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Assessment of Caffeine and Nicotine Contents in Tea Consumed in Yobe State, Nigeria using HPLC and GC-MS Methods

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Abstract

The investigation of caffeine and nicotine contents in tea samples sold in Yobe State Nigeria was carried using simplified HPLC and GC-MS analytical methods. The physical characteristics (pH, colour and taste) of the tea infusions were also analysed. The percentage composition of caffeine analysed by HPLC shows that the highest caffeine extract was obtained by sample B (1.02 %) while the lowest caffeine content was obtained by sample I (0.54 %). The study also shows that the highest nicotine concentration was exhibited by sample G (0.43) whereas, the lowest nicotine concentration of 0.06 was obtained by samples C. The nicotine content detected in the only green tea sample (A), was 0.15 mg/g. The caffeine compositions vary due to the combination of genetic make-up, environmental conditions, processing factors as well as brewing practices. The concentrations of nicotine in all the samples are below the maximum permissible limit of 0.5 mg/kg.

Keywords: Tea, Caffeine, Nicotine, HPLC, GCMS

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1. Introduction

Tea is among the popular beverages consumed globally (Cheng, 2004). Tea is produced from the soft shoots of the Camellia sinensis (L.) (Carloni *et al.*, 2013; Karak *et al.*, 2014; Gutman & Ryu, 1996). Based on its processing method, Tea is marketed as fermented (Black tea), unfermented (green tea) and partially-fermented (oolong tea) (Abidi *et al.*, 2020; Chen *et al.*, 2011) Tea is widely consumed for its therapeutic benefits, palatability and aroma (Cabrera, Artacho, & Gimenez, 2006). Some of the health benefits of tea consumption include lessening the risk of cardiovascular diseases, diabetes, cancer, obesity and inflammation (Jiang *et al.*, 2019; Karak & Bhagat, 2010; Liu *et al.*, 2020; Massounga Bora

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et al., 2018; Rho *et al.*, 2019) and increasing neuroprotective activities (Rho *et al.*, 2019).

Caffeine (1, 3, 7-trimethylxanthine), popularly consumed as stimulant is commonly obtained from tea leaves and other sources such as coffee and cola (Francis, 1999; Abidi *et al.*, 2020). Caffeine is pharmacologically active, it stimulates the central nervous system, mild diuretic, a natural pesticide, increases blood pressure, increases heart rate, stimulates gastric motility, algicidal, bactericidal (Jinno, 1996; Eaton, 2010). It also induces increased wakefulness, accelerates flow of thought, increases focus, and enhances general body coordination (Sethuraman *et al.*, 2013). Additionally, caffeine is used as an additive in pharmaceutical preparations along with acetylsalicylic acid, ascorbic acid, codeine, and paracetamol and other analgesic and antipyretic preparations (Hamad, 2010). It is also very widely consumed through a wide range of dietary products, like cocoa beverages, energy drinks, soft drinks etc. (Da Silva, 2011). Concentration of caffeine varies in specific plants species due to the differences in variety, change in climate and horticultural techniques. In tea, highest caffeine content is found in the youngest leaves, although, this can be affected by the processing conditions (Hecimovic *et al.*, 2011).

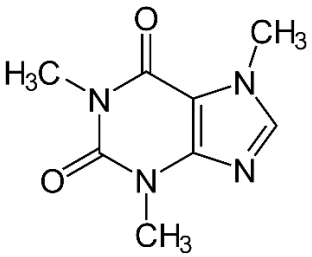
Caffeine does not stay for long in the brain and has less tendency to affect the major brain functions. However, frequent exposure to caffeine results to developing a tolerance to it. Too much caffeine consumption can lead to caffeine intoxication, which is characterized by nervousness, irritability, anxiety, tremulousness, muscle twitching (hyperreflexia), insomnia, headaches, respiratory alkalosis, and heart palpitations (Cappelletti *et al.*, 2015). In pregnant women, too much caffeine increases the risk of miscarriage, difficulty in birth and reduced baby weight (Sengpiel *et al.*, 2013). Studies have revealed that osteoporosis, high blood pressure, heart disease, heart burn, ulcers, severe insomnia and infertility can occur as a result of high caffeine consumption (Kim, 2014). Caffeine has diuretic effect which causes excretion of fluid through the kidney, which may lead to dehydration. This occurs when sufficient dose of caffeine is administered to people with caffeine intolerance (Spriet, 1995).

