Extraction and Characterization of Caffeine: A Biochemical Compound Contained In Some Locally Consumed Tea Leaves (*Camellia Sinensis*)

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Abstract: - **This research work involved extraction and characterization of caffeine from a variety of tea leaves locally consumed, using liquid-liquid extraction method. The extraction process involved the following steps; the tea leaves were steeped/boiled at 75°c, followed by evaporation, decantation, filtration and recrystallization. The pure caffeine crystals yield from 30g of each of the four different tea leaves studied are: Chinese refined green tea 46mg, Mambila beverage tea type II 12mg, while Chinese green tea and Mambila beverage tea type I each has a value of 7mg. Literature studies indicates that, caffeine content of tea leaves differ with level of maturity and processing method employed on the tea leaves. The high caffeine content in CRGT makes it popular and most patronized by consumers. The methods applied for purity analysis on the caffeine crystals include; melting point determination and infrared spectroscopy (FTIR). The caffeine extracted boils at 237°c and the absorption bands generated from the sample caffeine is similar to those reported in literature.**

Keywords: **caffeine intake, psychological effects, refine green tea, diuresis, and infrared spectrophotometer**

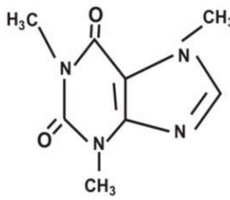
I. INTRODUCTION

Caffeine is an alkaloid found naturally in tea and coffee plants which acts as a stimulant of the central nervous system in humans, having the effect of temporarily warding off drowsiness and restoring alertness (Roberts and Barone, 1996). Other sources of caffeine include, cocoa beans, kola nuts, guarana berries, yerba mate and yaupan holly. Indeed it is found naturally in leaves seeds or fruits of many plant species. Caffeine's popularity as a natural stimulant is unparalleled. An estimated 80% of the world's population enjoys a caffeinated product daily. The caffeine content in a beverage varies depending on the ingredients and how the drink is prepared (Mumin *et al.*, 2006). Major regulatory bodies such as U.S. Department of Agriculture (USDA) and European Food Safety Authority (EFSA) define a safe caffeine intake as up to 300-400 mg per day for a healthy adult with no medical issues (Richling *et al.,* 2014). Due to its stimulating effects, it has been known to enhance alertness and focus, improved athletic performance, elevated mood and increased metabolism (Islam *et al.,* 2002; Thomas, 2008, Kevin, 2010 and Drug bank, 2013). When consumed in large doses, caffeine has been associated with anxiety, restlessness, and difficulty in sleeping. Some studies suggested that, drinking caffeine regularly even in moderate amounts; can cause chronic headaches and migraines (Gebely, 2017). Mumin *et al.,* 2006, reported that; Every time we drink tea, coffee, cocoa, chocolate or cola, we are giving our body a hit of caffeine. Alcohol and nicotine, along with caffeine are the three most widely used mood-affecting drugs in the world. The effects of caffeine on human being depend on concentrations. Consuming high dosage of this compound causes various physiological and psychological effects which include stimulation of the central nervous system, gastric acid secretion and diuresis (Zhang *et al*., 2005 and Yukawa, 2004).Lindsey, (2005) and Belay, (2011) reported that; caffeine has a tendency of rapidly and completely absorbed from gastrointestinal tract within very short period of time and get distributed in the body. It is not removed from the circulation until metabolized initially into paraxanthine and thyobromine then into derivative of uric acid and diaminourcil, which is eventually removed from the circulation. It is reported that, the plasma half-life of caffeine in man, that is; the time required for its level to be diminished by 50% via biotransformation and excretion is 5 to 6 hours (Lindsey and Kervigan, 2005).It should be noted that, when the peak of plasma level of caffeine concentration is 15 to 30M, effects such as mild anxiety, respiratory stimulation, cardiovascular effects, diuresis and increase in gastric secretion would be noticeable. When the levels are above 150M, a symptom of acute toxicity may appear, which include severe restlessness, over excitement, muscular tension, twitching and cardiovascular disturbance (Roberts, 1996, Aragao *et al.,* 2005 and Betty, 2017).

Chemical Structure and Properties of Caffeine

Some properties of caffeine are; pure anhydrous caffeine taste bitter, whitish in colour and odorless powder with melting point 235—237^{O}C (Royal society of chemistry, 2017). Caffeine is moderately soluble in water at ambient temperature (2g/100ml), but very soluble in boiling water (66g/100ml) with a chemical formula $C_8H_{10}N_4O_2$and chemical structure as shown in Figure 1. It is slightly soluble in petroleum ether and Benzene but moderately soluble in alcohols and acetone solvents. Pure caffeine sublimes at

178^{O}C and is weakly basic with a pH range of 6.5 to 10.4 requiring strong acid to protonate it (Vallambroso, 2006; and Belay, 2011).



