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Research Article

Effect of Extraction Period on Total Phenolics, Total Flavonoids, and Antioxidant Capacity of Ugandan *Camellia sinensis* (L) Kuntze, Black Primary Grades and Green Tea

Biryomumaisho Justus Murokore,[1,2] **Peter Vuzi California**,[1] **Alex Paul Wacoo**,[3] **Rapheal Wangalwa**,[4] **Clement Olusoji Ajayi**,[5,6] **Hannington Gumisiriza**,[7] **and Agnes Nandutu Masawi**[1]

[1]*Department of Biochemistry and Sports Science, School of Biological Sciences, College of Natural Sciences, Makerere University, P.O. Box 7062, Kampala, Uganda*
[2]*Department of Biochemistry, Faculty of Medicine, Mbarara University of Science and Technology, P.O. Box 1410, Mbarara, Uganda*
[3]*Department of Medical Biochemistry, School of Biomedical Sciences, College of Health Sciences, Makerere University, P.O. Box 7062, Kampala, Uganda*
[4]*Department of Biology, Faculty of Science, Mbarara University of Science and Technology, P.O. Box 1410, Mbarara, Uganda*
[5]*Department of Pharmacy, Faculty of Medicine, Mbarara University of Science and Technology, P.O. Box 1410, Mbarara, Uganda*
[6]*Pharm-Biotechnology and Traditional Medicine Centre, Mbarara University of Science and Technology, P.O. Box 1410, Mbarara, Uganda*
[7]*Department of Chemistry, Faculty of Science, Mbarara University of Science and Technology, P.O. Box 1410, Mbarara, Uganda*

Correspondence should be addressed to Biryomumaisho Justus Murokore; jbmurokore@must.ac.ug

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Introduction. Globally, the consumption and production of tea are on the rise because of its beneficial constituents. Scarce literature exists on the effects of extraction periods on the contents of the biologically important and protective phytochemicals such as phenolics, flavonoids, and antioxidants in locally produced teas in Uganda. *Aim*. This study determined the effects of extraction periods on the aqueous total phenolic content (TPC) of local *Camella sinensis,* black primary grades and green tea, and their ecological differences, their total flavonoid content (TFC), and antioxidant capacities (AOC). *Methods*. Samples of local tea were collected from Kigezi, Ankole, and Buganda regions, and those of green tea were purchased from a local supermarket in Uganda. Four- and 40-minute infusions were separately prepared for each sample. Total phenolic and flavonoid contents were determined using the Folin–Ciocalteu and aluminium chloride methods using garlic acid and quercetin as standards, respectively. Antioxidant content was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and ferric reducing assay power (FRAP) methods, using ascorbic acid as the standard. *Results*. Green tea had the highest total phenolic content both with four-minute (9.50 ± 0.25 mgGAE/g) and 40-minute (25.81 ± 1.13 mgGAE/g) extractions, followed by D1 (4.14 ± 0.33 mgGAE/g) at four minutes and PF (23.60 ± 2.37 mgGAE/g) at 40 minutes. Regionally, Kigezi (4.71 ± 0.09 and 22.13 ± 0.85 mgGAE/g) at four and 40 minutes, respectively, gave the highest TPC. In TFC, tea from Buganda ($4{,}371 \pm 0.00$ μgQE/g) was the highest. In DPPH and FRAP, GT ($93.82 \pm 0.03\%$, 39.04 ± 0.02 AAEμg/mL) was the best, followed by Buganda tea ($88.71 \pm 0.03\%$, 36.99 ± 0.01 AAEμg/mL), respectively. *Conclusion*. Longer extraction periods increase TPC in all teas. Green tea generates approximately twice the TPC generated by black tea in four-minute infusions. Green tea gives higher TPC, DPPH, and FRAP but less TFC than some black teas and is perhaps the best in terms of protection against oxidative damage to the body.

