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# Quantitative Analysis of Caffeine Content from Different Tea Growing Regions of Rwanda

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**Abstract:** In recent times, tea becomes highly known owing to its pharmacological potential properties such as antitumor, anti-oxidative, and anti-carcinogen activities. Especially, the quality of tea is assessed based on its caffeine content. Most people drink tea with the purpose of getting some caffeine amount in their body to diminish the increased cardiovascular disease, some chronic diseases, and cancer risks. The main study's objective was to determine the caffeine content in tea samples from different tea growing regions of Rwanda in order to identify the region that has tea with the highest amount of caffeine. This may help in classifying the tea regions and tea quality in Rwanda according to their amount of caffeine. In this study, eleven samples were obtained from National Agriculture Export development Board (NAEB). Extraction of the caffeine was done using hot water. Quantification of caffeine from tea samples was accomplished by using high-pressure liquid chromatography (HPLC); the mixture of methanol (20%), deionized water (79%) and acetic acid (1%) [(20:79:1)] was used as HPLC's mobile phase. The tea sample grown at Rubaya tea growing region was obtained to have the highest caffeine quantity of 802.927±40.04 ppm. This was followed by tea grown at Nyabihu which contained caffeine quantity of 798.937±19.74 ppm. The least caffeine quantity was obtained in tea sample taken from Shagasha region with caffeine quantity of 476.128±97.05. Our results indicated that the caffeine content from tea growing regions in Rwanda had a range from 476.128±97.05 to 802.927±40.04 ppm, also had different tea quality.

**Keywords:** Caffeine Content, RWANDA, Tea, Quantitative Analysis, Quality

## 1. Introduction

Tea is a tree that takes about 7 years to reach maturity in order to produce the leaves that are used in industry tea production. In the developing countries such as Pakistan, India, China, and in the Middle Eastern countries the tea favor had been grown rapidly in 1999. The major exporters of tea in the world are India and Kenya [1]. In Rwanda, teas are planted on hills at high altitude and on well-drained marshes. In Rwanda, the Northern, Western and Southern provinces are the main contributors regions of tea plantation on the total area that is approximately 12, 500 ha in [2]. Previous studies have shown that owing to its location, Rwanda is located within the equatorial belt and has an average rainfall of 2, 000mm per year [3]. The weather and altitude are one of the best factors that contribute to the amount and quality of caffeine content in tea. The varieties of tea production can differ considerably according to the variation of growing conditions, harvesting time, and production processing [4].

Caffeine is an organic compound which can be found in the different plant, soft drinks, and human urine. It is active pharmacological substance, and depends on the dose caffeine acts like a stimulant of the cardiac muscle, central nervous system and it is also a stimulant factor of respiratory system [5]. The black and green teas infusions have as a large number of antioxidants and primarily catechins that contain antimutagenic, anticarcinogenic and antitumor properties [6]. The tea leaves, coffee beans, cocoa beans, cola nuts and other plants contain the natural alkaloid compound that is caffeine (1, 3, 7-trimethylxanthine) molecule [2].

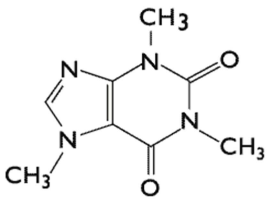


*Figure 1. Chemical structure of caffeine.*

Because of caffeine works like the central nervous system and metabolic stimulant factor, it has greater impacts on human's health according to the amount of consumed caffeine. The caffeine effects vary from person to another depending on the degree of tolerance of the body, health situation and body size of the person. Furthermore, caffeine improves its performance action in time of sleepiness and leads to insomnia [7].

The modifications of caffeine compound are released and turnover for several neurotransmitters by inhibiting presynaptic adenosine receptors [8]. Adenosine plays a generally protective role in the brain where it reduces neural activity levels. For example, adenosine may lead to induce torpor in animals during seasonally hibernate [9]. The adenosine molecular structure is totally similar to the structure of caffeine compound and has the capacity and the ability to bind to the receptors of adenosine molecule structure on the surface of cells and it does not make any activation to these receptors, thereby acting as a receptor's competitive inhibitor [10]. Adenosine molecule is located in a whole part of the body where it has a greater importance in the foundation of the body usage energy production that is adenosine triphosphate (ATP). Besides, caffeine is a special chemical compound to protect the brain from suppression of neural activity, and an increase of blood flow through the receptors that are located on vascular smooth muscle [11].