Nicotine is an alkaloid that is also obtainable from plant sources. Nicotine can be found in twelve (12) plant families among which are tobacco plants and tea leaves (Leete, 1983). Nicotine is well known to have serious systemic side effects in addition to being highly addictive. Researches have shown that nicotine is a human carcinogen affecting the lungs (Wassenaar *et al.*, 2013), Pancrease (Crowley-Weber *et al.*, 2003) and breast (Chen *et al.*, 2011). Nicotine has been formerly used as pesticide due to its strong insecticidal effect. This has been banned by the European Union in 2010 due to its toxicity. The lethal dose of nicotine is estimated to be 30 – 60 mg (0.5 – 1.0 mg/kg body weight) for adults and 10 mg for infants. Consequently, the maximum nicotine residue in food has been set to be 0.01 mg/kg.

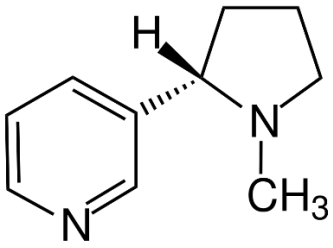
Many methods have been reported for the analysis of caffeine contents in tea. These include capillary electrophoresis (CE) (Kotani *et al.*, 2007), thin-layer chromatography (Vovk *et al.*, 2005), near infrared

spectrometry (Dou *et al.*, 2005) and Fourier transform near infrared spectrometry (Sinija & Mishra, 2009). On the other hand, the procedures for the determination of traces of nicotine include radioimmunoassay (RIA) (Castro & Monji, 1986), gas chromatography (GC) with nitrogen-phosphorus detector (NPD) (Sheen, 1988), GC with mass spectrometric (MS) detection (Siegmund *et al.*, 1999), GCMS/MS (Muller *et al.*, 2011), and liquid chromatography with mass spectrometric detection (LC-MS/MS) with solid phase extraction concentration (Liu *et al.*, 2013). The richness of tea extract in biomolecules and minerals leads it to be used as corrosion inhibitor of metals as mild steel (Dao *et al.*, 2023; Salghi *et al.*, 2017). Caffeine and nicotine served as good inhibitors of steel and copper in aggressive media (Messaoudi *et al.*, 2020; Elmsellem *et al.*, 2013; Espinoza-Vázquez and Rodríguez-Gómez *et al.*, 2016).

The present work is aimed at using a simple HPLC and GCMS procedures for determination of caffeine and nicotine in aqueous tea extracts from 10 commercial tea samples available in the local markets of Yobe State, Nigeria to ascertain the caffeine and nicotine contents in the infusions of the tea samples.



Caffeine molecule



Nicotine Molecule

2. Materials and Methods

2.1. Reagents

standards, ethanol, dichloromethane and other chemicals were purchased from Merck (Darmstadt, Germany). The stock solution of caffeine was prepared in methanol (1000 mg L^{-1}) and stored. Diluted standard solutions were prepared daily by diluting the stock solutions. Deionized water was used throughout.

2.2. Equipment/analytical tools

The analytical procedure was performed using a HPLC and GC-MS apparatus.

2.3. Tea sample extraction

Different tea brands were purchased from a local market at Damaturu town and used in this study. Ten (10) Different varieties of tea bags were boiled with 100 ml of distilled water for 10 minutes. This was allowed to cool for 5 minutes and then decanted into another beaker. Dichloromethane (15ml) was added

to the solution followed by 1% sodium sulphide; the mixture was shaken and transferred into separatory funnel. After a complete separation, the lower layer was evaporated to get the white crystals of caffeine. The extracted caffeine was analysed using HPLC and GC-MS.

2.4. Analysis of physical characteristics

Twenty grams (25 g) of each tea sample was soaked in 100 ml of distilled water and boiled. The solution was allowed to cool down to room temperature then filtered. The pH of the filtrates were measured using pH meter. Average of replicate (3) reading were recorded. Fresh portions of the filtrates were taken and observed for colour and taste.

2.5. HPLC analysis of caffeine and nicotine

The samples were grinded into fine powder. 1.0 mg was dissolved in water/acetonitrile mixture (90:10) and then votex mixed strongly for 1min. The solution was then filtered through 0.45μm filter paper or centrifuged at 3500rpm for 10minutes. The filtrate/supernatant was analyze using Agilent HPLC model 1200 series (or UV-Vis spectrophotometer) using a caffeine standard.

