ESI Preprints Not Peer-reviewed

# Theophylline and Caffeine Content in Black Tea Produced in Burundi

*Alice Ndayirukiye, PhD Student*
Doctoral school, University of Burundi, Department of Chemistry, Faculty of Sciences, CRSNE, Bujumbura, Burundi
*Prof. Ferdinand Ndikuryayo*
Institute of Applied Pedagogy, University of Burundi, Bujumbura, Burundi
*Prof. Pierre Claver Mpawenayo*
*Dr. Jeremie Ngezahayo*
*Prof. Godefroid Gahungu*
Department of Chemistry, Faculty of Sciences, CRSNE, University of Burundi, Bujumbura, Burundi

Doi: 10.19044/esipreprint.1.2026.p600

Approved: 22 January 2026
Posted: 24 January 2026



*Cite As:*
Ndayirukiye, A., Ndikuryayo, F., Mpawenayo, P.C., Ngezahayo, J. & Gahungu, G. (2026). *Theophylline and Caffeine Content in Black Tea Produced in Burundi*. ESI Preprints. https://doi.org/10.19044/esipreprint.1.2026.p600

## Abstract

To date, the specific levels of theophylline in Burundian tea remain unknown, and scientific information regarding caffeine content is limited. This study aims to address this gap by determining the content of these methylxanthines in different grades of black tea produced across the six main tea-growing regions of Burundi. Using High-Performance Liquid Chromatography (HPLC) based on the refernce NTC-ISO 20481 method, samples collected from a dry-season production by Office du Thé du Burundi (OTB) and Promotion pour la Théiculture à Mwaro (PROTHEM) were analyzed. The results revealed that the theophylline content ranged from 0.145% to 0.279%, while caffeine levels varied between 1.979% and 3.331%. The significant variations ($p < 0.05$) depending on both the region and the tea grade were also revealed. Generally, the PD and D1 grades were identified as the richest in methylxanthines, with OTB-produced teas showing higher concentrations than those from PROTHEM. These findings confirm that Burundian tea is comparable to premium East African teas and remains within safe limits for daily consumption. The results of this study should help consumers to control the amount of these methylxanthines according to their nutritional needs, while Burundian black tea companies could use them to better promote this product and better position themselves on the international market.

**Keywords:** Burundi tea, Caffeine, Theophylline, HPLC

## Introduction

Tea, derived from the leaves of the plant Camellia *sinensis*, is the most widely consumed beverage in the world after water (Sajilata et al., 2008). It is one of the three most consumed non-alcoholic beverages globally (Rahman et al., 2013), and its consumption has increased considerably over the years. Considered healthier than coffee and cocoa, tea is strongly recommended by the World Health Organization (Dutta, 2017) and remains one of the most affordable beverages available (A. Hicks, 2009).

The health benefits of tea are largely attributed to biologically active ingredients, particularly a subgroup of alkaloids known as methylxanthines (Monteiro et al., 2016). These compounds, which include caffeine, theobromine, and theophylline, are chemically very similar (Figure 1) and are mainly found in foods such as coffee beans, cocoa beans, and tea leaves (Srdjenovic et al., 2008; Rodriguez et al., 2015). They play an important role in human health (Fernández et al., 2002) by sharing stimulatory effects on the central nervous system, as well as on the gastrointestinal, cardiovascular, renal, and respiratory systems (de Sena et al., 2011).

Among these, caffeine was first isolated from tea in the early 1820 (Ashihara et al., 2008). It acts as a mild stimulant and is metabolized in the liver into paraxanthine (80%), theobromine (11%), and theophylline (4%) (Londzin et al., 2021). Theophylline (1,3-dimethylxanthine) has been used for many years as a bronchodilator for chronic respiratory diseases like asthma (Jafari et al., 2011) and recently proposed as an adjuvant in COVID-19 treatment due to its immunomodulatory properties (Montaño et al., 2022). However, side effects such as nausea and dizziness can occur, necessitating dose moderation (B. Zhu et al., 2015).

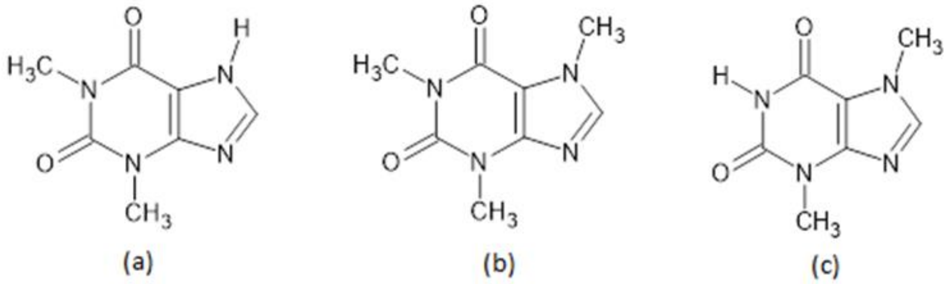


**Figure 1:** Chemical structures of (a) theophylline; (b) caffeine and (c) theobromine

Various factors, including soil, climate, plucking season, and processing methods, lead to variability in the methylxanthine content of tea (Dubuis et al., 2014 ; Turkmen & Velioglu, 2007 ; Baek et al., 2022).

In Burundi, the production and commercialization of tea across different grades are exclusively held by the Office du Thé du Burundi (OTB) and Promotion de la Théiculture à Mwaro (PROTHEM). Although Burundian tea is exported to many other countries, currently, these organizations rely primarily on organoleptic tests to assess black tea quality before marketing. To the best of our knowledge, the specific levels of theophylline in Burundian tea remain unknown, and scientific information regarding caffeine content is limited. While it is established that content varies with the plucking season, determining these levels in dry-season production using High-Performance Liquid Chromatography (HPLC) would provide significant added value.

This work aims to determine the amounts of theophylline and caffeine in different grades of black tea produced in Burundi, considering both the grade and the production region. To achieve these objectives, a modified HPLC method (NTC-ISO 20481, 2008) was employed. This study expects to classify tea-growing regions based on their methylxanthine content. Ultimately, Burundian tea growers, industrialists, and consumers will benefit from understanding the levels of these compounds, allowing for better awareness, regulatory measures, and a clearer positioning of Burundian black tea quality.

## Material and Methods

### *The sampling*

The study was conducted on black tea samples collected from six major tea-growing regions in Burundi. The sampling sites and the corresponding processing factories managed by the OTB and PROTHEM are geographically illustrated in Figure 2.

