ESTIMATION OF CAFFEINE IN PACKAGED TEA OF LOCAL MARKET OF DHARAN, NEPAL

A project work submitted to the Department of Chemistry, Central Campus of Technology, Tribhuvan University, in partial fulfillment of the requirements for the degree of Bachelor of Science in Chemistry

By:

Dinesh Timsina

Department of Chemistry

Central Campus of Technology

Institute of Science and Technology

Tribhuvan University, Nepal

2019

Board of examiner and certificate of Approval

This project work entitled "**Estimation of caffeine in packaged tea of local market of Dharan, Nepal**" by Dinesh Timsina (Roll No.: 500080003; T.U Regd. No.: 5-2-008-0098-2015) under the supervision of Mrs. Indumati Paudel, Central Campus of Technology, Hattisar, Dharan, Department of Chemistry, Nepal, is hereby submitted for the partial fulfillment of Bachelor of Science (B.Sc.) Degree in Chemistry. This project work had not been submitted in any other university or institution previously and has been approved for the award of Bachelors of Degree.

…………………………

Supervisor
Mrs. Indumati Paudel
(Asst. Professor)
Department of Chemistry
C.C.T., Dharan

…………..………………..

Co-Supervisor
Mr. Debendra Rai
Departmet of Chemistry
C.C.T, Dharan

………………………….

Internal Examiner
Department of Chemistry
C.C.T., Hattisar, Dharan
Tribhuvan University

…………..………………..

Asst. Professor Dr. Sujit K. Shah
MMMC, Biratnagar

………………………………….

Lalita Shrestha
Chairman
(Department of Chemistry)
Central Campus of Technology, Hattisar, Dharan
Date:

Recommendation

This is recommended that **Dinesh Timsina** (Roll No.: 500080003; Tribhuvan University Registration Number: 5-2-008-0098-2015) has carried out project work entitled "**Estimation of caffeine in packaged tea of local market of Dharan, Nepal**" as a partial fulfillment of fourth year of four year B.Sc. course under my supervision. To my knowledge, this work has not been submitted for any other degree.

…………………….....

Mrs. Indumati Paudel

(Asst. Professor)

Supervisor

Department of Chemistry

Central Campus of Technology Hattisar, Dharan, Sunsari

Declaration

I hereby declare that the project work entitled "Estimation of caffeine in packaged tea of local market of Dharan, Nepal" was done by me under the kind supervision and guidance of Asst. professor Mrs. Indumati Paudel and co-supervisor Mr. Debendra Rai, is being submitted to the Department of Chemistry, Central Campus of Technology, Dharan, Tribhuvan University, Nepal for the partial fulfillment of degree of Bachelor of Science in Chemistry.

…………………………

Dinesh Timsina
Central Campus of Technology
Hattisar, Dharan

Acknowledgement

I would like to express my heartfelt gratitude to my respected supervisor Asst. Professor Indumati Paudel and co-supervisor Debendra Rai, Teaching asst., Department of Chemistry, Central Campus of Technology, Dharan for their valuable guidance, suggestions, support and encouragement for this research work.

I would like to give my special thanks to my respected teachers Asst. Professor Manoj Khanal and Mrs. Lalita Shrestha for helping me during my entire research work.
Likewise, I am also thankful to lab officer Jha Sir and Mr. Ganga Siwakoti for helping and giving every support in the laboratory of CCT.

My special thanks to all my classmates for their help in preparing the final work. I especially thank my friends Aashish Bhattarai, Sandesh Chudal, Pratik Khatiwada, Bharat Adhikari, Rabin Bogati and Pooja Dangi for their support during the completion of this work. I am also very grateful to all those perceptions, observation and inputs have helped me directly or indirectly.

I also express my sincere thanks to Mr. Sunil Aryal and Mr. Pradeep Sangroula for their help and suggestions.

Last but not the least, I owe my deepest gratitude to my respected parents for making me stand in this position where I am now.

September, 2019 ..……………….

(Dinesh Timsina)

Abstract

Caffeine is a drug found in tea that stimulates the central nervous system of human. The aim of this research was to estimate the amount of caffeine present in various tea samples available in local market of Dharan, Nepal. Caffeine content in tea was estimated using UV-Vis spectrophotometer. Along with caffeine physical parameters (pH, Moisture, and Ash content) were also estimated and their correlation with caffeine was statistically analyzed using SPSS at 5% level of significance.

The highest amount of caffeine was found in Sencha tea (8.48%) and the lowest amount of caffeine was found in Haldibari tea (5.11%). Moisture content and ash content has shown positive correlation with caffeine at the 0.01 level (2-tailed). The obtained caffeine concentration of all tea samples followed mandatory standard of DFTQC, Nepal. According to this research, the recommended daily intake of tea consumption based on caffeine ranges from one and half cup to three cups per day. Intake of tea more than the recommended range may cause various health problems including caffeine intoxication.

Table of Contents Page

List of Tables

List of Figures

List of Abbreviations

Abbreviations	Full Form
AOAC	Association of Official Analytical Chemists
CCT	Central Campus of Technology
DFTQC	Department of Food Technology and Quality Control
HPLC	High Performance Liquid Chromatography
NTCDB	Nepal Tea and Coffee Development Board

1. Introduction

1.1 General introduction

Caffeine is an alkaloid belonging to the methylxanthines family. 1,3,7-trimethylxanthine is the systematic name of caffeine. Other names of caffeine are trimethylxanthine, theine, mateine, guaranine and methyl theobromine (Rehman and Ashraf, 2017). In its purified state, it occurs as a white powder with a bitter taste but odorless (Agyemang and Oppong, 2013). It is found in 60 plant species in varying quantities in the seeds, leaves or fruits. The most common sources of caffeine are coffee, cocoa beans, cola nuts and tea leaves (Tautua et al., 2014).

The distribution of caffeine in 23 species belongs to the genus *Camellia* in which *Cam. Sinesis* contains higher caffeine content than other species (Ashihara and Crozier, 1999). Varietal diversity, climatic changes in the growing areas, horticultural techniques and processing conditions result in variation of caffeine content in tea. In tea, youngest leaf has the highest concentration (Shrestha et. al, 2016). Nitrogen application in fertilizers can increase caffeine by as much as 40%. Seasonal variations, leaf position on the cutting, and genetic origin show similar effects (Spiller, 1998). The caffeine content of tea leaves varies with specific tea type, but the normal range goes from 2 – 5% (dry weight, w/w) together with small amount of theobromine (Atoma and Gholap, 2011).

In humans, caffeine acts as a central nervous system stimulant, hence it is used both recreationally and medically to reduce physical fatigue and restore mental alertness when unusual weakness or drowsiness occurs (Tautua et al., 2014). Several studies have reported the ability of caffeine to improve mood, attention, performance and speed at which information is processed and reaction time (Tfouni et al., 2017). Caffeine can induce hair growth. Dopamine concentration increases in the brain by caffeine which helps to ease depression. Caffeine increases stamina, protects the person from eyelid spasms, cataracts, reduces the risk of type 2 diabetes, parkinson’s disease and prevents several types of cancer including liver and colorectal cancer (Rehman and Ashraf, 2017).

Several studies have shown that persons consuming even low or moderate amounts of caffeine (100 mg/day) may develop a withdrawal syndrome with caffeine cessation with symptoms such as headaches, lethargy, muscle pain, impaired concentration and physiological complaints such as nausea or yawning (Chambers, 2009). Caffeine consumption immediately before or during exercise can cause harmful effect. It appears that caffeine may attenuate the normal physiological mechanisms that help increase myocardial blood flow that occurred during the increased demand of exercise. Caffeine blocks adenosine receptors, thus reducing the ability of the coronary arteries to improve their flow commensurate with the increased myocardial demand of exercise. This could perhaps result in supply-demand Ischemia (Pray et al., 2014).

Millions Kg of tea are produced and billion litres of brewed tea are consumed worldwide every year. According to Global Tea production, (2018), 5196 million kg of tea is produced in 2014 and 5305 million kg of tea is produced in 2015 throughout the world. 242, 250, 258, 266 and 273 billion litres of tea are consumed worldwide in 2014, 2015, 2016, 2017 and 2018 respectively (Statista, 2019). Nepal produced 24803.612 metric tons of tea and exported 15685 metric tons of tea in year 2017. This shows that Nepal consumed 9118.612 metric tons of tea along with little amount of imported tea in year 2017.

1.2 Statement of the problem

Caffeine has various effects on human health. Consumption of caffeine in excess amount can cause general toxicity, cardiovascular effects, effects on bone status and calcium balance, confusion, hallucinations, vomiting, changes in adult behavior, increased incidence of cancer and effects on male fertility. The problem is that we don’t know how much caffeine we are consuming and how much we should consume on daily basis.