1. Introduction

Camellia sinensis (L) Kuntze (tea) is a species of evergreen small shrub whose leaves and leaf buds are used to produce drinking tea. It belongs to the genus *Camellia* in the family Theaceae [1]. Phenolic compounds (including flavonoids) are the most abundant secondary metabolites in plants that serve as a supply source of health-beneficial properties such as antimicrobial and antioxidant activities in the human diet [2, 3]. During the preparation of black tea, tea leaves are macerated and oxidized by passing air through them, a process called fermentation, which turns them to brownish-black in colour. The fermentation process allows polyphenol oxidase (PPO) and peroxidase to oxidize the simple polyphenols, which are mainly catechins, promptly to more complex polyphenols, especially theaflavin and thearubigins [4]. It is passed through hot air, drying and making it ready for consumption. Heating inactivates polyphenol oxidase (PPO) and peroxidase [5]. It is sieved into different grades which include dust 1 (D1), pekoe dust (PD), pekoe fannings (PF), and broken pekoe (BP) as the primary ones [6]. On the other hand, in processing green tea, the green fresh leaf is macerated and dried through hot air, without fermentation, making it ready for consumption [7]. Green tea contains far more catechins than black tea, and these are the main nutritional and medicinal components, which are also responsible for its strong antioxidant properties. The four main catechins include epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG). EGCG is the most important phenolic in green tea and the highest in concentration (60%), followed by EGC (20%), ECG (14%), and EC (6%) [8]. Other important components of green tea include minerals such as chromium, magnesium, selenium and zinc, carotenoids, tocopherols, ascorbate, and caffeine, which is half of that in coffee in terms of concentration [9]. Consumption of green tea is preventive against cancers of the mouth, lungs, esophagus, stomach, colon, small intestine, kidney, pancreas, and mammary glands [10]. Green tea phytochemicals are anti-inflammatory (especially EGCG), antimicrobial, and anticarcinogenic because of their antioxidant properties. They control inflammation by decreasing protein denaturation and increasing the production of anti-inflammatory cytokines [11]. Both green and black teas exhibit antioxidant properties because of the polyphenols, which also reduce oxidative stress and activate DNA repair [12]. Epigallocatechin and epigallocatechin-3-gallate in green tea inhibit copper-catalyzed peroxidation of low-density lipoproteins (LDL) and scavenge free radicals responsible for lipid peroxidation in heart mitochondria. Green tea extracts reduce lipid peroxidation and protein nitration through the suppression of reactive oxygen and nitrogen species generation. These extracts also decrease NADPH oxidase activity, thereby lowering superoxide levels [13]. Antioxidants from green and black tea inhibit the in vivo formation of DNA adducts and induce antioxidant enzymes that quench the oxidative burden, minimizing damage to enzymes such as glutathione peroxidase, glutathione reductase, catalase, quinone reductase, and superoxide dismutase. In addition, green tea delays aging (just like a combination of vitamin C and E) through an antioxidant mechanism [14]. When black (oxidized) tea is orally administered, it binds aflatoxins in the GIT preventing their absorption and promoting their egestion along with feces, thereby protecting the body from aflatoxicity. On the other hand, catechins in green tea inhibit angiogenesis, DNA hypermethylation, proliferation, and metastasis of tumor cells, and enhance telomerase enzyme function, tumor suppressor genes, and augment tumor cell apoptosis [11]. In addition, through up-regulating superoxide dismutase and glutathione, they maintain the integrity of the mitochondria [12].

There are variations in the phenolic content and antioxidant capacities of different varieties of tea. For instance, Chinese, Indian, or Sri Lankan varieties have the antioxidant capacity that corresponds to the phenolic content which is higher than the Chinese ones [15, 16]. However, literature about the total phenolic and flavonoid contents and antioxidant capacities of Uganda tea is scanty. Moreover, methods of harvesting, processing, and/or preparation affect the nutritional/medicinal content and bioavailability [17, 18] of the drinkable infusion. The methods of extraction used may lead to more or less yield, determine the heavy metal content of the infusion, or degrade some important active ingredients [19].