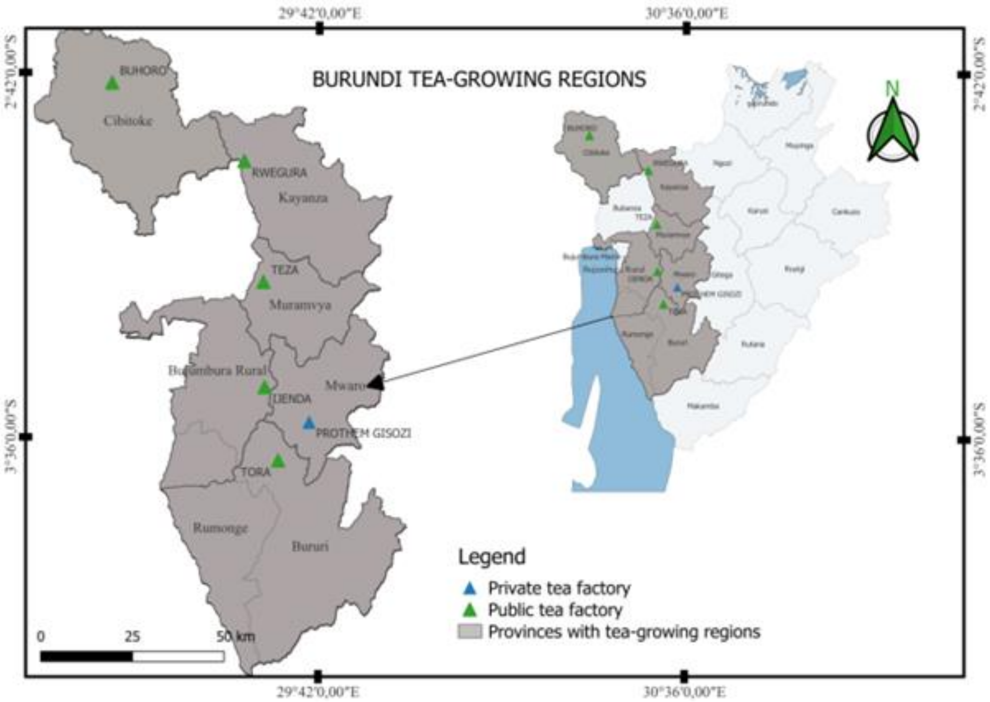


**Figure 2:** The location of tea processing factories in Burundi on map (the green triangles are used for the five OTB factories and the blue triangle for the PROTHEM factory).

**Table 1:** Distribution of samples by processing factories.

| Samples | TORA | TEZA | IJENDA | BUHORO | RWEGURA | GISOZI |
|---|---|---|---|---|---|---|
| PF1 | + | + | + | + | + | + |
| BP1 | + | + | + | + | + | + |
| PD | + | + | + | + | + | + |
| D1 | + | + | + | + | + | + |
| D | - | + | + | + | - | + |
| F1 | - | - | + | + | + | + |
| FS1 | + | - | - | - | - | - |
| FS | + | + | - | + | - | - |
| BMF | + | + | + | + | + | - |

*The + and - signs indicate the availability and non availability of the tea grade at the factory at the time of sampling (PF1 = Pekoe Fannings One, BP1= Broken Pekoe One, PD = Pekoe Dust, D1 = Dust One, D = Dust, F1=Fannings, FS1= Fannings One, BMF = Broken Mixed Fung).*

A total of 41 samples were collected during the dry season production period, specifically between June and August 2022. The distribution of black tea grades varied based on factory availability at the time of sampling. The detailed distribution of samples across regions and grades is summarized in Table 1.

***Choice of method***

The selection of High-Performance Liquid Chromatography (HPLC) was predicated on the requirement for a highly sensitive analytical method capable of simultaneously quantifying caffeine and theophylline, given that the latter typically occurs in trace amounts in tea (Baek et al., 2022).

***Chemicals and reagents***

The analytical standards used were pure theophylline (purity > 99%, SIGMA) and pure caffeine (Merck 2584, Germany). Reagents included analytical grade methanol and glacial acetic acid. Ultrapure water was obtained using a Puranity PU 15 UV/UF+ system.

***Instrumentation and HPLC conditions***

General laboratory equipment used for sample preparation included an analytical balance (SARTORIUS, QUINTIX35-1S, ®), a heated magnetic stirrer, centrifuge tubes, and a centrifuge (Eppendorf 5810R). Chromatographic analyses were conducted using an Agilent 1260 Infinity II UV HPLC system equipped with a Poroshell 120 EC-C18 column (4.6 times 100 mm) for separation. The mobile phase consisted of a mixture of water, methanol, and glacial acetic acid in a volumetric ratio of 79:20:1 (v/v/v), which was degassed in a Bransonic CPX3800 (CPX-952-818R) ultrasonic bath for 15 minutes prior to use. The optimized operating conditions included a flow rate of 0.9 mL/min, an injection volume of 10 muL, and a column temperature maintained at 40°C. Detection was performed via UV absorption at a wavelength of 272 nm, with a total run time set to 8 minutes per injection.

***Preparation of standard solutions***

Stock standard solutions (1000 ppm) of theophylline and caffeine were prepared by dissolving 0.10030 g and 0.10067 g of the respective pure standards in 100 mL of ultrapure water. From these stock solutions, a series of five diluted working standards were prepared to establish calibration curves Table S1, Supporting Information (SI). The linearity of the method was verified by plotting peak areas against concentrations (Figure S1 and Table S2, SI).

***Preparation of samples***

The extraction protocol was based on the ISO method for caffeine determination (NTC-ISO 20481, 2008), with modification to the extraction time informed by existing literature (M. B. Hicks et al., 1996 ; López-Martínez et al., 2003a ; X. Zhu et al., 2004). To optimize the solid-liquid extraction time for simultaneous determination, 5 g (± 0.01 mg) of a tea sample (Grade D1) and 5 g of magnesium oxide were added to 500 mL of distilled water in a flask. Three independent extraction runs were subsequently conducted under boiling conditions for durations of 30, 45, and 60 minutes, respectively. Upon completion, the solutions were cooled to room temperature, and a 50 mL aliquot from each run was centrifuged at 1510 rpm for 15 minutes. The resulting supernatant was filtered through a 0.20 µm microfilter into a vial for HPLC analysis, and the results of this preliminary optimization (Table S3, SI) indicated that 60 minutes was the optimal extraction time.

