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Antioxidant Activity, Theaflavin, Total Polyphenol, and Catechin Composition of *Camellia sinensis* Processing Effluents from Various Factories in Kenya

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Abstract:- Research into the antioxidant efficacy and sustainability implications of tea waste, a by-product of the rapidly growing global beverage industry, is increasingly necessary. This study scrutinized samples taken from various factories to explore their capacity for free radical scavenging and potential public health and environmental benefits. Analysis of the samples and reference antioxidant (BHT) revealed a notable dose-dependent rise in free-radical-scavenging action, implying a positive concentration-dependent antioxidant efficacy. Variations in antioxidant activity occurred from tea wastes sourced at various factories, alluding to geography having a pivotal effect on the biological contents and antioxidative capacity. Of particular interest were Boito's and Chelal's cyclone fluff samples that evinced relatively lower potency than those from other factories. Tombe factory's cyclone sample demonstrated a superior capacity for scavenging free radicals at different concentrations, suggesting that particular production or cultivation processes augmented its effectiveness. Notably, some tea waste samples even equalled the potency of BHT (a synthetic antioxidant) when augured to its highest level. Furthermore, this study revealed disparities in Theaflavin and Thearubigin content due to factors like maturation period and processing methods guiding these concentrations. We identified tea waste samples possessing remarkable polyphenol concentration, thereby providing valuable insights for consumers and industries. Further investigations on the chemical composition of tea waste phytocompounds are paramount due to their acclaimed health-promoting properties and the factors influencing their concentrations and antioxidant efficacy.

Keywords:- *Thearubigin; Catechin; Theaflavin, Polyphenol, Free Radicals.*

I. INTRODUCTION

➢ *Background*

Tea (*Camellia sinensis*) is a widely consumed beverage with extensive global popularity, produced in massive quantities across regions like Kenya, China, India, and Sri Lanka, among others [1]. Despite its wide acclaim for its aroma, flavour, and purported health benefits, the large-scale production of tea and its processing brings forth a significant environmental issue: the generation of tea wastes. While the focus has largely remained on the processed leaves used for brewing, it is imperative to note that a substantial proportion of biomass, including spent tea leaves, stems, and flushes, is usually discarded during the manufacturing process [2,3]. This effluent presents an environmental burden and often ends up in landfills, contributing to resource wastage and increased greenhouse gas emissions [4].

In recent years, the paradigm has gradually shifted toward the eco-sustainable management of agro-industrial byproducts, including tea wastes [5–7]. One of the most promising areas of research in this realm is the exploration of the antioxidant potential of these waste materials [8,9]. Antioxidants are molecules capable of preventing or slowing the oxidative damage in cells caused by free radicals [10]. These radicals can damage cellular components, including DNA, lipids, and proteins, leading to a variety of chronic diseases such as cardiovascular diseases, neurodegenerative disorders, inflammation, and cancer. This makes the search for potent antioxidant compounds, especially from natural sources, crucial in health and nutrition research [8,9,11]. As such, the potential utility of antioxidant-rich substances from tea waste in pharmaceuticals, nutraceuticals, and even in the food industry is vast and warrants rigorous investigation, hence this study [11–13].

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Existing research has substantiated that polyphenolic phytocompounds such as catechins, theaflavins, and tannins are primarily responsible for the antioxidant activity of tea [14,15]. However, the general misconception has been that these compounds predominantly reside in the leaves, rendering the remaining biomass less useful. Contrarily, recent studies have exhibited that these bioactive compounds are not limited to the leaves alone but are also significantly present in the waste materials [16]. Moreover, several extraction techniques, including solvent-based methods and supercritical fluid extraction, have been developed to efficiently isolate these antioxidants from tea waste [17]. Notably, various assays like 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, Ferric reducing antioxidant power (FRAP), and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) have been applied to quantify the antioxidant potential of tea waste extracts [17]. Furthermore, a growing body of literature has demonstrated that the application of these extracts has been effective not only in *in vitro* studies but also *in vivo*, signifying their pharmacological relevance [8,10].