Due to such effects of caffeine in human health, its study is very important. No studies of caffeine in tea are done in Dharan Municipality. Thus to determine the amount of caffeine present in locally available tea is very important. This study will help those people who consume locally available tea of Dharan municipality.

1.3 Objectives of the study

1.3.1 General Objective

a) To estimate the amount of caffeine present in different packaged tea available in local market of Dharan.

1.3.2 Specific Objective

b. To perform comparative study of caffeine present in packaged tea of Dharan.

c. To determine the consumable amount of tested tea samples.

d. To study the relation of caffeine with ash, moisture and pH of tea.

1.4 Significance of the work

The main significance of the study is to estimate the amount of caffeine present in different tea samples available in the local market of Dharan. The study on caffeine is very important in our daily life due to its presence in tea which is consumed by almost all people. Caffeine can cause various health problems if consumed in higher amount. Thus, the concentration of caffeine in tea should be known. In Dharan municipality no studies has been done related to presence of caffeine in tea. Thus my study will be very beneficial for people as this study will determine the amount of caffeine present in various tea samples. Hence, we can determine which tea in what amount is good for health.

1.5 Limitations of the work

There are different types of tea consumed in Dharan. Different brands of tea are available in local market of Dharan. This study is based only on few samples of tea. Thus this study is unable to include all types of tea found in local market of Dharan. Hence criteria of this study are limited.

2. Literature review

2.1 Tea

Tea is one of the most widely consumed beverages in the world next to water with a per capita consumption of ~120 ml/day (McKay and Blumberg, 2002). Its popularity is increased due to sensorial properties, relatively low price, stimulation effects and potential health benefits (Baptista, 1998). Medicinal effects of tea have been widely investigated, having a long, rich history with its first references nearly 5000 years ago (Wheeler and Wheeler, 2004).

Tea is prepared as an infusion with the leaves of *Camellia sinensis*, a plant cultivated over 30 countries across the world that belongs to the Theaceae family (Lopez, 2011). There are mainly two varieties of tea plants: C. *sinesnsis* var. *sinensis,* a small-leaved, bush like plant indigenous from China, which grows in several countries of Southeast Asia experiencing a cold climate and C. s*inensis* var. *assamica,* a large-leaved plant discovered in the Asam region of India which grows in several countries with semitropical climate (Mejia et al., 2009).Tea plants prefer a warm and humid climate with plenty of rainfall and also like diffused light and weak acidic and well-drained soil. The well-grown tea plants provide high-quality tea shoots, which vary with tea cultivars and the environmental conditions such as the type of soil and altitude and climate of the tea plant growing area. A Chinese idiom says, “A higher mountain yields higher quality tea” which indicates that the mountain conditions are optimum to tea plant growth, especially the growth of high-quality flush (Ho et al., 2009). Moreover, tea quality is also determined by the processing techniques employed. For instance, the same fresh tea leaves can be processed to black tea, oolong tea and green tea by fermentation, semifermentation, and no fermentation respectively. Those basic three types of tea have different quality characteristics including color, aroma, taste and appearance.

The fresh tea leaves are usually used for tea manufacturing and are harvested by hand plucking or mechanical plucking. Compared to mechanical plucking, hand plucking is more labor intensive and time consuming and less efficient but with higher uniformity. The well-known high-quality green teas are mostly produced from hand-plucking fresh tea leaves in China. Fresh tea leaves could be harvested during different seasons in a year, which changes with the climate of tea growing area. In China, the leaves are plucked in spring, summer and autumn; in winter the tea bush is dormant. After being plucked the fresh tea leaves are sent immediately to tea factories for manufacturing.

According to the different ways of processing, especially the extent of fermentation tea is usually divided into three basic types: green tea (nonfermented), oolong tea (semifermented) and black tea (fully fermented). Alternatively, with the combination of the ways of processing and the characteristic quality of manufactured tea, tea is classified into six types: green tea, yellow tea, dark tea (containing brick tea and pu-erh tea), white tea, oolong tea and black tea (Ho et al., 2009). The so-called fermentation in tea processing is not the anaerobic breakdown of an energy-rich compound (as a carbohydrate to carbon dioxide and alcohol or to an organic acid) but in essence is mainly the oxidative polymerization and condensation of catechins catalyzed by endogenous polyphenol oxidase and peroxidase. The oxidation products such as theaflavins and thearubigins contribute to tea color and the taste of black tea (Balentine et al., 2012).

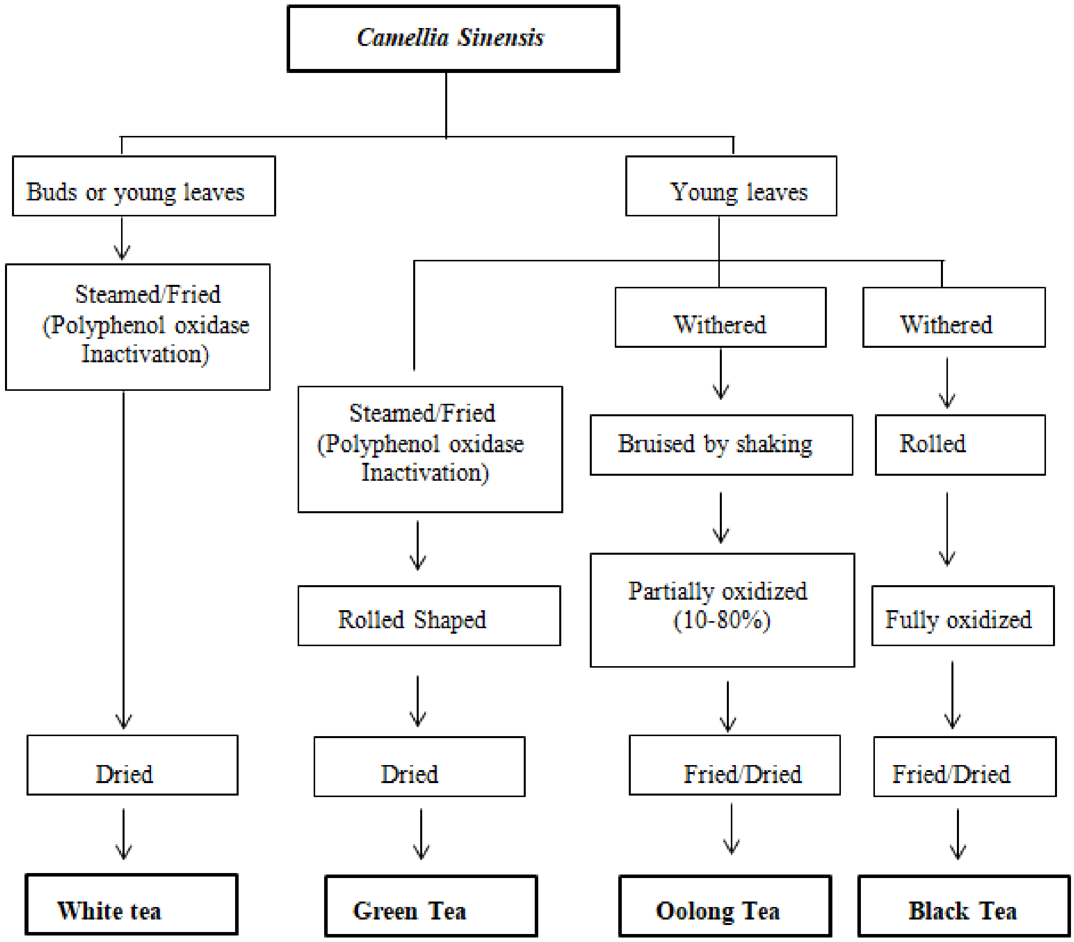

Fig. 2.1 Schematic representation of tea processing (Source: Dias et al., 2013)

About 78% of the world tea production is black tea, 20% green tea and 2% oolong tea which is majorly produced in Fujian, China (Shi et al., 2018). Nepal produces approximately 16.29 million kilograms of tea per annum. It accounts for only 0.4% of the total world tea output (Himal Mandap Journeys, 2017). Nepal grows mainly three types of tea, i.e. *Camellia asamica* and *C. asamica spp lasiocalyx* for CTC and Camellia *sinensis* for orthodox tea. The biodiversity, topography and organic matter richest soil place Nepal Tea in a unique position. The abundance of hairy growth (pubescence) on the underside of the leaf, on the bud and sometimes even on the stalk gives Nepali Orthodox Tea its fine ‘tippy’ quality and precious flavours. Similarly, CTC tea of Nepal is famous for its strong, bright and full bodied liquor. Indeed, Nepal’s Tea is the symbol of quality from the Himalayas (NTCDB, 2019). Orthodox tea refers to the process where tea is hand or machine-rolled. Most teas like green tea, oolong tea, white tea and hand rolled tea fall under orthodox tea. In Nepal, orthodox tea is produced and processed in the mountainous regions of Nepal at an altitude ranging from 3,000 – 7,000 feet above the sea level. There are six major districts, primarily in the eastern regions of Nepal that are known for the producing quality orthodox tea, which are Ilam, Panchthar, Dhankuta, Terhathum, Sindhupalchok and Kaski (Himal Mandap Journeys, 2017).