### *Sample analysis*

Following optimization, all 41 tea samples were prepared using the protocol described above, with the solid-liquid extraction time fixed at one hour. Standards and samples were injected into the HPLC system under the conditions described in the instrumentation and HPLC conditions section. Compounds were identified by retention time and quantified using the best-fit linear regression equation from the calibration curves.

### *Statistical analysis*

Statistical processing was carried out using SPSS software (*IBM SPSS Statistics for Windows*, 2020). An Analysis of Variance (ANOVA) was performed to assess the significance of differences between regions and grades. The Duncan multiple range test (Duncan, 1955) was applied to identify homogeneous groups and statistically significant differences ($p < 0.05$).

## Results

### *Calibration and calculation of content*

The linearity of the analytical method was confirmed through calibration curves for both analytes, which are displayed in Figure S1 (Supporting Information). The strong correlation observed allows for the direct determination of concentrations based on peak areas.

The final content of theophylline and caffeine in the tea samples was calculated using the following equation:

$$Content(\%) = \left[ \frac{conc.(mg/L) \times V(L)}{m(g) \times 1000(mg)} \right] \times 100$$

Where:

Conc. is the concentration of theophylline or caffeine derived from the calibration curve (g/L);
V is the volume of water used for the solid-liquid extraction (L);
m is the mass of the tea sample (g).

***Average theophylline content***

The quantitative results for theophylline across the 41 black tea samples are summarized in Table 2. Detailed statistical data, including the results of the Duncan multiple range test for significant differences, are provided in Table S4 (SI). To facilitate a visual comparison of the variations across different regions and grades, the trends in theophylline content are illustrated in Figure 3.

***Average caffeine content***

The average caffeine concentrations determined for the different grades of black tea produced in Burundi are presented in Table 3 and Table S5 (SI). Similar to theophylline, the distribution and specific trends of caffeine content across all tea-growing regions are depicted in Figure 4.

***Comparative analysis of extraction method***

To evaluate the influence of the preparation method, the methylxanthine content obtained via the standard ISO extraction was compared with the infusions prepared using tap water for five minutes. The raw data from these three comparative tests are listed in Table S6 (SI), while the aggregated average values are summarized in Table 4.

**Table 2.** Average theophylline content (% w/w) in different grades of tea from the same region.

| Grade | TORA | TEZA | IJENDA | MABAYI | RWEGURA | GISOZI |
|---|---|---|---|---|---|---|
| PF1 | $0.196\pm0.011^{b}$ | $0.207\pm0.003^{c}$ | $0.205\pm0.007^{bc}$ | $0.203\pm0.003^{d}$ | $0.188\pm0.003^{c}$ | $0.145\pm0.009^{c}$ |
| BP1 | $0.201\pm0.006^{b}$ | $0.204\pm0.005^{c}$ | $0.189\pm0.001^{d}$ | $0.227\pm0.003^{d}$ | $0.206\pm0.013^{b}$ | $0.165\pm0.003^{ab}$ |
| PD | $0.202\pm0.003^{ab}$ | $0.230\pm0.011^{ab}$ | $0.214\pm0.013^{ab}$ | $0.279\pm0.017^{a}$ | $0.238\pm0.014^{a}$ | $0.169\pm0.009^{ab}$ |
| D1 | $0.216\pm0.002^{a}$ | $0.220\pm0.002^{b}$ | $0.204\pm0.003^{bc}$ | $0.219\pm0.021^{cd}$ | $0.233\pm0.004^{a}$ | $0.177\pm0.004^{a}$ |
| D | - | $0.220\pm0.011^{b}$ | $0.199\pm0.003^{cd}$ | $0.257\pm0.010^{b}$ | - | $0.173\pm0.003^{ab}$ |
| F1 | - | - | $0.206\pm0.004^{bc}$ | $0.220\pm0.007^{cd}$ | $0.203\pm0.004^{b}$ | $0.161\pm0.009^{b}$ |
| FS | $0.198\pm0.003^{b}$ | $0.241\pm0.006^{a}$ | - | $0.231\pm0.002^{c}$ | - | - |
| FS1 | 0.213±0.001* | - | - | - | - | - |
| BMF | $0.212\pm0.006^{a}$ | $0.205\pm0.001^{c}$ | $0.218\pm0.001^{a}$ | $0.170\pm0.002^{e}$ | $0.174\pm0.003^{c}$ | - |
| P-Value | 0.002 | 0.000 | 0.001 | 0.000 | 0.000 | 0.001 |

The sign - indicates that the grade is not produced. In the same column, (a-e)the same superscripts for grades from the same region indicate that theophylline contents are statistically identical. *No comparison.