Caffeine

Figure 1. Chemical structure of caffeine

Study has shown that, caffeine contents of tea leaves and coffee beans vary widely with differences in species. Within identified species, there is a wide range of amounts of caffeine content (Silvarolla and Fazuoli, 2004).

Table 1. Caffeine levels of some tea varieties and food products

Type of tea/ food products	Caffeine level
Black tea	3.57%
Green tea	3.11%
Earl grey tea	4.27%
chocolate	0.074%
Soft drink	0.02%

Source: Moroydor *et al*., 2013 and Pradeep, *et al*., 2015

The effects of environment such as local climate (humidity/ sunlight) soil and agricultural factors may be less important than generic variation in the control of caffeine contents in both tea leaves and coffee beans (Belay, 2011).This study aims at determining caffeine contents from various tea leaves locally consumed as beverage using liquid-liquid extraction method. A comparative analysis of the caffeine levels will also be investigated.

II. MATERIALS AND METHODS

Collection of samples for analysis

Tea leaves of different types and colors were bought from Damaturu main market. Each type was collected and kept in a separate clean, dry polythene bag and transported to the laboratory for further treatment. The tea leaves types used for this research were given the following codes for easy identification for the purpose of this work. The reddish brown type is Mambila beverage tea type I (MBT I), Appendix IA; the dark brown type is Mambila beverage tea type II (MBT II), Appendix IIA; the green tea; which is Chinese green tea (CGT), Appendix IB and the Chinese refined green tea (CRGT), Appendix IIB. Each of these tea types constitute a sample.

Materials required

Erlenmeyer flask, separating funnels, Whatman no.1 filter papers, heating mantle, crucible dish, melting point testing machine (SMP-10), Infrared spectrophotometer(M530), beakers, calcium carbonate, anhydrous sodium sulphate, dichloromethane, 30g of each tea leave type and distilled water.

Caffeine extraction and analysis

Liquid-liquid extraction technique was used for the extraction of caffeine from each tea leave type as reported by Pradeep, 2015 and Andra & Wilson, 2013. The analysis of crude caffeine recovered was carried out using FTIR, synthesis of caffeine salicylate from the recovered caffeine and melting point determination as reported by Williams and Katherine, 2011; Rachel *et al.,*2003, Komes *et al.*, 2009and Pradeep, 2015.

Percent recovery of crude caffeine (%)

$$= \frac{weight\ of\ caffeine\ recovered\ (g)}{weight\ of\ tea\ \ sample\ (g)}\ x\ 100$$

III. RESULTS AND DISCUSSION

Fine caffeine crystals were extracted from tea leaves of various sources using dichloromethane as a solvent for the extraction. Table 2 shows the quantity of caffeine extracted from a variety of tea leaves both in milligrams and percentage as well as that of pure caffeine tablet for comparison. The Chinese refined green tea has the highest amount of caffeine, 46.0mg (0.153%); followed by Mambila beverage tea type II with 12.0mg (0.04%). The Chinese green tea type and Mambila beverage tea type I surprisingly recorded equal amounts of caffeine despite coming from different sources. Each has a caffeine content of 7.0mg which is equivalent to 0.023% of the mass used. The results suggest that, Chinese green tea and Mambila beverage tea type I would have a mild stimulating effect compared to the other two varieties of tea leaves mentioned. The values obtained for the duo is far below what was reported for green and black tea leaves by Moroydor *et al.*, 2013; but was slightly higher than the value reported for soft drinks by Pradeep, 2015.

Table 2: Quantity of caffeine recovered from different tea leaves

Sample type	Amount of caffeine recovered(mg)	Caffeine percentage (%)
MBT-I	7.00	0.023
MBT-II	12.0	0.040
CGT	7.00	0.023
CRGT	46.0	0.153

The yield of caffeine from the liquid-liquid extraction technique is good which shows the efficiency of the method used. However, Chinese refined green tea would have strong stimulating effect than

Table 3: Melting point of isolated caffeine and caffeine salicylate

Sample	Melting point (^{O}C)
Caffeine crystal	237.0
Caffeine salicylate	135.0

Mambila beverage tea type II as indicated by the results. It's not a surprise then that, the former variety is often more patronized by local drivers and keke nape riders in the community. It is this hidden strong stimulating effect they go after. Caffeine content of beverages from various source varies with soil conditions, climate and generic variations as reported by Belay, 2011 and Match-tea.com, 2015. The significant differences observed in caffeine content of the various tea leaves studied could be attributed to these factors.