In 2018 about 84 billion servings of tea or more than 3.8 billion gallons of tea was consumed in USA only. This shows the popularity of tea around the world. About 84% of all tea consumed was black tea, 15% was green tea and the small remaining amount was oolong, white and dark tea (Statista, 2019).

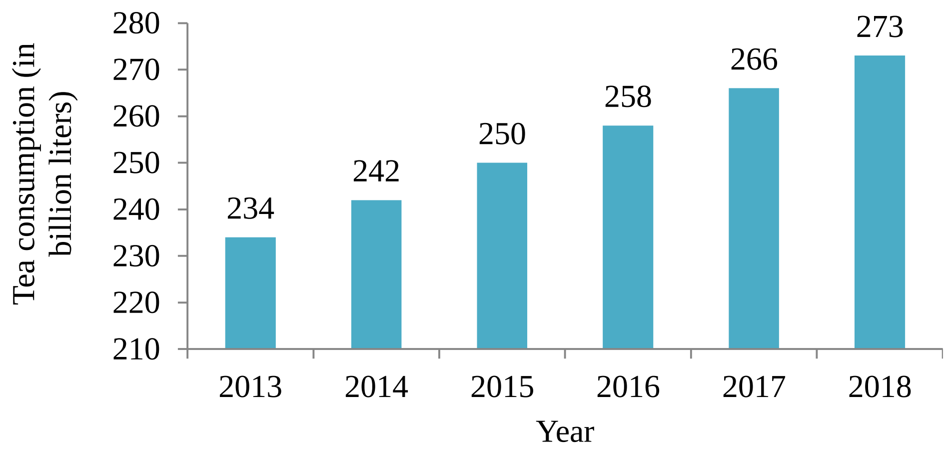

Fig. 2.2 Annual tea consumption worldwide (Source:Statista, 2019)

2.1.1 Statistical data of Tea production

2.1.1.1 Worldwide Tea production

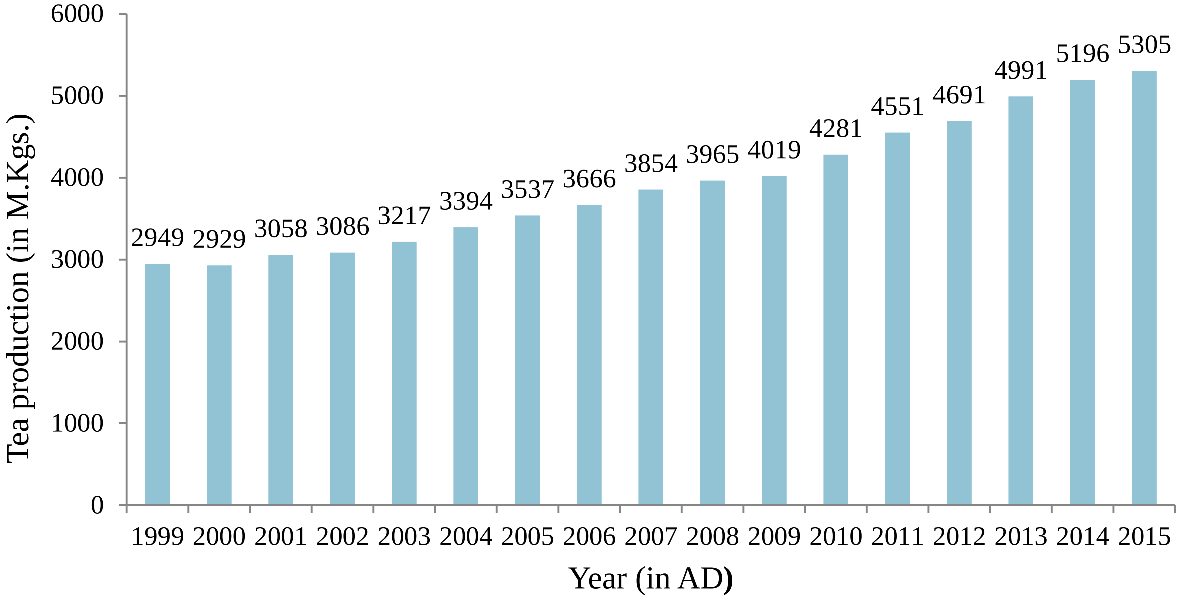

Fig. 2.3 Worldwide Tea production (Source: Global Tea production, 2018)

2.1.1.2 Tea production in Nepal

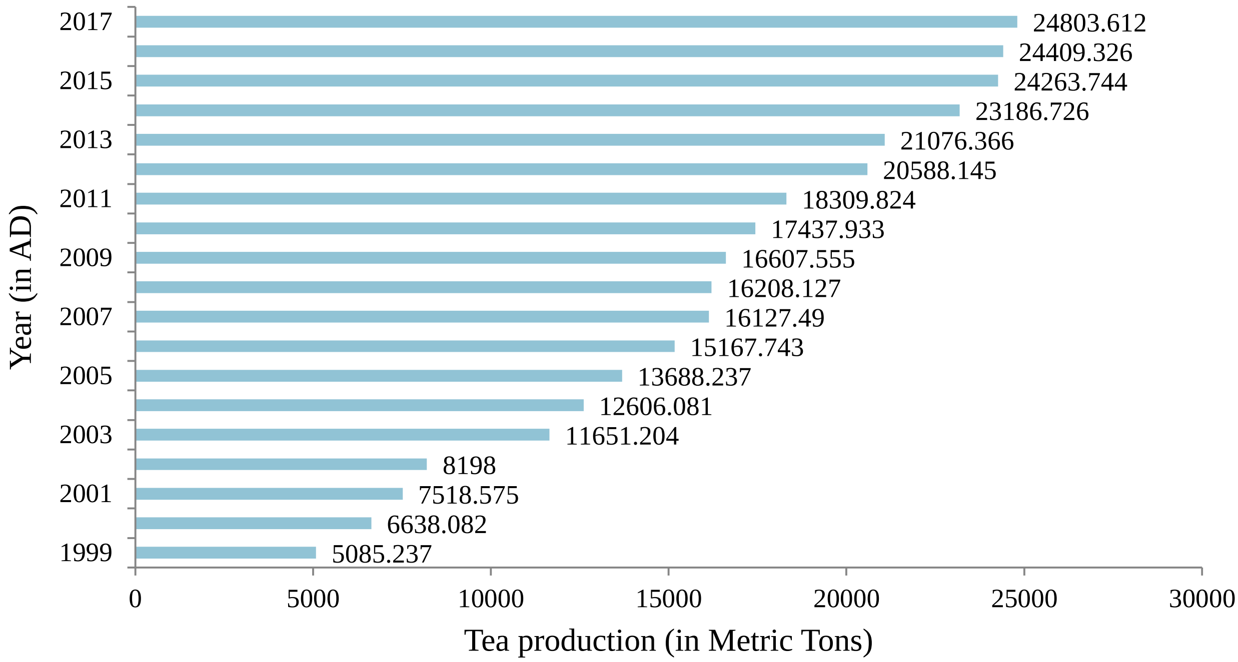

Fig. 2.4 Tea production in Nepal (Source: NTCDB, 2019)

2.1.2 Types of Tea

2.1.2.1 Green Tea

China is the center of origin of green tea production. Green tea is produced in over 20 countries in tropical, sub-tropical and temperate regions. Green tea is sourced from *Camellia sinensis* (L.) O. Kuntze (Theaceae), the same species from which oolong, white, black and pu-erh teas are derived. Green tea is a non-fermented and less oxidized tea. Health benefits of green tea are due to Catechin polyphenols and caffeine. Caffeine contributes to green tea's stimulant properties, while the amino acid theanine contributes to its relaxing properties. Variation of catechin and caffeine content occurs between green tea types depending on environmental growth conditions, processing, storage and preparation (Ahmed and Stepp, 2013). The amount of caffeine present in green tea ranges from (1-5) % by dry weight (Uzunalic et al., 2004).

Table 2.1 Chemical composition of green tea leaves

Constituent	Percentage(% of dried leaf)
Polyphenols	37.0
Carbohydrates	25.0
Caffeine	3.5
Protein	15.0
Amino acids	4.0
Lignin	6.5
Organic acids	1.5
Lipids	2.0
Ash	5.0
Chlorophyll	0.5

Source: (Sinija and Mishra, 2008)

2.1.2.2 Oolong Tea

Oolong tea is produced by a partial oxidation of the leaf, intermediate between the process for green and black tea (Blumberg, 2003). Oolong tea contains sweet scent of green tea with the delicate aroma of black. Oolong teas range from greenish rolled oolongs which have a light flowery flavor to dark brown-leafed oolongs which produce much earthier flavors, some with a hint of peach (Smith, 2010). The flavor of oolong tea has been reported to be related to a combination of various compounds such as catechins (bitterness), amino acids (freshness), soluble sugar (sweetness), theaflavins (briskness) and thearubigin (mellowness). Many of the main taste compounds in oolong tea, such as alkaloids, 11 flavan-3ols, 8 organic acids and esters, and 3 theaflavins, have been identified by different methods (Liu et al., 2018). According to tests done by (Komatsu et al., 2003), the amount of caffeine present in 15 grams of oolong tea is found to be 77 mg.