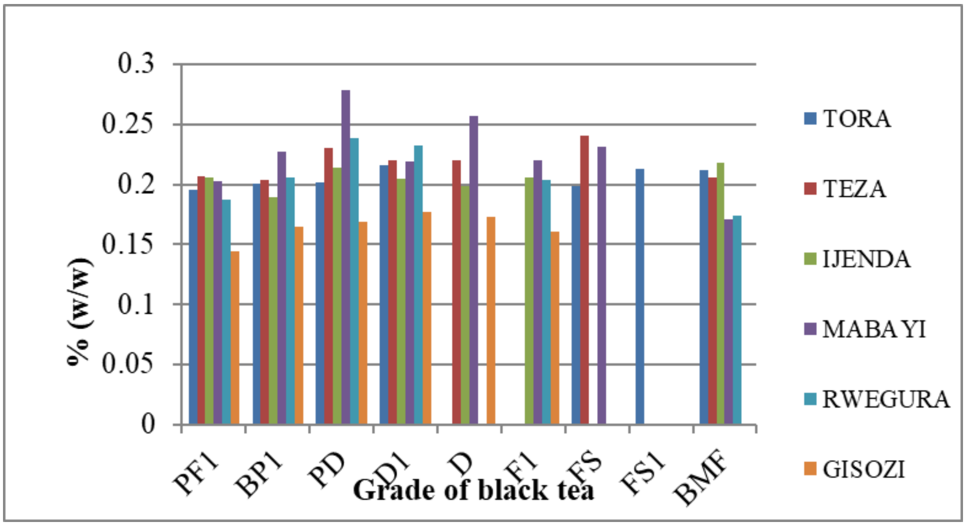


**Figure 3:** Theophilline content as a function of grade of black tea from the six regions.

**Table 3:** Average caffeine content (% w/w) of different grades of tea from the same region.

| Grade | TORA | TEZA | IJENDA | MABAYI | RWEGURA | GISOZI |
|---|---|---|---|---|---|---|
| PF1 | 2.898±0.022[c] | 2.926±0.012[ab] | 2.627±0.018[d] | 3.004±0.026[d] | 2.397±0.017[e] | 2.528±0.021[bc] |
| BP1 | 2.944±0.020[b] | 2.825±0.031[bc] | 2.687±0.018[c] | 2.923±0.039[e] | 2.443±0.023[d] | 2.509±0.021[c] |
| PD | 2.527±0.018[f] | 2.822±0.024[bc] | 2.756±0.021[b] | 3.331±0.055[a] | 3.213±0.034[a] | 2.703±0.023[a] |
| D1 | 2.706±0.018[e] | 2.987±0.025[a] | 2.881±0.016[a] | 3.126±0.015[b] | 2.724±0.019[c] | 2.705±0.021[a] |
| D | - | 2.947±0.152[a] | 2.853±0.015[a] | 3.059±0.017[c] | - | 2.583±0.030[b] |
| F1 | - | - | 2.672±0.019[c] | 2.774±0.026[f] | 3.006±0.020[b] | 2.460±0.014[d] |
| FS | 2.817±0.018[d] | 2.766±0.015[c] | - | 2.729±0.016[f] | - | - |
| FS1 | 3.068±0.021* | - | - | - | - | - |
| BMF | 2.921±0.018[bc] | 2.293±0.023[d] | 2.586±0.023[e] | 1.979±0.015[g] | 2.232±0.018[f] | - |
| P-Value | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |

The sign - indicates that the grade is not produced. (a-g) in the same column, the same superscript (a-g) for grades from the same region indicate that theophylline contents are statistically identical. *No comparison.

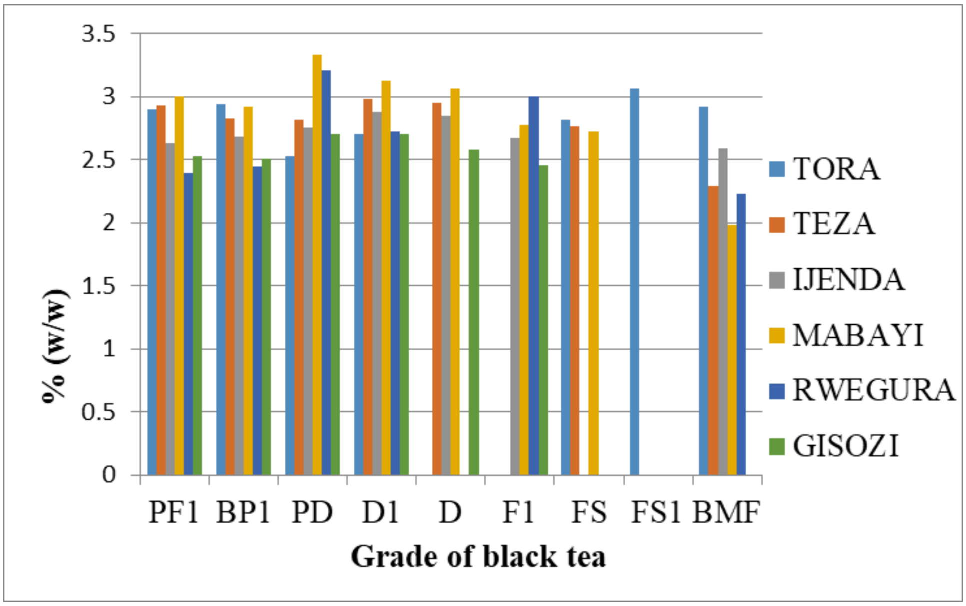


**Figure 4:** Caffeine content as a function of grade of black tea from the six regions.

**Table 4:** Average value of theophylline and caffeine content (% w/w) determined from tea infusions*.

| Sample | Theophylline | Caffeine |
|---|---|---|
| PD MABAYI | 0.307±0.008 (0.279±0.017)[a] | 3.034±0.017 (3.331±0.055)[b] |
| PF1 GISOZI | 0.211±0.004 (0.145±0.009)[a] | 2.334±0.001 (2.528±0.021)[b] |

* Prepared using tap water infusion. a ISO 20481 based theophylline content value from this study and b ISO 20481 based caffeine content value from this study.

## Discussion

### *Method validation and linearity*

The reliability of the analytical method was established prior to sample analysis. The calibration curves displayed in Figure S1 (SI) demonstrate excellent linearity, implying a strong correlation between peak area and concentration for the standard solutions ($R^2$ = 0.99996 for theophylline and $R^2$ = 0.99998 for caffeine).

Regarding extraction efficiency, the results of the theophylline extraction test yielded contents of 0.270%, 0.272%, and 0.282% for 30, 45, and 60 minutes, respectively. This aligns with previous studies indicating that the maximum extraction time of caffeine by water is one hour (Mourya et al., 2019). These results confirm that xanthines can be extracted and assayed simultaneously, in accordance with established protocols (de Sena et al., 2011 ; Horžić et al., 2009).

***Theophylline content and variation***

The results summarized in Tables 2 and S4 show that the average theophylline content of different grades of black tea produced in all tea-growing regions varies between 0.145% and 0.279%. Specifically, MABAYI black tea grade PD exhibits the highest theophylline content (0.279 pm 0.017%), while the lowest (0.145 pm 0.009%) is found in GISOZI black tea grade PF1.

Based on the average theophylline content, the classification of tea grades in ascending order for each region is as follows:

TORA: PF1 < FS < BP1 < PD < BMF < FS1 < D1
TEZA: BP1 < BMF < PF1 < D < D1 < PD < FS
IJENDA: BP1 < D < D1 < PF1 < F1 < PD < BMF
MABAYI: BMF < PF1 < D1 < F1 < BP1 < FS < D < PD
RWEGURA: BMF < PF1 < F1 < BP1 < D1 < PD
GISOZI: PF1 < F1 < BP1 < PD < D < D1

Analysis of variance (ANOVA) revealed very significant differences ($p < 0.05$) between the contents of tea grades within the same region and within the same grade across different regions. However, the Duncan post-hoc test indicates that PD and D1 grades have statistically identical contents in all tea-growing regions except for MABAYI.

Overall, as summarized in Figure 3, the so-called "fibre-free first-line" grades found in all regions are generally rich in theophylline. Notably, the BMF grade (fibrous second-line) is also rich in theophylline across all regions. Furthermore, theophylline content of tea grades produced by OTB factories is generally higher than those produced by PROTHEM, with significant statistical differences.