10μL of the extract was injected into the HPLC where separation was carried out using an RP C18 column (100 mm x 2.1 mm x 5 μm). The mobile phase composed of water: acetonitrile (90:10 *v/v* 0.15 % formic acid) ISO. The mobile phase flow rate was set at 1.50 mL min^{-1} and column temperature was maintained at 35 °C. Corresponding caffeine content was detected at 205 and 272nm while nicotine was detected at 213 and 261nm where the target was identified by retention time matching with the sample standard.

2.6. GCMS Analysis

0.01g of the sample was dissolved in 10mL of dichloromethane solvent, votex mixed strongly for 2 minutes and then centrifuged at 3,000rpm for 10 minutes. The clear supernatant was collected into a TSP microvial for GCMS analysis. 1 μL of the sample was injected into the GC. The sample was analyzed using Agilent GC (7890B), equipped with 30 m x 250 μm x 0.25 μm Column; coupled with Agilent MSD (5977A MSD). The carrier gas helium was set at flow rate of 1 ml/min. The GC oven was initially set at 70 °C, for 3 min then ramped at 10 °C/min to 280 °C and held for 9 min. Equilibration time, MSD Transfer Line, MS Source and MS Quad were set at 230 ^{0}C and 150 ^{0}C respectively. The identification and characterization of chemical compounds in various samples were based on GC retention time. The mass spectra were compared with those of standards available in NIST mass spectrum libraries. The percentage composition of the sample constituents were expressed as a percentage by peak area.

3. Results and Discussions

3.1. Physical characteristics

Table 1 shows the results of the physical characteristics of the tea samples. The pH values show that the solutions of all the tea samples are acidic. The lowest pH value and therefore the most acid was sample G (4.08).

Table 1. Physical characteristics of tea samples

S/N	Name of tea samples	pH	Taste	Colour
1.	Ahmad Green Tea (A)	5.20	Bitter	Light green
2.	Black tea Ahmad Tea (B)	5.84	Strongly better	Dark brown
3.	Glen black tea (C)	4.96	Bitter	Dark brown
4.	Hillway (D)	5.12	Strongly better	Brown
5.	Richmond (E)	4.96	Mild bitter	Brown
6.	Highland tea (F)	4.36	Bitter	Dark brown
7.	Lipton (G)	4.08	Strongly better	Brown
8.	Top tea (H)	4.98	Strongly better	Brown
9.	Akbar (I)	5.08	Bitter	Light brown
10.	Kerkho gold (J)	5.13	Bitter	Dark brown

The highest pH value was obtained by sample B (5.84). The only green tea, sample A, shows a pH value of 5.20. Tea leaves naturally contain various organic acids, including tannic acid, citric acid, and quinic acid. These acids contribute to the overall acidity of the brewed tea. The specific composition and concentration of these acids can vary depending on factors such as the tea plant species, growing conditions, and processing methods (Gondal *et al.*, 2016). The pH level of the tea solution give an indication of its acidity. Generally, tea tends to be slightly acidic, with pH levels typically ranging from around 4 to 6 (Gotti *et al.*, 2009). The tastes of the tea samples ranged from bitter to strongly bitter. Sample E was found to be mildly bitter While samples B, D, and H were strongly bitter. The bitter taste of aqueous tea extracts can be attributed to several compounds present in the tea leaves (Papieva *et al.*, 2011). Most of these compounds are Catechins, Theaflavins and Thearubigins, Caffeine, Tannin and Alkaloids. The variation in the bitterness of tea could be attributed to the type of tea, the brewing temperature and duration, and the specific composition of polyphenols in the tea leaves (Guth & Grosch, 1993). The colours of the tea samples as presented in table 1 ranged from brown to dark brown except for sample A which appears green. The colors of aqueous tea extracts can vary significantly depending on several factors, including the type of tea, the processing method, and the brewing conditions. The dark colours exhibited by the black tea samples could be due to complete oxidation of the tea leaves during processing whereas green tea do not undergo any oxidation (Sultana *et al.*, 2008).

3.2. Caffeine content

Figure 1 shows the percentage caffeine composition in the tea samples analyzed. The highest caffeine extract was obtained by sample B (1.02 %) while the lowest caffeine content was obtained by sample I (0.54 %).