2.1.2.3 Black Tea

Black tea is produced by the enzymatic oxidation of tea flavinols (Spiller, 1998). This process results in the oxidation of simple polyphenols to more complex condensed polyphenols that gives black tea its bright red color and brisk astringent flavor. The degree of fermentation determines the characteristics of the finished tea product (Harbowy et al., 2012). Orthodox and CTC (crush, tear and curl) are the processing methods of black tea. Rather than rolling and twisting the leaves in the orthodox manner, tea leaves are cut into small pieces by machines resulting pebbly granules which is also called as CTC tea. These teas can brew quickly and are more convenient for filling tea bags (Kohler, 2015). Black tea contains (1-5) % of caffeine of its dry weight. Caffeine content in tea depends upon type, brand and brewing method. Thus amount of caffeine present in same type of tea is variable (Atoma and Gholap, 2011).

2.1.2.4 White Tea

White tea is minimally processed that much less oxidation occurs. As soon as the buds are plucked they are allowed to wither and air dry in the sun or in a carefully controlled environment. Some buds may be steamed or exposed to low heat to help dry them more quickly to stop oxidation (Teatulia, 2018). The main bioactive constituents of white tea include amino acids, polyphenols, and methylxanthines. White teas with large portion of buds contain more caffeine than those with a large portion of mature leaves, as the young tips are highest in caffeine. White teas made of tips are very mild in flavor and contains high caffeine in brewed cup of tea (White tea reviews, 2015). There are some types of white tea that contains higher caffeine content than green tea, but the opposite also occurs. This is due to the fact that the chemical composition is influenced not only by differences in the processing techniques, but also by the geographical origin of growth, climate, soil, botanical variety, harvest time, horticultural practices and even brewing conditions (Dias et al., 2019).

2.2 Caffeine

Caffeine is a white crystalline xanthine alkaloid that acts as a stimulant drug. It was first extracted from coca beans into purest form, a white powder in the 1820s by a Germen Scientist name Fredrich Ferdinand Range (Ambadekar et al., 2016). 1, 3, 7-trimethyl xanthine is the systematic name of caffeine ($C_8H_{10}N_4O_2$). Other name of caffeine is trimethyl xanthine, theine, mateine, guaranine and methyl theobromine. It is odorless white powder or needles. It is less soluble in water, moderate in ethyl acetate, pyrimidine, pyrrole and acetone and highly soluble in petroleum ether, ether, benzene and chloroform (Rehman and Ashraf, 2017). X-Ray crystallographic analysis of equimolar suspension of (-)-gallocatechin-3-O-gallate (GCg) and caffeine has shown that the resulting complex was capable of showing $\pi - \pi$ interactions between the A, B rings of GCg and the two six-membered rings of caffeine. The nitrogen atoms in the structure of caffeine are all planar (in sp2 orbital hybridization), resulting in the aromatic characteristics of caffeine. This has shown more light on the stereochemical structure and intermolecular interaction at caffeine (Agyemang and oppong, 2013).

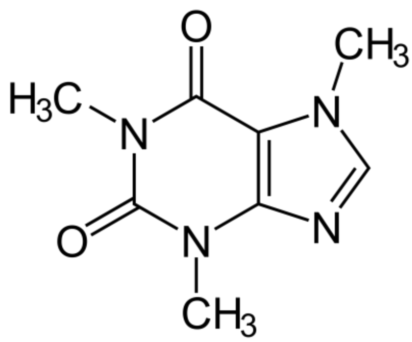

Fig. 2.5 Structure of Caffeine molecule

Table 2.2 Physical properties of Caffeine

Molecular weight	194.19 g
Melting point	236ºC
Sublimation point	178 ºC
pH	6.9 (1% solution)
Specific Gravity	1.2
Volatility	0.5%
Solubility in water	2.17%
Density	1.2 g/cm^3

(Source: Motora and Beyene, 2017)

Caffeine occurs naturally in over 60 plants species including tea, coffee and cola. Caffeine is found in varying quantities in seeds, leaves and fruit of plants. Tea leaves from *Camelia sinensis* produce secondary metabolites including caffeine in the defense of the plants against invading pathogens including insect, bacteria, fungi and viruses (Friedman et al., 2005). Caffeine Content in tea varies with tea types but normal range goes from (2-5) % by dry weight (Jun, 2009). The range of caffeine levels in the tea plant is affected by all of the parameters that bring about variation in plant composition. Nitrogen application in fertilizers can increase caffeine by as much as 40%. Seasonal variations, leaf position on the cutting, and genetic origin show similar effects. For instance, var. *sinensis* is slightly lower in caffeine than var. *assamica* (Spiller, 1998). Caffeine can be produced by natural sources as well as by synthetic method in the industry. It is used as a flavoring agent in baked goods (Rehman and Ashraf, 2016). Tea and coffee are the common source of caffeine. Although coffee and tea both contains caffeine they have different effects on our health. This is due to the presence of different amount of caffeine (Demir et al, 2015).

Caffeine is metabolized into three metabolites into the liver; they are Paraxanthine (84%), theobromine (12%) and theophylline (4%). Caffeine from various food stuffs are absorbed by small intestine within 45 minutes of ingestion and then distributed throughout all tissues of the body. Following ingestion, caffeine is rapidly and essentially completely absorbed from the gastrointestinal tract into the bloodstream. Maximum caffeine concentrations in blood are reached within 1–1.5h following ingestion. Caffeine can be absorbed rectally as well. Cytochrome P450 oxidase enzyme is responsible for caffeine metabolism in the liver.

Absorbed caffeine is readily distributed throughout the entire body. It passes across the blood–brain barrier, through the placenta into amniotic fluid and the foetus, and into breast milk. Caffeine has also been detected in semen. The liver is the primary site of caffeine metabolism. In adults, caffeine is virtually completely metabolized to 1methylxanthine and 1-methyluric acid from the paraxanthine intermediate. Only 1–5% of ingested caffeine is recovered unchanged in the urine. Infants up to the age of 8–9 months have a greatly reduced ability to metabolize caffeine, excreting about 85% of the administered caffeine in the urine unchanged (Nawrot et al., 2002; Nehling, 2018). Evidence from both scientific reviews and specific studies on consumption of caffeine generally concludes that daily consumption of 300 mg/day or about three cups of tea is safe, even for more sensitive segments of the population, such as young children and pregnant women (Wolde et al., 2014).

2.2.1 Health effects of Caffeine

2.2.1.1 Health Benefits

Caffeine is the world most widely consumed psychoactive drug but unlike many other psychoactive substances, it is both legal and unregulated in nearly all parts of the world. In humans caffeine acts as a central nervous system stimulant, temporarily warding off drowsiness and restoring alertness. Low doses of caffeine, 15-60 mg/serve (about as much as in a single cup of tea) improve long-term memory. Low to medium does (about two cup's worth) improves attention, memory and coordination (Aisha B.M.A. et al, 2012). Synthetic caffeine, chemically identical to natural caffeine is added to products such as yogurt, chocolate, energy drinks, soft drinks as well as some pharmaceuticals (Heckman et al., 2010). Being widely consumed psychoactive drug, it improves digestion and heart function, boosts energy level and may also decrease the risk of type 2 diabetes, parkinson's disease and various types of cancer (Demir et al., 2015). Caffeine can be converted into items that we enjoy in everyday lives-sodas, cosmetics and pharmaceutical products. Diet pills and cosmetics companies are the largest consumer of caffeine. It can be also used in manufacturing body wash, soap, lip-balm, facial scrub and several other products such as caffeine lipstick (Shinde and shinde, 2017).

Caffeine is used as a drug on the basis of its effect on respiratory, cardiovascular and the central nervous system. It is included with aspirin in some preparations for treatment of headaches as it decreases cerebral eye blood flow. It is included with ergotamine in some antimigraine preparations, the object being to produce a mildly aggregable sense of alertness. Caffeine is administered in the treatment of mild respirator depression caused by central nervous system depressants such as narcotic. Caffeine may also be used in the treatment of acute circulatory failure. In either beverage or in nonprescription tablet form, it may be used to relieve fatigue since it increases the amount of urine flow. In fact there are about 2000 non-prescription and about 1000 prescription drugs containing caffeine (Wanyika et al., 2010).