The level of theophylline in Burundian black tea (0.145% to 0.279%) is relatively higher than data available in scientific literature, where reliable ranges are often 0.02–0.04% (Graham, H.N., 1984a ; Szymański et al., 2022) or as low as 0.00248% in South Korean tea (Baek et al., 2022). However, our results are comparable to Kenyan black tea (0.2331%) reported by Szymański et al. (2022). These variations can be ascribed to different manufacturing processes and processing methods (Turkmen & Velioglu, 2007).

***Caffeine content and variation***

As presented in Table 3 and S5 (SI), the average caffeine content varies between 1.979% and 3.331%. MABAYI PD grade black tea showed the highest caffeine content (3.331±0.055%), while the lowest was observed in the same region for BMF grade (1.979±0.015 %).

In general, the average caffeine content of the different grades allows us to establish a classification in ascending order for each of the six tea-growing regions:

TORA: PD < D1 < FS1 < PF1 < BMF < BP1
TEZA: BMF < FS < PD < BP1 < PF1 < D < D1
IJENDA: BMF < PF1 < F1 < BP1 < PD < D < D1
MABAYI: BMF < FS < F1 < BP1 < PF1 < D < D1 < PD
RWEGURA: BMF < PF1 < BP1 < D1 < F1 < PD
GISOZI: F1 < BP1 < PF < PD < D < D1

The results plotted in Figure 4 clearly show that, generally, PD and D1 grades are the richest in caffeine across most regions. The analysis of variance reveals a p-value much lower than 0.05, confirming that caffeine content varies significantly between grades in the same region. Nevertheless, the Duncan test identified some statistically identical contents, such as BP1 and PF1 exclusively in the TEZA and GISOZI regions. Similar to theophylline, OTB tea grades showed higher caffeine levels than PROTHEM grades.

The caffeine results are in line with global literature. Burundian tea levels (1.979%–3.331%) are comparable to teas consumed in Spain (1.8–3%) (Sanchez, 2017) and the UK (2.2–2.8%) (Khokhar & Magnusdottir, 2002) , as well as recent multi-country studies showing ranges of 2.37–3.3% (Szymański et al., 2022).

***Influence of preparation method and health implications***

A comparison was made between the standard ISO 20481 method and a simple infusion (Table 4) using two samples (MABAYI PD and GISOZI PF1). The results show that theophylline content is higher in the infusion (0.211%–0.307%) than in the extract obtained using the ISO method (0.145%–0.279%).

This may be attributable to the nature of the water used. The acid-base properties of theophylline allow better extraction in slightly alkaline tap water compared to neutral distilled water (Bruneton, 2009). Mineral ions ($Ca^{2+}$ and $Mg^{2+}$) in tap water promote the formation of water-soluble complexes by replacing the hydrogen on the N-7 position of the theophylline imidazole ring, whereas caffeine remains stable in pure water (Zareef et al., 2020); (Astill et al., 2001).

Despite the extraction efficiency Table S7 (SI), the amount of theophylline per cup (250 ml) remains low (6.97 mg – 11.93 mg), which is well below the therapeutic doses of 13 mg/kg/24h for adults (Matte et al., 1982).

Regarding caffeine, concentrations range from 38.85 mg/cup for lighter varieties to 58.88 mg/cup for stronger ones. According to the European Food Safety Authority (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA, 2015)According to the EFSA (2015), the safe daily intake for a healthy adult is 400 mg/day. This means consumers can safely enjoy up to 6 cups of the stronger Burundian tea or 10 cups of the lighter varieties daily. This aligns with UK consumption patterns (92–146 mg/day) (Khokhar & Magnusdottir, 2002), suggesting Burundian tea is a safe and flavorful source of methylxanthines.

## Conclusion

This study successfully determined the theophylline and caffeine contents in various grades of black tea produced across the six main tea-growing regions of Burundi. Using a modified HPLC method (NTC-ISO 20481), we demonstrated that methylxanthine levels vary significantly depending on both the geographical region and the grade of tea.

Our findings reveal that Burundian black tea produced in dry season contains theophylline levels ranging from 0.145% to 0.279% and caffeine levels from 1.979% to 3.331%. Generally, the PD and D1 grades were identified as the richest in both compounds across most regions, while significant differences were observed between teas produced by OTB and PROTHEM. From a consumer perspective, we found that infusion with tap water extracts theophylline more efficiently than distilled water, likely due to mineral interactions. Furthermore, despite the robust methylxanthine content, Burundian black tea remains well within safe consumption limits established by the EFSA, allowing healthy adults to consume up to 6 cups of the strongest grades daily.

As a supplement to this study, an investigation was undertaken on other bioactive compounds acting on human health. By taking into account factors such as the geographical location, the picking period, and the soil conditions of Burundi's tea-growing sites, we will be able to quantify these compounds. Further studies could build upon this foundational work by exploring the specific agronomic and technological factors that influence these chemical profiles, ultimately contributing to the optimization of tea quality in Burundi.

## Acknowledgements

Alice Ndayirukiye would like to express her gratitude to the two Burundian tea companies, OTB and PROTHEM, who allowed to obtain the samples unconditionally. To the Burundian Bureau of Standardization and Quality Control for providing access to the chemical analysis laboratory.

**Conflict of Interest:** The authors reported no conflict of interest.

**Data Availability:** All data are included in the content of the paper.

**Funding Statement:** The authors did not obtain any funding for this research.

**References:**

1. Ashihara, H., Sano, H., & Crozier, A. (2008). Caffeine and related purine alkaloids : Biosynthesis, catabolism, function and genetic engineering. *Phytochemistry*, *69*(4), 841-856. https://doi.org/10.1016/j.phytochem.2007.10.029
2. Astill, C., Birch, M. R., Dacombe, C., Humphrey, P. G., & Martin, P. T. (2001). Factors Affecting the Caffeine and Polyphenol Contents of Black and Green Tea Infusions. *Journal of Agricultural and Food Chemistry*, *49*(11), 5340-5347. https://doi.org/10.1021/jf010759+
3. Baek, G.-H., Yang, S.-W., Yun, C.-I., Lee, J.-G., & Kim, Y.-J. (2022). Determination of methylxanthine contents and risk characterisation for various types of tea in Korea. *Food Control*, *132*, 108543. https://doi.org/10.1016/j.foodcont.2021.108543
4. Bruneton, J. (2009). *Pharmacognosie, phytochimie, plantes médicinales* ((4e éd.)). 75008 Paris.
5. de Sena, A. R., de Assis, S. A., & Branco, A. (2011). *Analysis of Theobromine and Related Compounds by Reversed Phase High-Performance Liquid Chromatography with Ultraviolet Detection : An Update (1992–2011)*.
6. Dubuis, E., Wortley, M. A., Grace, M. S., Maher, S. A., Adcock, J. J., Birrell, M. A., & Belvisi, M. G. (2014). Theophylline inhibits the cough reflex through a novel mechanism of action. *Journal of Allergy and Clinical Immunology*, *133*(6), 1588-1598. https://doi.org/10.1016/j.jaci.2013.11.017
7. Duncan, D. B. (1955). *Multiple range and multiple F tests. Biometrics, 11(1), 1-42.* [Logiciel].
8. Dutta, P. (2017, septembre 11). *WHO Encourages Tea Drinking for a New Generation*. World Tea News. https://www.worldteanews.com/Features/who-encourages-tea-drinking-new-generation
9. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). (2015). Scientific Opinion on the safety of caffeine. *EFSA Journal*, *13*(5). https://doi.org/10.2903/j.efsa.2015.4102
10. Fernández, P. L., Pablos, F., Martín, M. J., & González, A. G. (2002). Study of Catechin and Xanthine Tea Profiles as Geographical