Traditionally, tea preparation for consumption in Uganda and most other parts of the world follows the four-minute pattern of infusion before sieving [20]. There is an increasing interest in growing and producing tea in Uganda but scanty literature on the nutritional/medicinal contents, especially the phenolic and flavonoid content and antioxidant capacities. Most available literature gives results based on procedures involving organic solvents, yet for consumption, tea is usually prepared using boiling water for around four minutes. Steeping for longer than four minutes may cause intolerable amounts of metallic ions to drain into the tea [21], although maximum phenolic extraction from green tea occurs in 40 minutes [22]. The current study determined the effect of the extraction period (four and 40 minutes) on the aqueous total phenolic content of primary black grades, green tea, and teas from different regions in Uganda. The study further determined differences in total yield, flavonoid contents, and antioxidant properties of locally produced Ugandan black teas from different regions and green tea (*Camellia sinensis*) in water.

2. Materials and Methods

2.1. Materials. Gallic acid, anhydrous sodium carbonate, Folin–Ciocalteu reagent, quercetin, DPPH, aluminium chloride, sodium nitrate, methanol, diabasic phosphate buffer, monobasic phosphate buffer, potassium ferricyanide, trichloroacetic acid, ascorbic acid, and Whatman paper No. 1 were purchased from Sigma Aldrich (Steinheim, Germany). All these chemicals were of analytical grade.

2.2. Tea Sample Collection. Black tea primary grade samples were collected once a week for three days from three tea-growing regions of Uganda: Kigezi, Ankole, and Buganda,

from the industries that cooperated, one per region. The factories that cooperated were willing to give only samples of the four primary grades, i.e., D1 (dust 1), PD (pekoe dust), PF (pekoe fannings), and BP (broken pekoe). Green tea samples were purchased from Megha Supermarket in Kampala city, each from a different batch.

2.3. Sample Preparation. The extract was prepared using an infusion method in which 1 g of each sample was measured into a beaker, followed by the addition of 100 mL of boiling distilled water. The paste was frequently shaken for 4 minutes or kept at boiling temperature (95°C) in a water bath for 40 minutes, and then sieved using Whatman paper, No. 1.

2.4. Quantification of Total Phenolic Content. The total phenolic content in the Ugandan *Camellia sinensis* (primary grades of black tea and green tea) was estimated using the modified Folin–Ciocalteu method with gallic acid (cat no. 6546548769138) as the standard [23]. To 0.05 mL of the sample filtrate, 9.85 mL of distilled water was added, followed by 0.05 mL of Follin–Ciocalteu reagent, followed by 0.05 mL of 7% Na_2CO_3. The resultant solution was incubated in the dark for colour development at 25°C for 90 minutes. The absorbance of the solution was read at 730 nm wavelength using a Hitache, U2001 spectrophotometer, in the Department of Biochemistry, Makerere University, Uganda. The TPC in one gram of the sample was calculated and expressed as mg gallic acid equivalent (GAE) per gram of the dry sample. Total phenolic content was calculated from the standard curve constructed from gallic acid working solutions of 2, 4, 6, 8, and 10 μg/mL concentrations with a linear regression equation of $y = 33.596x - 0.0099, R^2 = 0.9862$.

2.5. Determination of Yield and Total Flavonoid Content (TFC) of Aqueous Extracts. The extract was prepared using an infusion method in which 1 g of each sample was extracted by boiling distilled water (100 mL) for 4 minutes and sieved using Whatman paper No. 1. The extract was dried at 40°C in an oven and weighed in a Petri dish to determine the yield.

Total flavonoid content was determined following the method of Zhishen et al. [24], using quercetin as the reference standard. The working solutions of 1, 100, 200, 400, and 500 μg/mL in methanol were used to construct a standard curve with a linear regression equation of $y = 0.006x - 0.0054$ and $R^2 = 0.999$. From each sample dry extract, 1 mg/mL solution was prepared in hot water. Each sample (1 mL) was pipetted into a cylinder in triplicate with the blank being methanol. This was followed by the addition of 3 mL of methanol, 0.2 mL aluminium chloride ($AlCl_3$), and 0.2 mL sodium nitrate ($NaNO_3$) and topped up with 5.6 mL of methanol to the mark. This was incubated for 15 minutes and the absorbance was read at 435 nm wavelength using a JENWAY, 6705 UV/Vis spectrophotometer. The results were presented in μg quercetin equivalents/g, distilled water (μg QE/mL, d.w.), and μg quercetin equivalents/g, dry sample (μgQE/g, dry sample), while variation in a set of data was analysed through the one-way analysis of variance. The difference among the means was considered at 95% confidence level using the post hoc methods of Tukey's multiple comparison through GraphPad Prism 8.0.1 software.