The American Alliance for health stated that there are three ways that caffeine may provide ergogenic effect. First, the metabolic theory suggests that caffeine provides improved endurance due to an increased utilization of fat as fuel and a sparing effect on carbohydrate utilization. Secondly, caffeine may increase the calcium content of skeletal muscle and enhance the strength of muscle contraction. Lastly caffeine has a direct effect on the central nervous system as a stimulant and this helps muscle recruitment (Powers, 2004). Caffeine is a powerful ergogenic aid that may be beneficial in training and athletic performance. It is used by athletes to enhance performance since it mobilizes fats from stores, a process that normally does not become maximal until intense activity is underway. It can exert its effects on both the central nervous system and the peripheral tissues, resulting in a number of physiological effects that might improve performance. Caffeine has been shown to increase speed and power. It also allows athletes to train longer. Caffeine stimulates the brain which contributes to clearer thinking and greater concentration. Studies have shown that caffeine doesn't directly improve maximal oxygen capacity but assists in the process of resisting fatigue (McDaniel et al., 2010).

2.2.1.2 Health Risks

The effect of caffeine is short-lived because it removes quickly from the brain and it tends not to negatively affect concentration or higher brain functions. However, continued exposure to caffeine leads to developing a tolerance to it. Too much caffeine can result in caffeine intoxication which is characterized by nervousness, irritability, anxiety, tremulousness, muscle twitching (hyperreflexia), insomnia, headaches, respiratory alkalosis, and heart palpitations. If caffeine is consumed in high amount by pregnant women then it can lead to miscarriage, difficult birth and delivery of low weight babies. Food stuffs containing more than 150 mg/L of caffeine must provide a warning message on the label (Pradhan et al, 2017). It is now widely believed that habitual daily use of caffeine

>500–600mg (four to seven cups of coffee or seven to nine cups of tea) represents a significant health risk and may therefore be regarded as ‘abuse’. Sustained abuse may in turn result in ‘caffeinism’, which refers to a syndrome characterized by a range of adverse reactions. Excessive caffeine intake (>400mg/day) may increase the risk of detrusor instability (unstable bladder) development in women. For women with preexisting bladder symptoms, even moderate caffeine intake (200–400mg/day) may result in an increased risk for detrusor instability (Arya et al, 2000). Study on effect of caffeine on blood pressure indicate that caffeine induces an increase in systolic (5-15 mmHg) and diastolic (5-10 mmHg) blood pressure, most consistently at doses >250 mg/person, in adults of both sexes irrespective of age, race blood pressure status, and habitual caffeine intake (Nawrot et al., 2003). The mayo clinic state that consuming more than 500-600 mg of caffeine a day may lead to insomnia, nervousness, restlessness, irritability, an upset stomach, a fast heartbeat and muscle tremors (Mayo clinic, 2017). One study showed that women who consumed 31-250 mg of caffeine/day had a 1.5-fold increase in the odds of developing fibrocystic breast disease and women who drank over 500 mg/day had a 2.3-fold increase in the odds of developing cysts (Boyel et al., 1984). Beta-adrenergic agonists medicine (used for curing breathing problems) in combination with caffeine will increase cardiovascular stimulation. Overdose may present with high serum caffeine levels and with symptoms of arrhythmias, delirium, seizures and metabolic acidosis (Guilbeau et al., 2012). Some people have over-sensitivity to the caffeine molecule, which causes allergic-like reactions in the body such as hives and pain. Although not a true allergy many report very negative symptoms after consuming even the smallest amounts (Lega et al., 2003).

2.2.2 Methods for determination of Caffeine

Caffeine can be extracted by various methods such as water extraction, supercritical carbon dioxide extraction and organic solvent extraction. Solvent as chloroform, methyl chloride, ethanol, Acetone and ethyl acetate are commonly used for solvent extraction of caffeine (Shinde and shinde, 2017). HPLC is one of the popular techniques used for the determination of caffeine in different mixtures. HPLC is a high priced and resource consuming technique, so it is not available in most universities in developing countries (Tautua et al, 2014). Another method which is used widely for determining the amount of caffeine is UV/visible Spectrophotometry. Demissie et al., (2019) and Rehman and Ashraf (2016) has also estimated caffeine in tea using UV Visible Spectrophotometer.

3. Materials and Methods

3.1 Laboratory Setup

The entire work has been performed in the laboratories of Central Campus of Technology, Dharan.

3.2 Experimental Materials

3.2.1 Chemicals

- Anhydrous Caffeine powder (obtained from HiMedia, Assay: 98-101.5%)
- Buffer tablets of pH 4 and pH 7

3.2.2 Instrument

- UV-Vis Spectrophotometer (Labtronics, Model: LT-291)
- Muffle furnace (Accuma, India)
- Hot air oven
- pH meter (Deluxe, Model: LT-10)
- Digital balance (Phoenix Instrument)

3.2.3 Others

- Filter paper (Whatman Grade 42, diameter 125mm, pore size 3 µm)
- Distilled water (Single distilled water)

3.3 Sampling

Simple random sampling technique was used for sampling. Samples were collected from local market of Dharan. Sample size was 100 g for each sample. Following tea samples were collected from local market of Dharan.

Table 3.1 Tea sample specifications

Sample Name	Manufacturer	Mfd. Date	Type	Raw Materials
Red Label	Unilever Nepal Ltd	April, 2019	CTC (Black Tea)	Tea Leaves
Tokla	Nepal Tea Development Corporation	April, 201	CTC (Black Tea)	Tea Leaves
Haldibari	Haldibari Tea Industries	February, 2019	CTC (Black Tea)	Tea Leaves
Sencha	Ilam Tea House	November, 2018	Sencha Green Tea (Steam Non-fermented)	Tea Leaves
Green Pearl	Ilam Tea House	October, 2018	Green Tea (Rosted)	Tea Leaves
Rakura	Himal Tea Industries Pvt. Ltd	March, 2019	Green Tea	Tea Leaves
Black Tea	Ilam Tea House	September, 2018	Orthodox Black Tea	Tea Leaves
White Tea	Ilam Tea House	September, 2018	White Tea	Tea Leaves
Nama Tea	Namo Pkg. Udhyog	February, 2019	Green Tea	Tea Leaves

3.4 Methodology

3.4.1 Raw material collection

Total nine different types of manufactured tea samples of Green, Black and White tea were purchased from local market of Dharan. Sample was collected from different stores located in different area of Dharan. Sample size was 100 gram for each sample and simple random sampling technique was used for sampling.

3.4.2 Determination of Moisture content (AOAC, 2000)

A crucible was placed in a hot air oven at 105ºC for 1 hour. It was weighed. Then small amount of tea was placed in the crucible, covered with lid and weighed. The crucible was placed in a hot air oven at 105ºC with its lid removed and dried for 1 hour. The crucible was taken out, immediately covered with the lid, cooled in a desiccator and weighed.

Wt. of empty crucible = G

Wt. of crucible + tea (before heating) = B

Wt. of crucible + tea (after heating) = F

% of moisture content (M) = $\frac{(B-F)*100}{(B-G)}$

3.4.3 Determination of Ash content (AOAC, 2000)

A crucible was dried in the muffle furnace at 550±25ºC for 4 hours. The crucible was placed in the desiccator, cooled and weighed. Small amount of tea was dried in the hot air oven at 150ºC for 3 hour. Then the sample was put in the muffle furnace at 550±25ºC for 4 hours. Then crucible as taken out from furnace, placed in the desiccator, cooled to room temperature and weighed.

Wt. of empty crucible = G

Wt. of crucible + tea (before heating) = B

Wt. of crucible + tea (after heating) = F

% of ash (A) $\frac{(F-G)*100}{(B-G)}$

3.4.4 Determination of pH (AOAC, 2000)

2 gram of tea was taken into a beaker. 100 ml of boiling distilled water was added to it. The mixture was allowed to stand for 5 minutes with stirring. pH was measured by using pH meter precalibrated in the buffer 4 and 7.

3.4.5 Estimation of Caffeine

Estimation of caffeine is as per the method described by Shar et al., (2017) with slight modification.

3.4.5.1 Wavelength selection

Wavelength of 271 nm was used for analysis of caffeine which is as per method described by Shar et al., (2017).

3.4.5.2 Preparation of standards

Caffeine stock standard solution of 500µg/ml was prepared by dissolving 0.05 gram of caffeine in 100 ml of distilled water in 100 ml volumetric flask. Working standard solutions of 0.5, 1, 10 and 20 µg/ml was prepared by suitable dilution of stock solution.