Tracers. *Journal of Agricultural and Food Chemistry*, *50*(7), 1833-1839. https://doi.org/10.1021/jf0114435
11. Graham, H.N. (1984a). *Tea : The plant and its manufacture; chemistry and consumption of the beverage. In : Spiller, G.A., ed., The Methylxanthine Beverages and Foods : Chemistry, Consumption and Health Effects, New York, Alan R. Liss, pp. 29–74).*
12. Hicks, A. (2009). *Current Status and Future Development of Global Tea Production and Tea Products**.
13. Hicks, M. B., Hsieh, Y.-H. P., & Bell, L. N. (1996). Tea preparation and its influence on methylxanthine concentration. *Food Research International*, *29*(3-4), 325-330. https://doi.org/10.1016/0963-9969(96)00038-5
14. Horžić, D., Komes, D., Belščak, A., Ganić, K. K., Iveković, D., & Karlović, D. (2009). The composition of polyphenols and methylxanthines in teas and herbal infusions. *Food Chemistry*, *115*(2), 441-448. https://doi.org/10.1016/j.foodchem.2008.12.022
15. *IBM SPSS Statistics for Windows* (Version 27.0). (2020). [Logiciel]. IBM Corp.
16. Jafari, M. T., Rezaei, B., & Javaheri, M. (2011). A new method based on electrospray ionisation ion mobility spectrometry (ESI-IMS) for simultaneous determination of caffeine and theophylline. *Food Chemistry*, *126*(4), 1964-1970. https://doi.org/10.1016/j.foodchem.2010.12.054
17. Khokhar, S., & Magnusdottir, S. G. M. (2002). Total Phenol, Catechin, and Caffeine Contents of Teas Commonly Consumed in the United Kingdom. *Journal of Agricultural and Food Chemistry*, *50*(3), 565-570. https://doi.org/10.1021/jf010153l
18. Londzin, P., Zamora, M., Kąkol, B., Taborek, A., & Folwarczna, J. (2021). Potential of Caffeine in Alzheimer's Disease—A Review of Experimental Studies. *Nutrients*, *13*(2), 537. https://doi.org/10.3390/nu13020537
19. López-Martínez, L., López-de-Alba, P. L., García-Campos, R., & De León-Rodríguez, L. M. (2003). Simultaneous determination of methylxanthines in coffees and teas by UV-Vis spectrophotometry and partial least squares. *Analytica Chimica Acta*, *493*(1), 83-94. https://doi.org/10.1016/S0003-2670(03)00862-6
20. Matte, J., Lapointe, M., & LeBel, M. (1982). Guide posologique de la theophylline et ses sels. *Canadian Family Physician*, *28*, 111-115. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2306292/
21. Montaño, L. M., Sommer, B., Gomez-Verjan, J. C., Morales-Paoli, G. S., Ramírez-Salinas, G. L., Solís-Chagoyán, H., Sanchez-Florentino, Z. A., Calixto, E., Pérez-Figueroa, G. E., Carter, R.,

Jaimez-Melgoza, R., Romero-Martínez, B. S., & Flores-Soto, E. (2022). Theophylline : Old Drug in a New Light, Application in COVID-19 through Computational Studies. *International Journal of Molecular Sciences*, *23*(8), Article 8. https://doi.org/10.3390/ijms23084167
22. Monteiro, J. P., Alves, M. G., Oliveira, P. F., & Silva, B. M. (2016). Structure-Bioactivity Relationships of Methylxanthines : Trying to Make Sense of All the Promises and the Drawbacks. *Molecules*, *21*(8), Article 8. https://doi.org/10.3390/molecules21080974
23. Mourya, S., Bodla, R., Taurean, R., & Sharma, A. (2019). Simultaneous estimation of xanthine alkaloids (Theophylline, Theobromine and Caffeine) by High-Performance Liquid Chromatography. *International Journal of Drug Regulatory Affairs*, *7*(2), 35-41. https://doi.org/10.22270/ijdra.v7i2.315
24. NTC-ISO 20481. (2008). *Instituto Colombiano de Normas Técnicas y Certificación (ICONTEC) 2008 Café y Productos del Café Determinación del Contenido de Cafeína Usando Cromatografía Líquida de Alto Desempeño (HPLC), NTC-ISO 20481 (Colombia : Instituto Colombiano de Normas Técnicas y Certificación).*
25. Rahman, M. M., Kalam, M. A., Salam, M. A., & Rana, M. R. (2013). Aged leaves effect on essential components in green and oolong tea. *International Journal of Agricultural Research, Innovation and Technology*, *3*(2), Article 2. https://doi.org/10.3329/ijarit.v3i2.17845
26. Rodriguez, A., Costa-Bauza, A., Saez-Torres, C., Rodrigo, D., & Grases, F. (2015). HPLC method for urinary theobromine determination : Effect of consumption of cocoa products on theobromine urinary excretion in children. *Clinical Biochemistry*, *48*(16), 1138-1143. https://doi.org/10.1016/j.clinbiochem.2015.06.022
27. Sajilata, M. g., Bajaj, P. R., & Singhal, R. s. (2008). Tea Polyphenols as Nutraceuticals. *Comprehensive Reviews in Food Science and Food Safety*, *7*(3), 229-254. https://doi.org/10.1111/j.1541-4337.2008.00043.x
28. Sanchez, J. (2017). Methylxanthine Content in Commonly Consumed Foods in Spain and Determination of Its Intake during Consumption. *Foods*, *6*(12), 109. https://doi.org/10.3390/foods6120109
29. Srdjenovic, B., Djordjevic-Milic, V., Grujic, N., Injac, R., & Lepojevic, Z. (2008). Simultaneous HPLC Determination of Caffeine, Theobromine, and Theophylline in Food, Drinks, and Herbal Products. *Journal of Chromatographic Science*, *46*(2), 144-149. https://doi.org/10.1093/chromsci/46.2.144