2.6. Measurement of Antioxidant Capacity

2.6.1. DPPH (2,2-Diphenyl-picrylhydrazyl) Free Radical Scavenging Method. Free radical scavenging activity was determined using the method of percentage loss of the original colour concentration. From a solution of 1 mg/mL prepared by addition of hot water to each sample, 1.0 mL solution was pipetted into a 10 mL measuring cylinder. This was followed by the addition of 1 mL of 4% DPPH and 2 mL of methanol. Incubation was carried out in the dark for 15 minutes at 25°C for colour to develop. Methanol solution was used as a blank. The percentage inhibition of DPPH was determined by measuring absorbance at 517 nm using a JENWAY 6705 UV/Vis spectrophotometer and calculated following the formula:

$$\%\ \text{inhibition of DPPH activity} = \left[\frac{(A_0 - A_1)}{A_0}\right] \times 100, \quad (1)$$

where A_0 = absorbance of DPPH (blank) and A_1 = absorbance of infusion-DPPH solution [25].

2.6.2. Determination of the Reducing Power of Water Extracts; the FRAP Assay. The reducing power of the different grades of black tea and green tea was determined using the ferric reducing assay power method (FRAP) as described by Benzie and Strain [26]. Briefly, phosphate buffer was prepared by mixing 37.50 mL of 0.2 M dibasic sodium phosphate with 62.5 mL of 0.2 M monobasic sodium phosphate and diluted to 100 mL with distilled water. To 0.75 mLs of the tea sample extract solution (1 mg/mL), 0.5 mL of phosphate buffer (0.2 M, pH = 6.6) was added, followed by potassium ferricyanide [$K_3Fe(CN)_6$] (1%, w/v). The mixture was incubated for 20 minutes at 50°C. The reaction was stopped by adding 0.75 mL of trichloroacetic acid (10%) and centrifuged at 3000 rpm for 10 minutes. To 1.5 mL of the resultant supernatant solution was added 1.5 mL of distilled water and a freshly prepared solution of iron chloride ($FeCl_3$) (0.1%, w/v). The absorbance was read at 700 nm on a JENWAY 6705 UV/VIS spectrophotometer. High absorbance of the reaction mixture indicates high reducing power. Standard ascorbic acid solution at concentrations 1.95, 3.91, 7.82, 15.63, 31.25, and 62.50 μg/mL was treated similarly and used to obtain a calibration curve. The calibration curve equation was $y = 0.0334x + 0.2891$, and $R^2 = 0.9753$. It was used to determine the reducing power of the extracts. The results obtained were expressed as ascorbic acid equivalents per mL (AAE) μmol/mL [27].

2.7. Data Management and Analysis. All quantitative data (Supplementary 1) were analysed using the GraphPad Prism 8.0.1 software. Data were expressed as the mean ± standard

error of mean (SEM) while variation in a set of data was analysed through the one-way analysis of variance. The difference among the means was considered at 95% confidence level using the post hoc methods of Tukey's multiple comparison wherever applicable.

3. Results

3.1. Total Phenolic Content of Different Black Tea Grades and Green Tea. All the samples yielded more TPC after 40 minutes than after four minutes of hot water extraction ($p < 0.05$). Green tea *C. sinensis* (9.50 mgGAE/g) had the highest TPC followed by D1 (4.141 ± 0.22 mgGAE/g), PD (3.81 ± 0.28 mgGAE/g), PF (3.69 ± 0.22 mgGAE/g), and BP (3.39 ± 0.37 mgGAE/g), as shown in Table 1. Four minutes of extraction yield of green tea was approximately two-fold the rest.

On the other hand, among the 40-minute extracts, all the black tea grades and green tea, *C. sinensis*, yielded much higher TPC compared to the four-minute extracts ($p < 0.0001$). The highest was still GT (25.81 ± 1.13 mgGAE/g), followed by PF (23.6 ± 2.37 mgGAE/g), D1 (21.50 ± 0.79 mgGAE/g), BP (20.94 ± 1.17 mgGAE/g), and PD (19.32 ± 1.73 mgGAE/g).