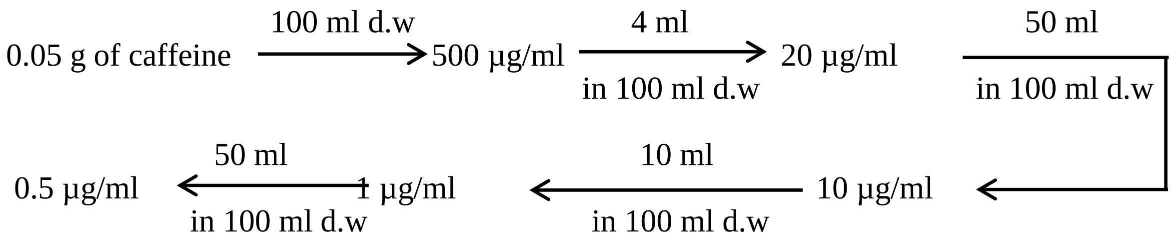

Fig. 3.1 Preparation of caffeine standards

3.4.5.3 Preparation of sample

2 gram of tea sample was weighed and put into 250 ml beaker. 100 ml of boiling distilled water was added and allowed to stand for five minutes with stirring; the solution was cooled and filtered into conical flask. 1 ml of brewed tea filtrate was pipetted out into clean 100 ml volumetric flask and volume was made up to the mark by adding distilled water. The diluted filtrate was filtered again and used for measuring absorbance.

3.4.5.4 Standard calibration curve

Absorbance of working standard solutions of caffeine was measured at 271 nm using UV-Vis Spectrophotometer ((Labtronics, Model: LT-291). Absorbance vs Concentration was plotted in graph to obtain standard calibration curve.

3.4.5.5 Calculation of Caffeine

Absorbance of different tea samples at 271 nm were measured and corresponding caffeine concentration was calculated using standard calibration curve.

4. Results and Discussion

Table 4.1 pH, Moisture and Ash content of Tea samples

S.N	Tea Sample	pH	% Moisture	% Ash
1	Red Label	4.623±0.019	3.401±0.226	14.711±0.213
2	Tokla	3.823±0.021	2.673±0.199	7.671±0.190
3	Haldibari	4.677±0.005	1.182±0.141	7.623±0.259
4	Sencha	4.743±0.005	3.701±0.329	11.276±0.249
5	Green Pearl	4.617±0.012	7.106±0.045	8.408±0.152
6	Rakura	4.677±0.005	2.453±0.418	7.807±0.303
7	Black Tea	4.190±0.008	5.321±0.397	6.939±0.662
8	White Tea	4.053±0.009	4.836±0.347	8.308±0.315
9	Nama Tea	4.083±0.005	0.943±0.062	5.575±0.217

Note: results are presented as mean±S.D.

4.1 pH content

Result obtained in this study showed that Sencha tea contains highest pH level (4.743) and Tokla tea contains lowest pH level (3.823). pH of all the tea samples are found to be almost similar with each other. Generally teas are found to be slightly acidic. From the above results we can see Tokla tea is acidic in nature. Thus, consuming Tokla tea in large proportion may increase our stomach acidity resulting in various types of health problems.

4.2 Moisture content

The highest moisture content is found in Green Pearl (7.106%) and lower moisture content is found in Nama Tea (0.943%). According to Department of Food Technology and Quality Control (DFTQC), the mandatory standard for moisture content in tea is less than 4%. Moisture content in Red Label, Tokla, Haldibari, Sencha, Rakura and Nama Tea was found to be within the mandatory standard of DFTQC while Green pearl, Black Tea and White Tea contains higher amount of moisture than the standard. This may be due to the high humidity of the processing environment as dried tea picked up moisture during the holding time between production and analysis. Shorter drying period due to carelessness of workers during manufacturing process can also increase moisture content in tea. Defect on packaging of tea may be another reason for higher moisture content since moisture can be absorbed through leaked area during storage.

4.3 Ash content

The highest ash content is found in Red Label (14.711%) and the lowest ash content is found in Nama Tea (5.375%). The mandatory standard for ash content in tea according to DFTQC ranges 5-8%. Tokla, Haldibari, Rakura, Black Tea and Nama Tea contains ash within the range of standard provided by DFTQC while Red Label, Sencha, Green Pearl and White tea deviated from the standard and contains higher amount of ash content. The deviation may be due to the presence of higher amount of minerals in those teas. According to (Adnan et al., 2013) no fermentation process during tea production and cultivation of tea in minerals rich soil can increase minerals amount of tea resulting increase in ash content. Thus we can say that tea leaves used for making Red Label may be cultivated in soil containing higher mineral content than others. Adulterations during tea processing may also have probability for higher ash content.

4.4 Standard calibration curve of caffeine

The standard calibration curve of caffeine obtained by UV-Vis Spectrophotometer is shown in figure 4.1.

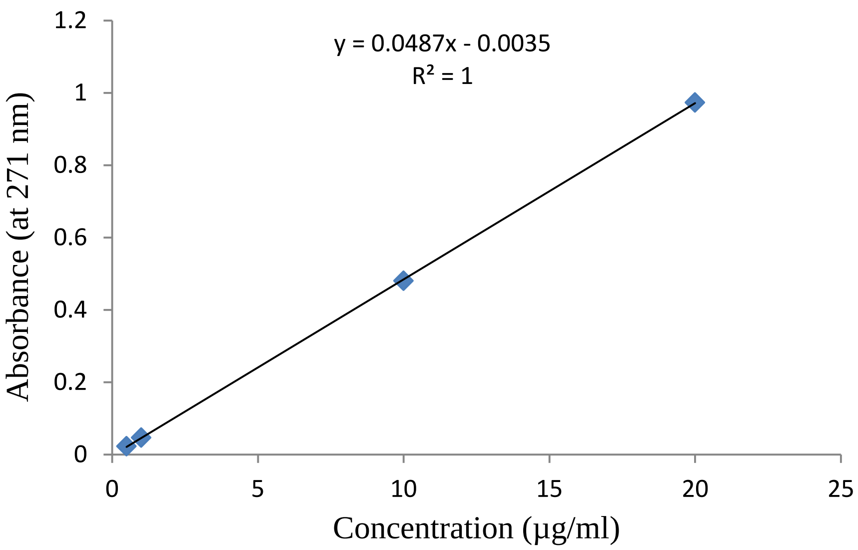

Fig. 4.1 Standard calibration curve of caffeine

The standard curve was found to show a linear increase in absorbance with an increase in concentration of caffeine solution. The equation from absorbance and concentration was found to be $y = 0.0487x – 0.0035$, which is a linear equation. The fitting of linear trend line in the data set shows a high goodness of fit (R^2) value of 1.

4.5 Caffeine content

The caffeine content in various tea samples are given in table 5.

Table 4.2 Caffeine content in various tea samples

S.N	Tea Sample	Absorbance	Concentration (mg/L)
1	Red Label	0.496±0.004	1026.4
2	Tokla	0.602±0.006	1244
3	Haldibari	0.495±0.016	1022.9
4	Sencha	0.823±0.007	1697.8
5	Green Pearl	0.558±0.009	1153
6	Rakura	0.623±0.013	1287.1
7	Black Tea	0.561±0.016	1159.1
8	White Tea	0.540±0.054	1116.7
9	Nama Tea	0.580±0.008	1198.8

The highest caffeine content is found in Sencha tea (1697.8 mg/L) and the lowest caffeine content is found in Haldibari tea (1022.9 mg/L). According to the mandatory standard of DFTQC, caffeine content in tea should be not less than 2% by its dry weight. Thus caffeine contents of the entire tea sample are within the standards of DFTQC. There is no any vast difference in caffeine concentration between the tea samples. According to Uzunalic et al., (2004) caffeine content is green tea ranges from 1-5% but these result slightly deviated resulting highest caffeine content of 1697.8 mg/L in green tea. This may be due to different growing and processing conditions for different tea. Variation of caffeine in tea is due to the fact that the chemical composition is influenced not only by differences in the processing techniques but also by the geographical origin of growth, climate, soil, botanical variety, harvest time, horticultural practices and even brewing conditions which is shown by the research of (Dias et al., 2019).

Statistical analysis of moisture content and ash content has shown a linear regression with caffeine concentration which are shown in appendix ‘A’ and ‘B’ respectively. From the appendix ‘A’ we can see that the pearson correlation coefficient for moisture content and caffeine is 1.00 which is significant ($p< 0.01$ for a two tailed test) based on 9 complete observations. Research of Alqarni et al., (2018) also shows the strong positive linear relationship between moisture content and caffeine content of tea. During drying and roasting process of tea, caffeine may be lost in some proportion due to applied heat. Since lower the drying and roasting time, greater will be the moisture content resulting in no loss of caffeine as compared to the tea dried for longer period of time. Thus there is strong positive linear relationship between caffeine and moisture content of tea. From the appendix 'B' we can see that the pearson correlation coefficient for ash content and caffeine is 0.994 which is significant ($p< 0.01$ for a two tailed test) based on 9 complete observations. Positive linear relationship between ash content and caffeine content is also shown by the research of Cizernicka et al., (2017).