Today, most of the world's people, including Rwandese consume caffeine daily in tea product and soft drink. Due to the wide consumption of tea in Rwanda and problems caused by high intake of caffeine in the body and pharmaceutical drug of caffeine, the most importance of this research work was to establish the precise amount of the caffeine content in Rwandan tea. The principal aim of this study was to determine the caffeine content in tea samples collected from different tea growing regions by using HPLC to categorize the tea growing regions and tea quality according to their amount of caffeine content in Rwandan tea.

## 2. Materials and Methods

### *2.1. Area of Study and Collection of Data*

The collection of tea samples was done from the tea grown in different regions of Rwanda (Figure 2). Those different tea growing regions are Sorwathe and Mulindi located in the Northern Province, Pfunda, Nyabihu, Rubaya, Gisakura, Gisovu and Shagasha which are located in the Western Province, Kitabi, Mata which are located in Southern Province and were found at NAEB. The tea samples were only black tea [12].

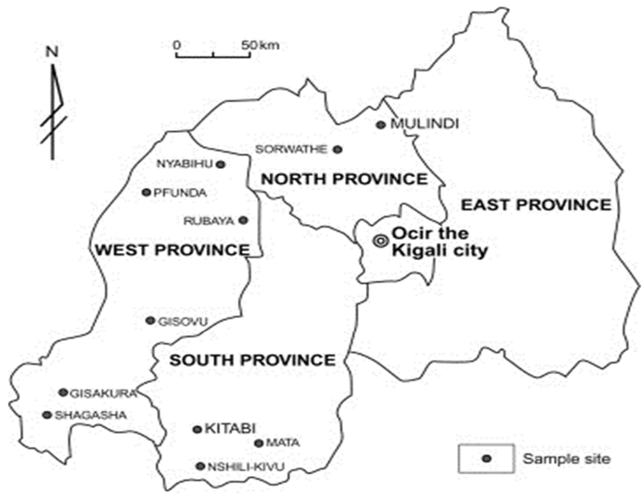


*Figure 2. Location map of tea growing region of Rwanda [12].*

### *2.2. Determination of Caffeine from Tea Using HPLC*

#### *2.2.1. Experimental Design*

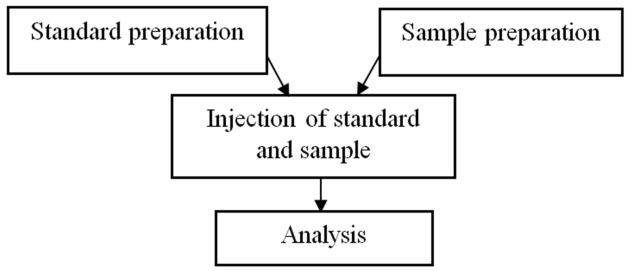


*Figure 3. Steps of quantification of caffeine from tea by HPLC.*

#### *2.2.2. Standards Preparation*

To prepare 100 ppm of standard, 0.01g of caffeine standard was measured and diluted into 100 ml of deionized water. Then after 5, 10, 15 and 20 mL portions of the stock 100 ppm caffeine solution were pipetted and placed into 25 mL volumetric flasks, finally, 25 ml flasks were filled to the mark with the mobile phase (Figure 3).

#### *2.2.3. Sample Preparation*

Two grams of tea was weighed and transferred to 200 mL of boiled water in volumetric flask of 250 mL. Once, water was boiled, and then tea was prepared as normal and filtered to remove the residues material when the tea had brewed for 5. minutes. After cooling, it was transferred to the 250 mL flask and diluted up to the mark with deionized water.

#### *2.2.4. Dilution and Preparation for Injection*

Dilution of the tea was prepared by pipetting 10. mL into 200 mL flasks and diluting to the volume with deionized water. At the end of sample preparation, the dilution factor was 145. The instrument was validated using a cross-laboratory reference standard bean sample analyzed for phytic acid both at KIST and North Dakota State University (USA).

#### *2.2.5. Analysis*

The standards of caffeine compound and the samples were analyzed by the HPLC analytical system with UV/V detector. An isocratic system consisting of methanol deionized water and acetic acid (20:79:1) was used as the mobile phase. The system was applied to the standards and samples at the 1.0 mL/min as flow rate of this experimental analysis. A five micron Nucleosil 100-5 C-18 column (125 ×4.60 mm) was used to quantify caffeine. The wavelength was 254 nm and retention time was 15 minutes, owing to the method has been developed and validated in our earlier study [13].