30. Szymański, M., Korbas, J., & Szymański, A. (2022). Methylxanthines release from various teas during extraction with water. *Acta Poloniae Pharmaceutica*, *78*, 781-788. https://doi.org/10.32383/appdr/145572
31. Turkmen, N., & Velioglu, Y. S. (2007). Determination of alkaloids and phenolic compounds in black tea processed by two different methods in different plucking seasons. *Journal of the Science of Food and Agriculture*, *87*(7), 1408-1416. https://doi.org/10.1002/jsfa.2881
32. Zareef, M., Mehedi Hassan, M., Arslan, M., Ahmad, W., Ali, S., Ouyang, Q., Li, H., Wu, X., & Chen, Q. (2020). Rapid prediction of caffeine in tea based on surface-enhanced Raman spectroscopy coupled multivariate calibration. *Microchemical Journal*, *159*, 105431. https://doi.org/10.1016/j.microc.2020.105431
33. Zhu, B., Haghi, M., Goud, M., Young, P. M., & Traini, D. (2015). The formulation of a pressurized metered dose inhaler containing theophylline for inhalation. *European Journal of Pharmaceutical Sciences*, *76*, 68-72. https://doi.org/10.1016/j.ejps.2015.04.016
34. Zhu, X., Chen, B., Ma, M., Luo, X., Zhang, F., Yao, S., Wan, Z., Yang, D., & Hang, H. (2004). Simultaneous analysis of theanine, chlorogenic acid, purine alkaloids and catechins in tea samples with the help of multi-dimension information of on-line high performance liquid chromatography/electrospray–mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis*, *34*(3), 695-704. https://doi.org/10.1016/S0731-7085(03)00605-8

## Appendix

**Table S1:** Concentration (mg/L) and volume (µL) of the stock solutions used to prepare 25 mL of the diluted standard solutions.

| | **Theophylline** | | **Caffeine** | |
|---|---|---|---|---|
| Number | Conc.$^{a}$ | V. $^{b}$ | Conc. $^{a}$ | V. $^{b}$ |
| 1 | 2 | 50 | 10 | 250 |
| 2 | 8 | 200 | 50 | 1.250 |
| 3 | 16 | 400 | 100 | 2.500 |
| 4 | 32 | 800 | 200 | 5.000 |
| 5 | 64 | 1600 | 400 | 10.000 |

a Concentration; b Volume taken.

**Table S2:** Concentration (mg/L) and peak area (mAU) values for standard solutions.

| Theophylline | | Caffeine | |
|---|---|---|---|
| Conc.$^{a}$ | Aire $^{b}$ | Conc.$^{a}$ | Aire $^{b}$ |
| 2.384 | 32.017 | 9.371 | 153.22 |
| 7.888 | 99.561 | 50.361 | 766.393 |
| 15.999 | 199.087 | 100.897 | 1522.386 |
| 31.966 | 394.997 | 201.235 | 3023.389 |
| 64.022 | 788.343 | 399.141 | 5983.951 |

a concentration (in mg/L); b Peak area in (mAU)

**Table S3:** Value of the concentration (Conc.) of theophylline in the extract and its content at the extraction time (T) for the sample tea used.

| **Time (min)** | **Mass(g)** | **Conc. (mg/L)** | **Content (% w/w)** |
|---|---|---|---|
| 30 | 5.00239 | 27.011 | 0.270 |
| 45 | 5.00269 | 27.189 | 0.272 |
| 60 | 5.00570 | 28.245 | 0.282 |

**Table S4:** Average theophylline content (% m/m) for the same grade of black tea.

| Grade | TORA | TEZA | IJENDA | MABAYI | RWEGURA | GISOZI | P-Value$^{\&}$ |
|---|---|---|---|---|---|---|---|
| PF1 | 0.196±0.011$^{a}$ | 0.207±0.003$^{a}$ | 0.205±0.007$^{a}$ | 0.203±0.003$^{a}$ | 0.188±0.003$^{b}$ | 0.145±0.009$^{c}$ | 0.000 |
| BP1 | 0.201±0.006$^{b}$ | 0.204±0.005$^{b}$ | 0.189±0.001$^{c}$ | 0.227±0.003$^{a}$ | 0.206±0.013$^{b}$ | 0.165±0.003$^{d}$ | 0.000 |
| PD | 0.202±0.003$^{d}$ | 0.230±0.011$^{bc}$ | 0.214±0.013$^{cd}$ | 0.279±0.017$^{a}$ | 0.238±0.014$^{b}$ | 0.169±0.009$^{e}$ | 0.000 |
| D1 | 0.216±0.002$^{ab}$ | 0.220±0.002$^{ab}$ | 0.204±0.003$^{b}$ | 0.219±0.021$^{ab}$ | 0.233±0.004$^{a}$ | 0.177±0.004$^{c}$ | 0.000 |
| D | - | 0.220±0.011$^{b}$ | 0.199±0.003$^{c}$ | 0.257±0.010$^{a}$ | - | 0.173±0.003$^{d}$ | 0.000 |
| F1 | - | - | 0.206±0.004$^{b}$ | 0.220±0.007$^{a}$ | 0.203±0.004$^{b}$ | 0.161±0.009$^{c}$ | 0.000 |
| FS | 0.198±0.003$^{c}$ | 0.241±0.006$^{a}$ | - | 0.231±0.002$^{b}$ | - | - | 0.000 |
| FS1 | *0.213±0.001* | - | - | - | - | - | - |
| BMF | 0.212±0.006$^{a}$ | 0.205±0.001$^{b}$ | 0.218±0.001$^{a}$ | 0.170±0.002$^{c}$ | 0.174±0.003$^{c}$ | - | 0.000 |

The - sign indicates that the grade is not produced. & Indicates the p-value of a statistical analysis of the average contents of the same grade in all tea-growingregions. In the same line, the same superscripts for grades in all regions indicate that caffeine levels are statistically identical. *No comparison.