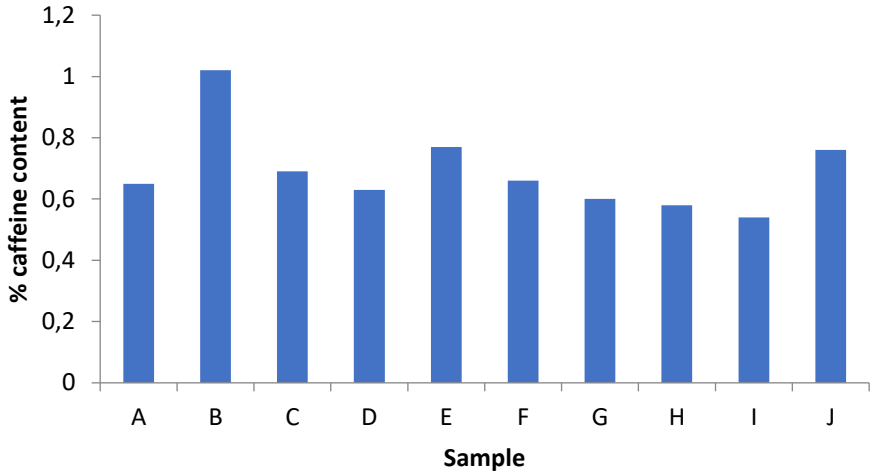


Figure 1. Percentage Caffeine Contents in the Tea Samples

The percentage caffeine composition for the other samples follows the order E (0.77 %) > J (0.76 %) > C (0.69 %) > F (0.66g) > A (0.65 %) > G (0.60 %) > D (0.63 %) > G (0.060 %). The variation in caffeine content in tea leave samples could be as a result of a combination of genetic, environmental, and processing factors, as well as brewing practices (Tritsch *et al.*, 2022).

3.3. Nicotine content

Nicotine contents of tea samples consumed in Yobe state, Nigeria are shown in figure 2. The highest nicotine samples were exhibited by sample E (0.38 mg/g) and G (0.43). The lowest nicotine concentrations of 0.06 and 0.08 were obtained by samples C and B respectively. The nicotine content detected in the only green tea sample, 0.15 mg/g was in sample A. The result indicates significant amounts of nicotine in all the samples. From the result, it is evident that the nicotine content in most of the black tea samples are higher than the amount measured in the green tea sample. This trend corresponds to the average nicotine concentrations reported by Ikka *et al.* (2018). The highest nicotine concentration observed in this study less than amount (0.6 mg/kg) reported by Thrane *et al.* (2015). All the detected levels of nicotine in this study are lower than the maximum allowed level of 0.5 mg/kg (EFSA, 2023). The variation in nicotine levels in the samples could be due to the differences in processing methods and tea varieties. Nicotine concentration increases with increase in the degree of fermentation (Thrane *et al.*, 2015) or the genetic variation in nicotine synthesis in tea plants (Ikka *et al.*, 2018).

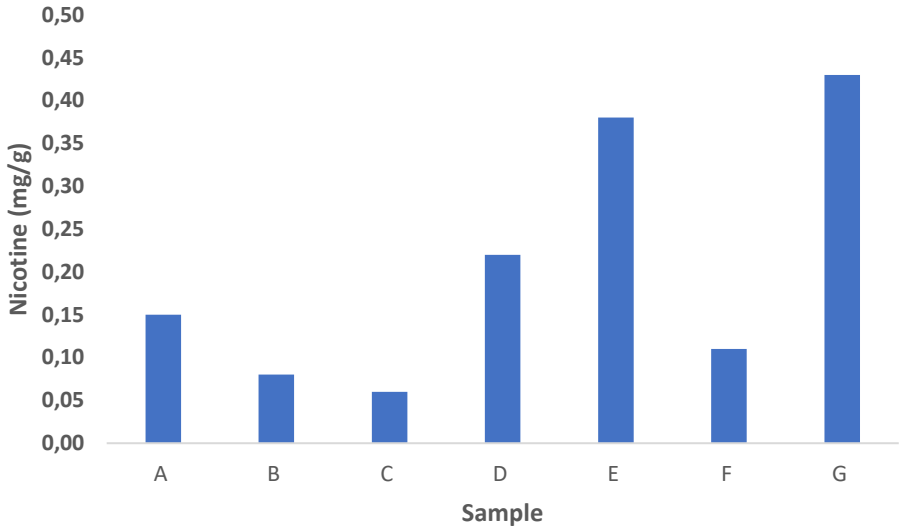


Figure 2. Concentration of Nicotine in Tea Samples

Conclusion

The tea samples have low caffeine contents which vary as a result the differences in their genetic properties, environmental conditions and processing methods. It also noteworthy that the concentrations of nicotine in all the samples are below the EFSA maximum permissible limit. Therefore, the analysed samples are fit for making tea beverages with low caffeine and nicotine toxicity risks.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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