3.2. Total Phenolic Content for Tea from Different Regions. Since D1 proved the best in TPC yield from all regions, we used it for comparison. So, Table 2 presents regional differences in D1 phenolic contents for 4- and 40-minute extractions and that of green tea. Among the four-minute extractions of D1, Kigezi tea (4.71 ± 0.09 mgGAE/g dry weight), it gave the highest TPC, while among the green teas, GT2 (10.82 mgGAE/g dry weight) gave the highest TPC. Likewise, the 40-minute extractions of D1 followed the same pattern: Kigezi (22.13 ± 0.85 mgGAE/g dry weight) gave the highest, and among the green teas, GT2 (26.26 ± 1.13 mgGAE/g dry weight) gave the highest. All the TPC yields for D1 in four-a minute extraction time from the different regions were significantly lower than for green tea ($p < 0.00$). However, they were not significantly different (Ankole Vs. Buganda ($p = 0.95$), Kigezi Vs Ankole ($p = 0.64$)), except Kigezi and Buganda (Kigezi Vs. Buganda ($p = 0.02$)). GT2 yielded a significantly higher TPC than GT1 ($p = 0.03$) in the four-minute extractions. There was no significant difference in the 40 minutes extracts of black or green teas, ($p > 0.05$).

On the other hand, all the 40-minute TPC extractions yielded significantly higher TPC than their respective four-minute extractions ($p < 0.05$).

3.3. Regional Differences in Extract Yield and Total Flavonoid Content (TFC), μgQE/G and μgQE/G Dry Sample. Results of regional differences in extract yield and total flavonoid content (TFC) were presented in Table 3. Total yield was highest with green tea (73.5 mg/g), followed by D1 black tea from Buganda (61.5 mg/g), then Ankole (58 mg/g), and finally Kigezi (53 mg/g).

Green tea (17.79 ± 0.056 μgQE/g) yielded the lowest total flavonoid content, while black tea from the Buganda region (71.07 ± 0.0 μgQE/g) yielded the highest. Considering flavonoid content per gram of dry sample, D1 tea from Buganda ($4{,}371 \pm 0.00$ μgQE/g dry sample) had the highest, followed by tea from Kigezi ($1{,}323 \pm 23.00$ μgQE/g), green tea ($1{,}307 \pm 4.08$ μgQE/g dry sample), and finally Ankole ($1{,}145 \pm 0.00$ μgQE/g).

Results for % inhibition of DPPH activity of the different black tea grades and green tea are shown in Figure 1. They ranged from $85.29 \pm 0.12\%$ to $93.82 \pm 0.03\%$, compared to ascorbate, which was at $95.54 \pm 0.0\%$. Green tea exhibited the highest % inhibition of DPPH activity ($93.82 \pm 0.03\%$), which is almost as good as ascorbate. Kigezi tea exhibited the lowest DPPH activity ($85.29 \pm 0.12\%$) while Buganda tea exhibited the highest % inhibition of DPPH activity ($88.71 \pm 0.03\%$) among the black teas.

Figure 2 presents the ferric reducing assay power (AAEμg/mL) of Ugandan black tea from different regions and green tea. Green tea had the highest ferric reducing assay power 39.04 ± 0.02 AAEμg/mL, while that for black tea from different regions ranged from 36.2 ± 0.02 to 36.99 ± 0.01 AAEμg/mL. Tea from the Buganda region had the highest ferric reducing assay power (36.99 ± 0.01 AAEμg/mL) among the black teas.

4. Discussion

Several reports indicate that tea leaves are rich in anti-inflammatory, anticancer, antihypertensive, antibacterial antidiabetes, antiobesity, neurologic health, and antioxidant properties [28]. Most literature on TPC, TFC, and antioxidant capacity of teas globally is based on procedures involving organic solvents, yet for consumption, tea is usually boiled in water for four minutes to avoid the accumulation of intolerable amounts of metallic ions [21]. However, maximum phenolic extraction may occur in 40 minutes' steeping [22]. This study investigated the aqueous total phenolic contents and their ecological differences, total flavonoid contents, and antioxidant properties of the primary grades of black tea and green tea produced from different regions in Uganda.