According to Wolde et al., (2014) the caffeine consumption of 300 mg/day is safe. Based on the criteria for daily consumption of caffeine and the amount of caffeine in tea determined by this research, the consumption level of different tea are calculated which are given in table 6.

Table 4.3 Consumption level of tea

S.N	Name of Tea	Consumable quantity (in g/day)	Consumable quantity Aprox. (in cup of tea/day)
1	Red Label	5.84	3
2	Tokla	4.82	$2\,{}^{1}/_{2}$
3	Haldibari	5.87	3
4	Sencha	3.53	$1\,{}^{1}/_{2}$
5	Green Pearl	5.2	$2\,{}^{1}/_{2}$
6	Rakura	4.66	$2\,{}^{1}/_{2}$
7	Black Tea	5.18	$2\,{}^{1}/_{2}$
8	White Tea	5.37	$2\,{}^{1}/_{2}$
9	Nama Tea	5.0	$2\,{}^{1}/_{2}$

From these results we can see that maximum amount of consumption level of Sencha tea is 3.53 gram per day on the basis of caffeine intake. While the consumption level of Red Label and Haldibari tea is 5.84 and 5.87 gram per day respectively. A cup of tea is generally prepared by brewing 2 gram of tea into 250 ml of water. On this basis we can say that maximum level of consumption of Sencha tea is one and half cup per day approximately. Similarly Tokla, Green Pearl, Rakura, Black Tea, White Tea and Nama tea consumption level is approximately two and half cup per day. 3 cups of Red label and Haldibari tea per day are the maximum limit of consumption depending upon caffeine content. Consuming tea greater than this amount may cause various health problems which are discussed in the literature above.

5. Conclusion and recommendations

5.1 Conclusion

On the basis of research, following conclusions can be drawn:

1. Sencha tea has found to contain highest caffeine concentration (1697.8 mg/L) and Haldibari tea (1022.9 mg/L) has found to contain lowest caffeine concentration among the tested samples.
2. Depending upon specific tea, consumption level of tea without harming human health ranges from one and half cup to three cups per day.
3. Moisture and Ash content of tea has positive linear relationship with caffeine content.

5.2 Recommendations

The experiment can be further continued with the following recommendations

1. Study on estimation of caffeine content in large number of tea samples can be carried out.
2. Along with caffeine, study on antioxidant properties and other tea components like tannin and flavonoids can be carried out.

6. References

Adnan, M., Ahmad, A., Ahmed, A., Khalid, N., & Ahmed, I. (2013). Chemical Composition and Sensory Evaluation of Tea (Camellia Sinensis) Commercialized in Pakistan. *Pakistan Journal of Botany,45*(3), 901-907.

Ahmed, S., & Stepp, J. R. (2013). Green Tea. *Tea in Health and Disease Prevention,*19-31. doi:10.1016/b978-0-12-384937-3.00002-1

Alqarni, M. H., Salkini, M. A., & Abdel-Kader, M. S. (2018). Roasting Effect on The Caffeine Contents and Antioxidant Potential of Different Coffee Grades Available in The Saudi Market. *Indo American Journal of Pharmaceutical Sciences,5*(12), 16738-16745. doi:10.5281/zenodo.2505094

Ambadekar, S., Barabde, G., & Solkar, S. (2016). Simultaneous determination of caffeine and benzoic acid from chocolates by chromatographic techniques. *International Journal of Food Science and Nutrition,1*(6), 11-15.

Amos-Tautua, A., Martin, W. B., & Diepreye, E. (2014). Ultra-violet Spectrophotometric Determination of Caffeine in Soft and Energy Drinks Available in Yenagoa, Nigeria. *Advance Journal of Food Science and Technology,6*(2), 155-158. doi:10.19026/ajfst.6.2

Arya, L. (2000). Dietary caffeine intake and the risk for detrusor instability: A case-control study. *Obstetrics & Gynecology,96*(1), 85-89. doi:10.1016/s0029-7844(00)00808-5

Ashihara, H., & Crozier, A. (n.d.). *Biosynthesis and Metabolism of Caffeine and Related Purine Alkaloids*(Vol. 30). Advances in Botanical Research.

Atomssa, T., & Gholap, A. V. (2011). Characterization of caffeine and determination of caffeine in tea leaves using uv-visible spectrometer. *African Journal of Pure and Applied Chemistry,5*(1), 1-8.

Balentine, D. A., Wiseman, S. A., & Bouwens, L. C. (1997). The chemistry of tea flavonoids. *Critical Reviews in Food Science and Nutrition,37*(8), 693-704. doi:10.1080/10408399709527797

Baptista, J. A., Tavares, J. F., & Carvalho, R. C. (1998). Comparison of catechins and aromas among different green teas using HPLC/SPME-GC. *Food Research International,31*(10), 729-736. doi:10.1016/s0963-9969(99)00052-6

Blumberg, J. (2003). Introduction to the Proceedings of the Third International Scientific Symposium on Tea and Human Health. *The Journal of Nutrition,133*(10), 3244-3246. doi:10.1093/jn/133.10.3244s

Boyle, C. A., Berkowltz, G. S., Livols, V. A., Ort, S., Merino, M. J., White, C., & Kelsey, J. L. (1984). Caffeine Consumption and Fibrocystic Breast Disease: A Case-Control Epidemiologic Study. *72*(5), 1015-1019.

Caffeine: How much is too much? (2017, March 08). Retrieved August 10, 2019, from https://www.mayoclinic.org/healthy-lifestyle/nutrition-and-healthy-eating/in-depth/caffeine/art-20045678?reDate=09082019

Chambers, K. P. (2009). *Caffeine and health research*. New York: Nova Biomedical Books.

Czernicka, M., Zagula, G., Bajcar, M., Saletnik, B., & Puchalski, C. (2017). Study of Nutritional Value of Dried Tea Leaves and Infusions of Black, Green and White Teas From Chinese Plantations. *National Institute of Public Health,65*(3), 237-245.

Demissie, E., w, G., & Abebe, A. (2016). Uv/Vis Spectrometer Determination of Caffeine in Green Coffee Beans from Haraghe, Ethiopia, Using Beer-Lambert's Law and Integrated Absorption Coefficient Techniques. *Scientific Study and Research*, *17*(2), 109–123.

Dias, T. R., Teixeira, N. F., Alves, M. G., Oliveira, P. F., & Silva, B. M. (2013). White Tea (Camellia Sinensis (L.)): Antioxidant Properties And Beneficial Health Effects. *International Journal of Food Science, Nutrition and Dietetics (IJFS),2*(2), 19-26.

Friedman, M., Kim, S., Lee, S., Han, G., Han, J., Lee, K., & Kozukue, N. (2006). Distribution of Catechins, Theaflavins, Caffeine, and Theobromine in 77 Teas Consumed in the United States. *Journal of Food Science,70*(9), 550-559. doi:10.1111/j.1365-2621.2005.tb08304.x

Global Tea Production 2015. (2018, September 25). Retrieved August 10, 2019, from https://worldteanews.com/tea-industry-news-and-features/global-tea-production-2015

Government of NepalNational Tea and Coffee Development Board. (2019). Retrieved August 10, 2019, from http://www.teacoffee.gov.np/teainfo/statistics2

Guilbeau, J. R. (2012). Health Risks of Energy Drinks: What Nurses and Consumers Need to Know. *Nursing for Womens Health,16*(5), 423-428. doi:10.1111/j.1751-486x.2012.01766.x

Harbowy, M. E., Balentine, D. A., Davies, A. P., & Cai, Y. (1997). Tea Chemistry. *Critical Reviews in Plant Sciences,16*(5), 415-480. doi:10.1080/07352689709701956

Heckman, M. A., Weil, J., & Mejia, E. G. (2010). Caffeine (1, 3, 7-trimethylxanthine) in Foods: A Comprehensive Review on Consumption, Functionality, Safety, and Regulatory Matters. *Journal of Food Science,75*(3), 77-87. doi:10.1111/j.1750-3841.2010.01561.x

Ho, C., Lin, J., & Shahidi, F. (2009). *Tea and tea products: Chemistry and health-promoting properties*. Boca Raton: CRC Press.

Horwitz, W. (2000). *Official methods of analysis of AOAC International*. Gaithersburg, MD: Association of Official Analytical Chemists.