#### *2.2.6. Statistical Data Analysis*

All measurements were done in triplicate. One way ANOVA was used to compare the means.

## 3. Results and Discussions

### *3.1. Standard Result and Calibration Curve*

*Calibration curve*

The calibration curve was obtained using four different concentrations of caffeine standard 20 ppm to 80 ppm. The calibration curve was founded owing to the obtained experimental peak areas results with caffeine standard concentrations.

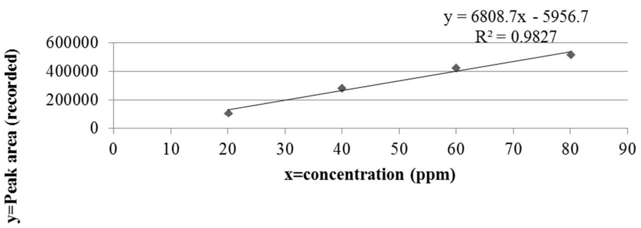


***Figure 4.*** *Calibration curve for caffeine standard.*

This standard curve of peak area vs. concentration showed a good linear relationship. The standard curve enabled the analysis of results using the equation to find a concentration of caffeine in unknown samples.

The equation was: $$\mathbf{y} = 6808.7\mathbf{x} - 5956.7 \quad (1)$$

***Table 1.*** *Where y = peak area, x = Caffeine concentration.*

| Y= intercept | m= Slope | ppm=Linear range | $R^2$ =correlation coef. |
|---|---|---|---|
| 5956.7 | 6808.7 | 20-80 | 0.9827 |

***Table 2.*** *Comparison of the means between the different regions.*

| Tea Regions | Concentration (ppm) | |
|---|---|---|
| SHAGASHA | 476.128±97.05$^{a}$ | Group I |
| PFUNDA | 480.963±81.24$^{a}$ | |
| MULINDI | 650.658±84.62$^{b}$ | Group II |
| KITABI | 712.233±82.31$^{bc}$ | Group III |
| SORWATHE | 716.961±22.82$^{bcd}$ | |
| GISOVU | 722.739±28.53$^{bcd}$ | Group IV |
| GISAKURA | 752.916±12.34$^{cd}$ | |
| MATA | 768.200±99.35$^{cd}$ | Group V |
| KARONGI | 779.302±40.27$^{cd}$ | |
| NYABIHU | 798.937±19.74$^{cd}$ | |
| RUBAYA | 802.927±40.04$^{e}$ | Group VI |

The mean values of the same letters are not notably different ($p>0.05$), n=3.

### *3.2. Discussion*

The highest amount of caffeine compound was obtained in tea sample grown at Rubaya with 802.927±40.04 ppm followed by tea grown at Nyabihu with 798.937±19.74 ppm. The least caffeine quantity was obtained in tea sample taken from Shagasha region with caffeine quantity of 476.128±97.05. From our result, the amount caffeine content in eleven tea growing regions samples are ranged from 476.128±97.05 ppm to 802.927±40.04 ppm. The highest caffeine quantity was detected in Rubaya followed by Nyabihu, Karongi, Mata, Gisakura, Gisovu, Sorwathe, Kitabi, Mulindi, Pfunda and Shagasha (Table 2).

*Conditions of climatic and soils*

Rwanda is the small size in surface area, characterized by a correspondent degree of agroecological diversity owing to its topography. Main five agro-ecological zones are Crest Zaire-Nile (Rubaya, Nyabihu, Karongi, Gisakura, Gisovu, Pfunda, Shagasha), Highlands of Buberuka (Sorwathe, Mulindi), Savannas of East, East and central plateau, and Granite ridge (Kitabi, Mata) [14].

Earlier studies revealed that caffeine content is related to environmental variability, processing manner, genetic, and origin of plant material [15]. According to these studies, our results have established that the caffeine content from tea varies widely from one region to another. Based on statistical analysis, the regions can be divided into 6 distinct groups. Group 1 includes Shagasha and Pfunda, Group 2 has Mulindi, Group 3 has Kitabi, Group 4 includes Sorwathe and Gisovu, Group 5 includes Gisakura, Mata, Karongi, and Nyabihu, Group 6 has Rubaya.