Results in Table 1 show that all the black tea primary grades (dust 1 (D1), pekoe dust (PD), pekoe fannings (PF), and broken pekoe (BP) and green tea had significantly higher total phenolic content after 40 minutes of hot water extraction than for four minutes. Forty minutes extracts of primary tea grades yielded about four times the total phenolic content yielded by the four minutes of extraction. However, similar treatment of green tea produced only two-fold the amount yielded by four-minute extraction. This suggests that 40 minutes extraction gives more phenolic content, and green tea is more beneficial than any black tea grade. This finding was expected since the longer the tea particles stay in hot water, the greater the solvent molecules' interactions with it and the greater the soluble phenolic molecules dissolved out [29]. Hence, for a better extraction outcome, it would have been better and more effective to increase the extraction time from the usual

Table 1: Effect of extraction period (minutes) on total phenolic content (TPC) of black tea grades and green tea.

Extraction time	Black tea grade				Green tea ($n = 6$)
	D1 ($n = 9$)	PD ($n = 9$)	PF ($n = 9$)	BP ($n = 9$)	
TPC (4 min)	$4.141 \pm 0.22^{a*}$	$3.81 \pm 0.28^{a*}$	$3.69 \pm 0.22^{a*}$	$3.39 \pm 0.37^{a*}$	$9.50 \pm 0.25^{b*}$
TPC (40 min)	21.5 ± 0.79^{a}	19.32 ± 1.73^{a}	23.60 ± 2.37^{a}	20.94 ± 1.17^{a}	25.81 ± 1.13^{b}

Abbreviated: DI = dust 1, PD = pekoe dust, PF = pekoe fannings, and BP = broken pekoe. Data are expressed as mean ± SEM; in rows, means that do not share a letter are significantly different ($p < 0.05$); in columns, mean with * is significantly different ($p < 0.05$) at 5% level of significance.

4 minutes to 40 minutes. Moreover, in countries such as India, black tea is boiled for more than 15 minutes before consumption [30]. However, the advantage of a short extraction time of 4 minute is that it doesn't allow the draining of heavy metals, such as aluminium and lead, common in prolonged extraction procedures, thus posing a health risk [21]. Fernando and Soysa [31] reported that 83% of tea brewed for 15 minutes had lead levels that were unsafe for pregnant and lactating mothers.

A four-minute hot water extraction of the primary tea grades in this study yielded 3.387 to 4.141 mgGAE/g, while green tea produced twice as much. This suggests that the health benefits of the extraction of the different tea primary grades may not be significantly different; hence, it may be okay to use any grade available. However, green tea appears to be twice as healthy. This could be one of the reasons why green tea (*C. sinensis*) is believed to be much more beneficial than black tea [32]. The phenolics in green tea, i.e., catechins are higher in concentration and twice as soluble in water as those in black tea when steeped for the usual four minutes. During the fermentation process of tea, about 70% of the catechins are oxidized and polymerized into various complex coloured compounds, e.g., thearubigin, theaflavin, and theabrownine [33], which reduces the solubility.

Results on differences in TPC by region and brand in Table 2 show that tea from Kigezi had the highest phenolic content, followed by that from Buganda. This indicates that tea from Buganda is of higher quality among the Ugandan teas. This could probably be because tea grown in highland areas such as Kigezi grows slowly, and the slow maturity enhances/heightens the quality and concentrations of phenolic content. Thus, the higher the altitude, the slower the growth rate and the greater the quality [34]. The Buganda region has a tropical type of climate. It is relatively warm and humid, favourable for the production of most crops including tea. The tropical climate in the Buganda region permits tea to grow quickly and be harvested when still soft, thus having high soluble content [33].

Results of regional differences in extract yield and TFC (Table 3) show that green tea yielded less total flavonoid content than D1 black tea from the Buganda region. This agrees with Yadav et al. [35] who reported more flavonoids in black tea than green tea. However, this study showed that black tea from the *Ankole* region yields less flavonoids than green tea, which is also in agreement with Hilal and Engelhardt [36], who reported that green tea contained more flavonoids than black tea. This indicates that there may be other factors that determine flavonoid content apart from it being the green or black type. These might include genetics or plant location.