Igea, J. M., & Lazaro, M. (2003). The perfect storm: The best conditions for making pollinic patients symptoms worse. *Allergy,58*(7), 680-681. doi:10.1034/j.1398-9995.2003.00199.x

Jun, X. (2009). Caffeine extraction from green tea leaves assisted by high pressure processing. *Journal of Food Engineering,94*(1), 105-109. doi:10.1016/j.jfoodeng.2009.03.003

Koehler, J. (2015, May 05). Tools: 6 Things to Know About Tea Bags. Retrieved August 10, 2019, from https://www.nationalgeographic.com/people-and-culture/food/the-plate/2015/05/05/tools-6-things-to-know-about-tea-bags/

Liu, P., Yin, J., Chen, G., Wang, F., & Xu, Y. (2018). Flavor characteristics and chemical compositions of oolong tea processed using different semi-fermentation times. *Journal of Food Science and Technology,55*(3), 1185-1195. doi:10.1007/s13197-018-3034-0

López, V., & Calvo, M. I. (2011). White Tea (Camellia sinensis Kuntze) Exerts Neuroprotection against Hydrogen Peroxide-Induced Toxicity in PC12 Cells. *Plant Foods for Human Nutrition,66*(1), 22-26. doi:10.1007/s11130-010-0203-3

Maidon, A. B., Mansoer, A. O., & H. S. (2012). Study Of Various Solvents For Caffeine Determination Using Uv Spectrophotometeric. *Journal of Applied Sciences Research,8*(5), 2439-2442.

McDaniel, L. W., McIntire, K., Streitz, C., Jackson, A., & Gaudet, L. (2010). The Effects Of Caffeine On Athletic Performance. *College Teaching Methods & Styles Journal,6*(1), 33-38.

McKay, D. L., & Blumberg, J. B. (2002, February). The role of tea in human health: An update. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11838881

Mejia, E. G., Ramirez-Mares, M. V., & Puangpraphant, S. (2009). Bioactive components of tea: Cancer, inflammation and behavior. *Brain, Behavior, and Immunity,23*(6), 721-731. doi:10.1016/j.bbi.2009.02.013

Motora, K. G., & Beyene, T. T. (2017). Determination of Caffeine in Raw and Roasted Coffee Beans of Iluabba Bora Zone, South west Ethiopia. *Indo American Journal of Pharmaceutical Research,7*(9), 463-470.

Nawrot, P., Jordan, S., Eastwood, J., Rotstein, J., Hugenholtz, A., & Feeley, M. (2003). Effects of caffeine on human health. *Food Additives and Contaminants,20*(1), 1-30. doi:10.1080/0265203021000007840

Nehlig, A. (2018). Interindividual Differences in Caffeine Metabolism and Factors Driving Caffeine Consumption. *Pharmacological Reviews,70*(2), 384-411. doi:10.1124/pr.117.014407

Nepali Tea - Beverage of the Himalayas - Himal Mandap Journeys. (2017, November 24). Retrieved August 7, 219, from http://www.himalmandaptreks.com/tea-nepal/

Perva-Uzunalić, A., Škerget, M., Knez, Ž, Weinreich, B., Otto, F., & Grüner, S. (2006). Extraction of active ingredients from green tea (Camellia sinensis): Extraction efficiency of major catechins and caffeine. *Food Chemistry,96*(4), 597-605. doi:10.1016/j.foodchem.2005.03.015

Pokhrel, P., Shrestha, S., Rijal, S. K., & Rai, K. P. (2016). A simple HPLC Method for the Determination of Caffeine Content in Tea and Coffee. *Journal of Food Science and Technology Nepal,9*, 74-78. doi:10.3126/jfstn.v9i0.16200

Powers, M. (2004). Safety, Efficacy, and Leqal Issues Related to: DIETARY SUPPLEMENTS. *Strategies,18*(1), 30-34. doi:10.1080/08924562.2004.10591112

Pray, L. A., Yaktine, A. L., & Pankevich, D. E. (2014). *Caffeine in food and dietary supplements: Examining safety: Workshop summary*. Washington, D.C.: The National Academies Press.

Rehman, R., & Ashraf, S. (2017). Spectrophotometric Determination of Caffeine in Selected Pakistani Beverages. *Journal of Food Processing & Beverages,5*(1), 823-828. doi:10.13188/2332-4104.1000019

Saberi, H. (2013). *Tea: A global history*. London: Reaktion.

Shahbandesh, M. (2018, May). Global tea consumption 2013-2021. Retrieved August 05, 2019, from https://www.statista.com/statistics/940102/global-tea-consumption/

Shar, Z., Shoaib, H., Anwar, N., Hussain, A., & Khan, K. (2017). Spectrophotometric Determination of Caffeine in Selected Pakistani Beverages. *Journal of Food Processing & Beverages,5*(1), 01-04. doi:10.13188/2332-4104.1000019

Shi, H., Liu, J., Tu, Y., Freter, C. E., & Huang, C. (2018). Oolong Tea Extract Induces DNA Damage and Cleavage and Inhibits Breast Cancer Cell Growth and Tumorigenesis. *Anticancer Research,38*(11), 6217-6223. doi:10.21873/anticanres.12976

Shinde, R. R., & Shinde, N. H. (2017). Extraction of Caffeine from Coffee and preparation of Anacin drug. *International Journal of Engineering Research and Technology,10*(1), 236-239.

Sinija, V. R., & Mishra, H. N. (2008). Green tea: Health benefits. *Journal of Nutritional & Environmental Medicine,17*(4), 232-242. doi:10.1080/13590840802518785

Spiller, G. A. (1998). *Caffeine*. Boca Raton: CRC Press.

Tea Fact Sheet. (n.d.). Retrieved August 6, 2019, from http://www.teausa.com/14655/tea-fact-sheet

Tfouni, S. A., Camara, M. M., Kamikata, K., Gomes, F. M., & Furlani, R. P. (2018). Caffeine in teas: Levels, transference to infusion and estimated intake. *Food Science and Technology,38*(4), 661-666. doi:10.1590/1678-457x.12217

Wanyika, H. N., Gatebe, E. G., Gitu, L. M., Ngumba, E. K., & Maritim, C. W. (2010). Determination of caffeine content of tea and instant coffee brands found in the Kenyan market. *African Journal of Food Science,4*(6), 353-358.

What is White Tea? (n.d.). Retrieved August 08, 2019, from https://www.teatulia.com/tea-varieties/what-is-white-tea.htm

Wheeler, D. S., & Wheeler, W. J. (2004). The medicinal chemistry of tea. *Drug Development Research,61*(2), 45-65. doi:10.1002/ddr.10341

White Tea Reviews, Varieties. (n.d.). Retrieved August 3, 2019, from https://ratetea.com/style/white-tea/5/

Wolde, T. (2014). Effects of caffeine on health and nutrition: A Review. *Food Science and Quality Management,30*, 59-65.

Yamamoto, S., Komatsu, T., Matsushita, H., Nakamori, M., Dodo, K., Wakabayashi, K., . . . Hosoda, K. (2003). Oolong tea increases energy expenditure. *Atherosclerosis,151*(1), 170-175. doi:10.1016/s0021-9150(00)80662-0

Yeboah, F. A. (2013). Caffeine: The wonder compound, chemistry and properties. *Topical Series in Health Science,37*(2), 27-37.

7. Appendices

Appendix A

Table A.1 Correlation between Moisture content and Caffeine content of Tea

		Caffeine	Moisture
Caffeine	Pearson Correlation Sig. (2-tailed)	1	1.000** .000
	N	9	9
Moisture	Pearson Correlation Sig. (2-tailed)	1.000** .000	1
	N	9	9

**. Correlation is significant at the 0.01 level (2-tailed).

Appendix B

Table B.1 Correlation between Ash content and Caffeine content of Tea

		Caffeine	Ash
Caffeine	Pearson Correlation Sig. (2-tailed)	1	.994** .000
	N	9	9
Ash	Pearson Correlation Sig. (2-tailed)	.994** .000	1
	N	9	9

**. Correlation is significant at the 0.01 level (2-tailed).

Photo Gallery

Fig. 1 Tea Samples

Fig. 2 Brewing Tea sample

Fig. 3 UV-Vis Spectrophotometer

Fig. 4 Muffle Furnace

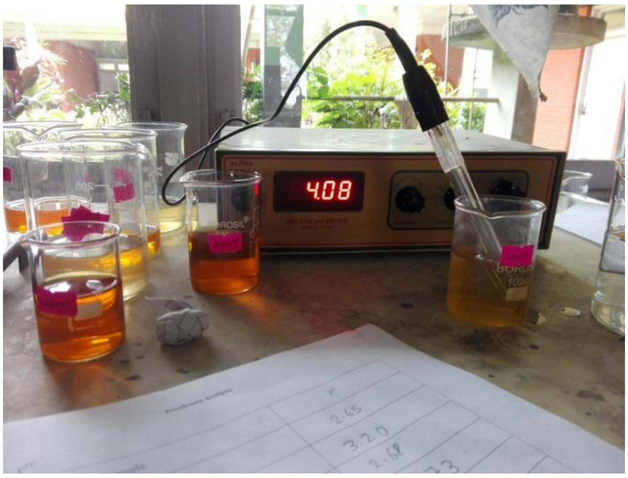

Fig. 5 pH meter

Fig. 6 Hot air oven

Fig. 8 Ash of Tea samples

Fig. 9 Standard solutions of caffeine