The studies on geography, have been demonstrated that weather and seasonal changes have effects on the amount of caffeine content from tea but it is hard to insulate the weather conditions. The variation of temperature may influence the quantity and quality of caffeine from tea [3] (Mäkelä, 2012). The annual average of temperatures in Rwanda is divided into four main groups: Bugarama valley ranged from 23-24°C, the eastern part lying on 20-21°C, and in higher elevations of the central plateau regions, where the temperatures are so chilly in range of 17.5-19°C and hills land's temperatures are under 17°C; Rwandan temperature and weather vary a little bit throughout the year [16].

The rainfall levels and climate changes in the region are very important to the quality and amount of caffeine content from tea and synchronized with the studies on climatic zones, have demonstrated the greater impacts of seasons on tea quality like having low caffeine depends on dry and rainy tea harvested seasons [17]. The range of rainfall annually is abundant between 1200 mm to 2700 mm as well as distributed, and reliable; the tea grown under these conditions has a quality and a good character of flavor. In Rwanda, the average annual rainfall is categorized into tree main groups that are: Crest Zaire-Nile (Rubaya, Nyabihu, Karongi, Gisakura, Gisovu, Pfunda, and Shagasha) has mean annual rainfall of 1401-1700mm; Highlands of Buberuka (Sorwathe, Mulindi) has 1061-1400mm; Granite ridge (Kitabi, Mata) also in range of 1141-1500mm [16]. Rainfall is one of the best factors that contribute to the high caffeine content in Rwandan tea.

Additionally, there are some factors that contribute to the variation amount of caffeine content in tea from different tea growing areas of Rwanda. The utilization of fertilizers compounds to some reduced total fertility rate of soil has a great influence on the amount of caffeine. Some of the farmers do not recover the price of organic fertilizers compounds whereas other farmers don't have the knowledge about how to use and the importance of fertilizers, nearby natural habitat, this can make a difference of caffeine content in tea from the same region (for example some from hills or marshes regions) [18]. The soil water deficit is one of the main climatic variables that influence the growth of the tea plant and water stress results in an accumulation of phenolic compounds after a period followed by a decline of them under prolonged water stress [19].

Based on the results have been found in our study and the factors which affect caffeine including Rwandan climate, abundant rainfall, and altitude above sea level. Rwandan tea growing region can be subdivided into two groups. The first is the tea planted on hillsides at high altitude (1900-2500) m, the second is tea planted on drained marshlands at an altitude ranged from 1550 m to 1800 m [14].

Studies have been also shown that the soil composition of some regions belongs to the sedimentary rock that has great change of caffeine concentration and quality [18]. The hilly tea growing regions are those which containing a high amount of caffeine content. Those hills regions have abundant rainfall, low temperature, high humidity, and acidic soils vary between pH 4.5 to 5. Where, the marshes regions have a high temperature, low humidity, soil pH of 5 to 5.5 and rainfall is low [20]. The result has shown that marshes tea growing regions have lower caffeine content than the hills tea growing.

The caffeine amount variation is due to the different factors that contribute to the tea growth as discussed above, and it depends on how the tea is plucked, processed, and brewed or stored, even if the tea growing region is the same [3]. However, Shagasha (1874 m), Gisakura (1931 m), Mata (2168 m), Mulindi (1880 m) and Pfunda (1695 m) are marshes tea growing regions, our results have shown that Shagasha tea growing region has the lowest caffeine content owing to its location within some of the factors that contribute to the decreasing of amount of caffeine content in tea plant.

Although the Rwanda has the favorable conditions the amount of caffeine content in tea from tea growing regions of Rwanda is low (802.927ppm) compared to tea from other regions around the world. Ethiopian tea has 20800 ppm [21], Kenyan black tea 23600 ppm and Chai maramoja tea from 73600 ppm [22], Zimbabwean Taganda black tea 17000 ppm [23]. It has high caffeine content than Sudanese black tea 473 ppm [24], assessed on mentioned factors that contribute to the high caffeine content in tea.

## 4. Conclusion

In this study, the caffeine content from different tea growing regions was done using HPLC method; this method was confirmed to be sensitive, precise, and accurate. According to the results found from the experiment done for the tea from different growing regions, the order of caffeine quantity was: Rubaya, Nyabihu, Karongi, Mata, Gisakura, Gisovu, Sorwathe, Kitabi, Mulindi, Pfunda, Shagasha respectively.

Further research on other important components such as polyphenols, flavonoids, catechins, and tannins could be carried out. It could be interesting to find out how the different seasons influence the caffeine content in the different regions.

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