Among the Black tea samples alone, however, the total flavonoid content in dry extract was highest in samples from the Buganda region, which was about three times higher than that from the Ankole and Kigezi regions. This may be the effect of altitude, just like in *Rosa* sp. consistent with Oprica and Roşu [37], who reported that total flavonoid content decreased with increasing altitude.

Results in Figure 1 showed that the percentage inhibition of DPPH activity was significantly different for all tea samples tested ($p < 0.05$) and found highest in green tea. This indicates that green tea is more effective in radical scavenging than black tea. This finding is consistent with Bartoszek et al., who reported that green tea is the best in DPPH % inhibition among teas [38]. Moreover, Anggraini et al. reported that % DPPH inhibition decreases when fresh tea is processed into Black tea [39]. Green tea had a lower % inhibition of DPPH activity than ascorbate, implying a lower free radical scavenging ability. This study found a higher value than that of Jeszka-Skowron et al., who reported a range of 60–80% [40]. This difference could be attributed to altitude, soil type, location, or genetic differences [41, 42].

Amongst the black teas, % inhibition of DPPH activity was highest in samples from Buganda region, just like % yield and TFC. This suggests that the higher the TFC, the higher the % inhibition of DPPH. This is in agreement with Gonbad et al., who reported that antioxidant capacity positively correlated with the flavonoid content [43]. Interestingly, tea samples from Kigezi yielded the highest TPC and lowest DPPH activity, while those from the Buganda region had the second highest TPC, highest % yield, TFC, and % inhibition of DPPH activity. This shows that TPC, TFC, and % inhibition of DPPH activity may not always be in correlation. Thus, other factors, especially altitude and climate, have a role to play in influencing these parameters [37, 43].

Results in Figure 2 showed that green tea had a higher ferrous reducing assay power than all the black tea from all regions. This implies that it is more effective in reducing the oxidation status of the body than black tea. This data is consistent with other previous studies [44, 45]. Among the black teas from different regions, the FRAP showed that antioxidant potential was the highest in samples from the Buganda region, followed by Kigezi and Ankole. Despite the high TPC in tea samples from the Kigezi highlands, the ferrous-reducing assay power remained low. This shows that altitude was not the only factor influencing phenolic content. Therefore, this study shows that although TPC

Table 2: Differences in total phenolic content (TPC) of black tea per region/green tea brand.

Extraction time	Black tea (D1) per region			Green tea	
	Kigezi tea	Ankole tea	Buganda tea	GT1	GT2
TPC (mgGAE/g); 4 min	$4.71 \pm 0.09^{a*}$	$3.57 \pm 0.46^{a*}$	$4.14 \pm 0.09^{a*}$	$8.17 \pm 0.25^{b*}$	$10.82 \pm 0.24^{c*}$
TPC (mgGAE/g); 40 min	22.13 ± 0.85^{bc}	16.42 ± 0.14^{a}	20.51 ± 0.94^{ab}	25.35 ± 1.13^{c}	26.26 ± 1.13^{c}

Data are expressed as mean ± SEM ($n = 3$); in rows, means that do not share a letter are significantly different ($p < 0.05$); in columns, mean with * is significantly different ($p < 0.05$) at 5% level of significance.

Table 3: Regional/brand differences in extract yield and total flavonoid content (TFC) in μgQE/g and μgQE/g dry sample.

Parameter	Black tea per region			Green tea
	Buganda tea	Ankole tea	Kigezi tea	
Extract yield (mg/g)	61.5	58	53	73.5
TFC (μg QE/mL)	71.07 ± 0.00^{a}	19.73 ± 0.00^{b}	24.96 ± 0.43^{c}	17.79 ± 0.056^{d}
TFC (μgQE/g dry sample)	$4{,}371 \pm 0.00^{a}$	$1{,}145 \pm 0.00^{b}$	$1{,}323 \pm 23.00^{c}$	$1{,}307 \pm 4.08^{d}$

Data are expressed as mean ± SEM ($n = 3$); in rows, means that do not share a letter are significantly different ($p < 0.05$) at 5% level of significance.

