg. This is about the amount in 1-2 cups of coffee. Consuming larger amounts during pregnancy or when breast-feeding is possibly unsafe. When consumed in larger amounts during pregnancy, caffeine might increase the chance of miscarriage and other problems. Also, caffeine can pass into breast milk, so nursing mothers should closely monitor caffeine intake to make sure it is on the low side. High intake of caffeine by nursing mothers can cause sleep disturbances, irritability, and increased bowel activity in breast-fed infants.

2.1.3 Anxiety disorders: Caffeine might make these conditions worse. Use with care.

2.1.4 Bipolar disorder: Too much caffeine might make this condition worse. In one case, a 36-year-old man with controlled bipolar disorder was hospitalized with symptoms of mania after drinking several cans of an energy drink containing caffeine, taurine, inositol, and other ingredients (Red Bull Energy Drink) over a period of 4 days. Use caffeine with care and in low amounts if you have bipolar disorder.

2.1.5 Bleeding disorders: There is concern that caffeine might aggravate bleeding disorders. Use caffeine with care if you have a bleeding disorder.

2.1.6 Heart conditions: Caffeine can cause irregular heartbeat in sensitive people. Use caffeine with caution.

2.1.7 Diabetes: Some research suggests that caffeine may affect the way the body uses sugar and might worsen diabetes. However, the effect of caffeinated beverages and supplements has not been studied. If you have diabetes, use caffeine with caution.

2.1.8 Diarrhea: Caffeine, especially when taken in large amounts, can worsen diarrhea.

2.1.9 Epilepsy: People with epilepsy should avoid using caffeine in high doses. Low doses of caffeine should be used cautiously.

2.1.10 Glaucoma: Caffeine increases the pressure inside the eye. The increase occurs within 30 minutes and lasts for at least 90 minutes after drinking caffeinated beverages.

2.2 High blood pressure: Consuming caffeine might increase blood pressure in people with high blood pressure. However, this effect might be less in people who use caffeine regularly.

2.2.1 Irritable bowel syndrome (IBS): Caffeine, especially when taken in large amounts, can worsen diarrhea and might worsen symptoms of IBS.

2.2.2 Weak bones (osteoporosis): Caffeine can increase the amount of calcium that is flushed out in the urine. If you have osteoporosis or low bone density, caffeine should be limited to less than 300 mg per day (approximately 2-3 cups of coffee). It is also a good idea to get extra calcium to make up for the amount that may be lost in the urine. Older women with an inherited disorder that affects the way vitamin D is used should use caffeine with caution. Vitamin D works with calcium to build bones.

3. BENEFITS OF CAFFEINE

Certain amounts of caffeine can have beneficial effects. Historically, small amounts of caffeine have been used to help control weight, alleviate pain, open up airways for improved breathing, and overcome chronic fatigue. Small amounts of caffeine are found to provide the following benefits:

- Can benefit people who are at high-risk for liver disease.
- Increases muscle strength.
- Increases metabolism by breaking down fat, freeing fatty acids and forcing them to be burned. (Caffeine is the most active ingredient in many diet pills.)
- Increases pain relief medication effects.
- Increases mental faculty.
- Reduces asthma symptoms.

4. Scope and limitation of the study: The study was limited to only determination of caffeine and acid content due to the lack of instruments like NMR and Mass spectroscopy.

5. Materials and Chemicals

5.1 Materials; Beaker, Pipette, Burette stand, Conical flask, Burner, Measuring flask, Separating funnel, Filter paper, Weight box and Analytical balance,HPLC,volumetric flasks and Spatula.

5.2 Chemicals; water, acetic acid, methanol, Lead acetate, Chloroform ,Tea samples, Water, Caffeine, NaoH and Phenolphthalein indicator.