**Tableau S5:** Average caffeine content (in % m/m) for the same grade of black tea.

| Grade | TORA | TEZA | IJENDA | MABAYI | RWEGURA | GISOZI | P-Value |
|---|---|---|---|---|---|---|---|
| PF1 | 2.898±0.022[b] | 2.926±0.012[b] | 2.627±0.018[c] | 3.004±0.026[a] | 2.397±0.017[e] | 2.528±0.021[d] | 0.000 |
| BP1 | 2.944±0.020[a] | 2.825±0.031[b] | 2.687±0.018[c] | 2.923±0.039[a] | 2.443±0.023[e] | 2.509±0.021[d] | 0.000 |
| PD | 2.527±0.018[e] | 2.822±0.024[c] | 2.756±0.021[d] | 3.331±0.055[a] | 3.213±0.034[b] | 2.703±0.023[d] | 0.000 |
| D1 | 2.706±0.018[d] | 2.987±0.025[b] | 2.881±0.016[c] | 3.126±0.015[a] | 2.724±0.019[d] | 2.705±0.021[d] | 0.000 |
| D | - | 2.947±0.152[ab] | 2.853±0.015[b] | 3.059±0.017[a] | - | 2.583±0.030 [c] | 0.000 |
| F1 | - | - | 2.672±0.019[c] | 2.774±0.026[b] | 3.006±0.020[a] | 2.460±0.014 [d] | 0.000 |
| FS | 2.817±0.018[a] | 2.766±0.015[b] | - | 2.729±0.016[c] | - | - | 0.002 |
| FS1 | 3.068±0.021* | - | - | - | - | - | - |
| BMF | 2.921±0.018[a] | 2.293±0.023[c] | 2.586±0.023[b] | 1.979±0.015[e] | 2.232±0.018[d] | - | 0.000 |

The - sign indicates that the grade is not produced. In the same line, the same superscripts for grades in all regions indicate that caffeine levels are statistically identical. *No comparison.

**Table S6:** Values for theophylline and caffeine content (% m/m) of 250 mL tea infusions prepared using the tap water.

| | | Theophylline | | Caffein | |
|---|---|---|---|---|---|
| Sample | M(g) [a] | Conc. (mg/L) [b] | Content | Conc. (mg/L) [b] | Content |
| PD MABAYI | 3.88074 | 47.711 | 0.307 | 236.859 | 3.052 |
| | 3.88074 | 46.403 | 0.299 | 234.172 | 3.017 |
| | 3.88074 | 49.020 | 0.316 | 235.515 | 3.034 |
| PF1 GISOZI | 3.31152 | 27.879 | 0.211 | 154.530 | 2.333 |
| | 3.31152 | 27.370 | 0.207 | 154.640 | 2.335 |
| | 3.31152 | 28.388 | 0.214 | 154.580 | 2.334 |

a Mass of the sample; b Concentration (mg/L).

**Table S7:** Concentrations (mg/L) and mass (in mg per cup of 250 mL) of theophylline and caffeine in tea infusions prepared using the tap water

| | Theophylline | | Caffein | |
|---|---|---|---|---|
| Sample | Conc (mg/L) | mass (mg) | conc (mg/L) | mass (mg) |
| PD MABAYI | 47.711 | 11.928 | 235.515 | 58.879 |
| PF1 GISOZI | 27.879 | 6.970 | 154.583 | 38.646 |

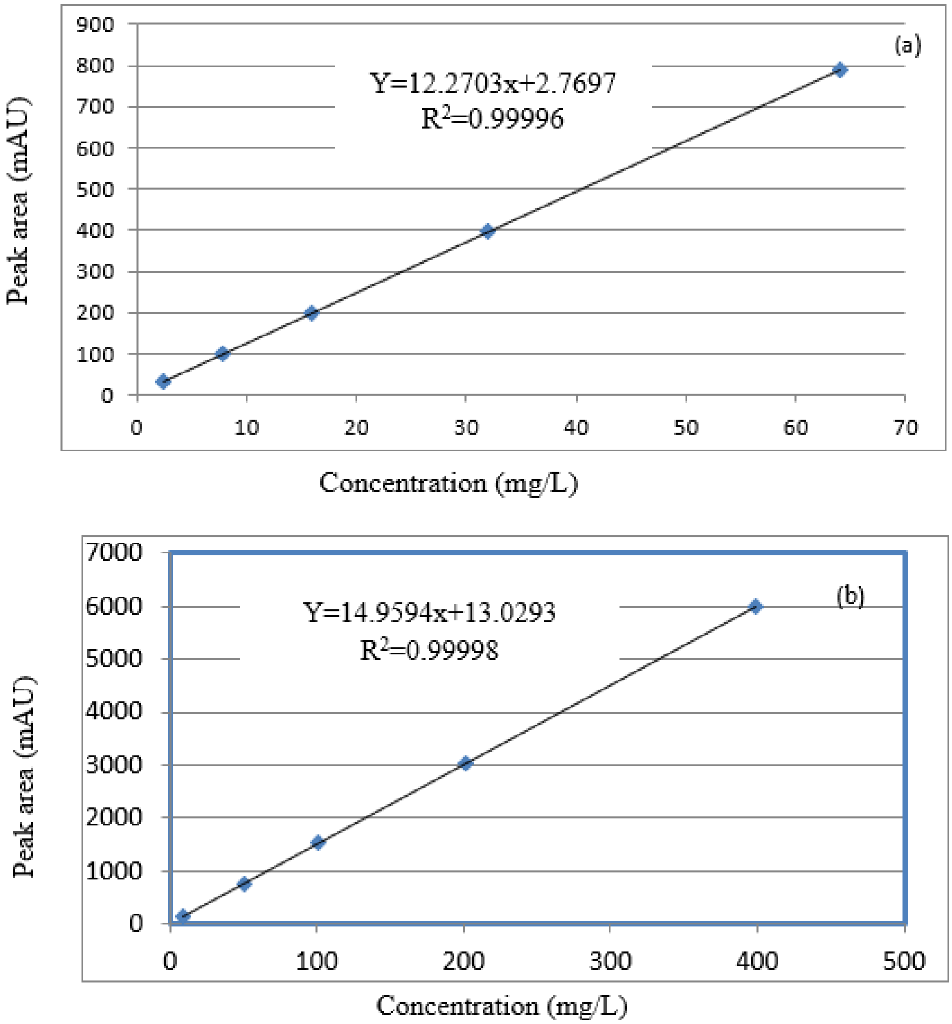


**Figure S1:** Calibration curves for (a) theophylline and (b) caffeine.