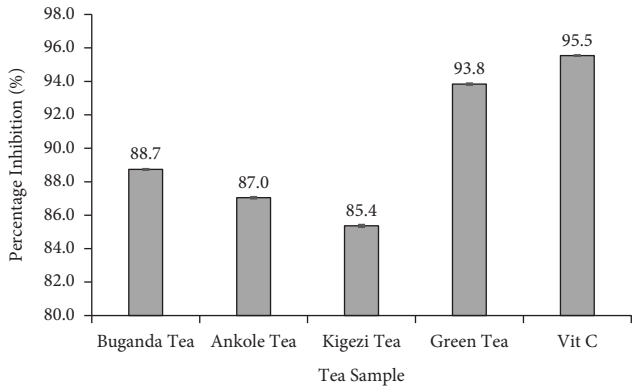

Figure 1: DPPH percentage inhibition of black tea from different regions and green tea.

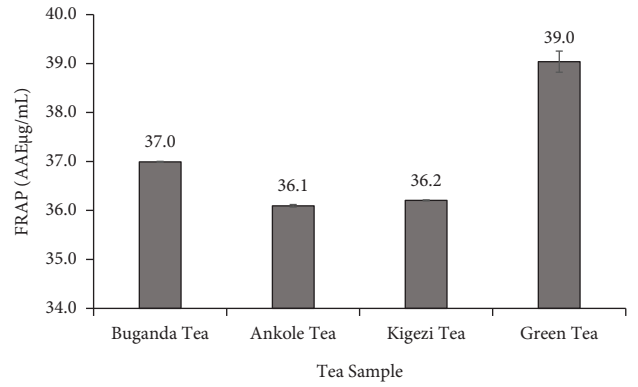

Figure 2: Ferric reducing assay power of black tea from different regions and green tea.

increases with altitude, the antioxidant activity or free radical scavenging power reduces with altitude, and this is consistent with findings by Chakraborty et al. [46]. This is due to changes in general climatic conditions, e.g., low temperature and atmospheric pressure, differences in radiation, and other environmental factors [41, 42]. This suggests that tea growers should prefer warm and humid, low-altitude areas for gardens to maximize production and quality of tea. In addition, green tea production should be more emphasized by governments for its great qualities and simplicity.

5. Conclusion

Green tea yields more than twice the TPC yielded by any black tea grade when extracted for only four minutes, but a slightly higher amount when both are extracted for 40 minutes. D1 is the best black tea grade. For the ordinary four-minute infusions, green tea is the best among teas, followed by D1, PD, PF, and BP, in the protective or nutritional content. TPC, TFC, % inhibition of DPPH activity, and FRAP may not always correlate naturally. These findings can be used to make tea choices and plan national allocations of tea gardens for maximum quality and production in Uganda and other nations, especially in consideration of altitude and climate.

5.1. Limitation. This study did not consider possible genetic variations among the different tea samples.

5.2. Recommendation. There is a need to develop a method that will extract the maximum total phenolic content while preventing the accumulation of heavy, unsafe metals in drinking tea. This will enable maximum utilization and the safety of tea. In addition, there is a need to evaluate how the nutritional values/activities change with increasing extraction period. Another study could investigate the impact of genetic variations in phenolic content in these regions.

Data Availability

The data for the effect of extraction period on total phenolic content, yield, total flavonoid content, % inhibition of DPPH, ferric reducing assay power (FRAP) of Ugandan

Camellia sinensis black primary grades, and green tea used to support the findings of this study are included within the article (Supplementary 1)

Ethical Approval

Approval to conduct the study was obtained from the Gulu University Research and Ethics Committee (GUREC) with the number: GUREC-110-18.

Conflicts of Interest

All authors declare that there are no conflicts of interest regarding the publication of this article.

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Supplementary Materials

Row results for effect of extraction period on total phenolic content, extraction yield, total flavonoid content, % inhibition of DPPH, ferric reducing assay power (FRAP) of Ugandan *Camellia sinensis* black primary grades, and green tea. (*Supplementary Materials*)

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