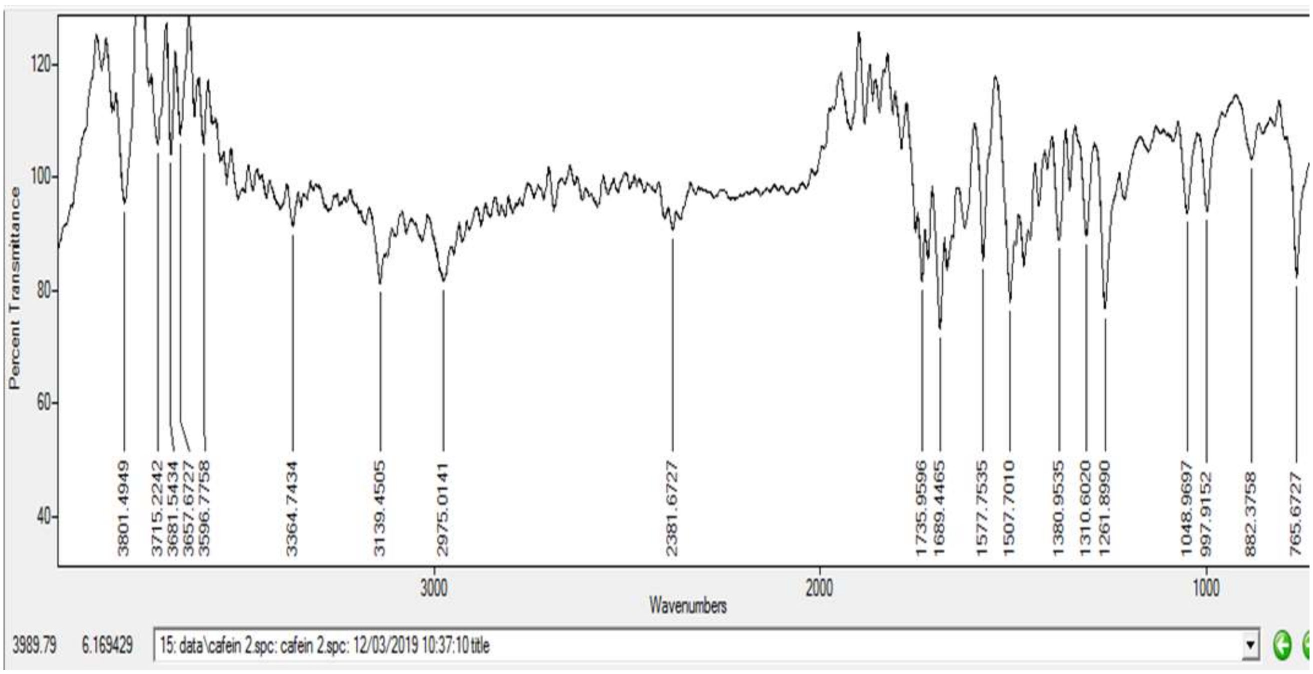


Figure 2: Fourier transform infrared spectroscopy spectrum of recovered caffeine sample

Among the tea samples studied, Mambila tea type I and Chinese green tea has low caffeine content; while the Chinese refined green tea has the highest caffeine content. Table 3 shows the melting points of pure white crystalline caffeine as isolated from different tea leave sources and its derived compound caffeine salicylate. The latter was found to melt at $237^{O}C$ while the former melts at $135^{o}c$ .It is worth noting that, the amount of caffeine recovered from each of these tea leaves could be more than the values obtained here; as the experimental procedure is characterized by series of crystallization, washing and recrystallization processes.

Table 4: characteristic Infrared-Absorption bands of isolated caffeine sample

S/No.	Bond type	Frequency (cm-1)
1.	C-H	2985.01
2.	C=C	1577.75
3.	C=O	1735.95
4.	C-N	1261.89
5.	C=N	1689.44

There may be significant loss of product during these procedures. Table 4 shows the frequency of IR absorption bands in each bond of caffeine molecule. The spectrum of caffeine depicted in Figure 2, was generated using FTIR spectroscopy. The Infrared spectrum indicates a high purity caffeine was produced.

IV. CONCLUSION

Varying amount of caffeine was obtained from a variety of tea leaves using liquid-liquid extraction method. Chinese refined green tea produced the highest quantity (46.0mg) of purified caffeine crystals, followed by Mambila beverage tea type II (12.0mg). While Mambila tea type I and Chinese green tea recorded the lowest values of 7.0mg each. The recovered pure caffeine crystals were characterized by determining IR spectrum and melting point values using FTIR and melting point apparatus. The results obtained agreed with that of reported literature values.

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APPENDICES

Appendix 1A: Mambila beverage tea type I.

Appendix IIA: Mambila beverage tea type II.

Appendix IB: Chinese green tea.

Appendix IIB: Chinese refined green tea.