6. Methods and Methodology

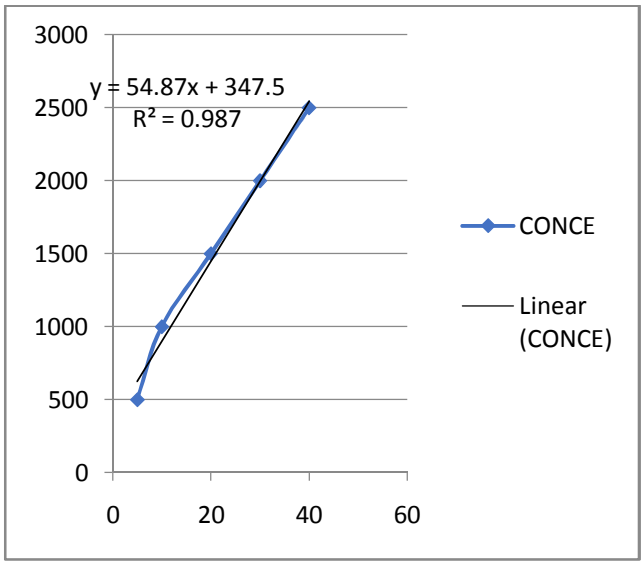
Determination of caffeine in tea samples and estimation of acid content present in tea leaves are the major objectives of this study. Tea samples from three different brands namely, Addis, Wushwush tea were taken in this study.

6.1 Standard solutions

Caffeine stock solution of 500 ppm was prepared by accurately weighing 50 mg of pure caffeine and quantitatively transferring it into 100 ml volumetric flask and making it to the mark with the mobile phase. Working standards of 5, 10, 20, 30, 40 ppm were prepared by serial dilution of the stock solution with the mobile phase.

6.2 Sample preparation and analyte determination

1.00 g of tea samples were weighed in triplicate and put into 125 ml beakers.50ml of boiling distilled water was added and let to stand for five minutes with stirring; the solution was cooled and filtered into conical flasks2.5 ml of the filtrate were pipette into clean 25ml volumetric flasks and made to the mark with the mobile phase. The standards and the samples were run in the HPLC system. The following were the HPLC conditions: Column, Reverse phase – ODS, 250 × 4.6 mm, flow rate, 1 ml/min, detector, photodiode array set at 278 nm, pressure, 150 khf/cm^2., mobile phase, water, acetic acid, methanol (79.9, 0.1 and 20) and sample volume, 5 µl. A calibration curve of peak areas versus concentration of the standards was plotted. The caffeine level of the various samples was calculated using the regression equation of the best line of fit.



Now we can calculate the concentration of caffeine using the following methods

Response factor=peak area/standard amount

Amount of the analyte= peak area/response factor

An injection containing tea at a concentration of 4 µg/mL is made and results in a peak area of 100.

The response factor of Addis tea: 100/4=25

An injection of the sample with the unknown concentration of tea has a peak area of 9000.

The amount of Addis tea present in the sample: 9000/25=360mg/mL

Then new we can culucalte from the equatıon,

Y=54.87X+347.5

360-347.5=54.87x

12.5/54.87=x x=0.23mg/ml of caffeine in Addis tea

And for wushwush tea the response factor is 50/4=12.5

An injection of the sample with the unknown concentration of tea has a peak area of 9500

9500/12.5=760

760-347.5/54.87=x which is the concentration of caffeine in wushwush tea

X=7.52mg/mL

7. RESULT AND DISSCUITION

Both tea samples are within the range of accepted standards of WHO. We can take both of them but, the difference in caffeine content between them id depending on the following factors.

- Cultivar or variety of plant grown
- Processing, especially roasting
- Blending of tea with other ingredients

Tea can be made from different parts of the tea plant, and these parts contain different quantities of caffeine. Leaf buds (tips) and younger leaves are higher in caffeine than older, mature leaves. This pattern can be explained by the fact that, for the tea plant, caffeine acts a natural insecticide, serving to protect the plant against being eaten by insects. Since the tips and tender young leaves are most vulnerable to insects, these parts of the plant are highest in caffeine; the older leaves are tougher and thus lower in caffeine.

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