While the commercial implications of the antioxidant activity of tea waste are promising, another important dimension worth considering is the economic viability. Cost-effective methods for the extraction and utilization of antioxidants from tea waste would contribute to the circular economy, offering a win-win situation where both environmental sustainability and commercial profitability are achieved [18]. It is also important to consider the regulatory landscape surrounding the incorporation of natural antioxidants into consumable products. Given that natural antioxidants are generally recognised as safe (GRAS) by the food and drug administration (FDA), they offer an advantage over synthetic alternatives like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), which have raised health concerns, including carcinogenicity, induction of autoimmunity, among others [19–21]. Therefore, this study investigated the antioxidant potential of various tea wastes obtained from various factories as potential sources of safe and efficacious antioxidant amalgams to combat oxidative stress and its associated complications.

II. MATERIALS AND METHODS

➢ *Collection and Processing of the Tea Waste Samples*

Samples of tea waste, encompassing fluffs, cyclones, cyclone fluffs, dry-offs, and fluff-dry mouths, were meticulously gathered from different processing facilities located in Kenya. These samples were carefully packaged in immaculate brown-hued glass receptacles and subsequently transported to the Tea Research Institute (TRI, KARLO), laboratories situated at Timbilil Estate, Kericho (latitude 0° 22`S, longitude 35^0 21'E, altitude 2180 M above sea level) for analysis. The samples were oven dried (Menmert, 854 Schwab, Germany) to a constant weight at a temperature of 103 ^{0}C and then finely milled using an electric blending device (Moulinex AR 1O43, China) to reduce the sample particle size. The powdered samples were in desiccators prior to characterisation.

➢ *Determination of 1,1-Diphenyl-2-picryl Hydrazyl Radical (DPPH) Free Radical-Scavenging Activity*

The experiment was conducted based on the methodology proposed by Brand et al.[22], with modifications by Moriasi *et al.* [23]. Concisely, 1 ml of 0.3 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH) was individually combined with 2.5 ml of each sample extract or Ascorbic acid at varying concentrations (3.125 µg/ml, 6.25 µg/ml, 12.5 µg/ml, 25 µg/ml, and 50 µg/ml). Each combination was prepared in triplicate. Following the preparation, the solutions were left to incubate for 15 minutes under dark conditions. After the incubation period, the absorbance levels of the solutions were recorded at a wavelength of 517 nm using a Shimadzu UV-VIS (1600) microprocessor double-beam spectrophotometer, with measurements taken against a blank within an hour. The designated negative control was composed of 2.5 ml of 0.3 mM DPPH and 1 ml of methanol.

To quantify the radical scavenging activity of DPPH (% RSA) for each extract, the formula delineated by Moriasi *et al.* [23] was employed (Eqn. 1).

$$\%\ \text{RSA} = \left[\frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}}\right] \times 100 \quad \text{Eqn. 1.}$$

Additionally, the EC_{50} values, representing the extract concentrations required to neutralize 50% of the DPPH radicals, were ascertained using linear regression plots correlating % RSA with concentration. This provided an assessment of the antioxidant potential of each extract.

➢ *Determination of Theaflavin and Total Polyphenol Concentration in the Tea Samples*

The theaflavin content of the tea samples was determined using the Flavognost method [24]. In brief, 9 grams of tea samples were steeped in 375 ml of boiling water from an overhead boiler in a tared flask, followed by agitation on a mechanical shaker for 50 minutes. After filtering through cotton wool and cooling to room temperature, 10 ml of the infusion was mixed with 10 ml of isobutyl methyl ketone (IBMK). This mixture was shaken for 10 minutes and left undisturbed until phase separation. From the upper layer, 2.0 ml was combined with 4.0 ml of ethanol and 2.0 ml of Flavognost reagent (2g diphenyl boric acid-2-amino ethyl ester in 100 ml ethanol). After thorough mixing and a colour development period of 15 minutes, absorbance was measured at 625 nm, using a 1BMK/ethanol (1:1 v/v) solution as the blank.

Thearubigins content was quantified using a previously described methodology [25]. Initially, 6.0 ml of a 1% aqueous solution of anhydrous disodium hydrogen orthophosphate was combined with an equivalent volume of cooled tea

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waste samples. This blend was subjected to an extraction process using 10 ml of ethyl acetate, achieved by vigorously shaking the mixture for 60 seconds. After allowing the mixture to settle, the aqueous phase was decanted. To the retained ethyl acetate layer, which encapsulated the theaflavin fraction, another 5.0 ml of ethyl acetate was introduced. Subsequently, a 10 ml aliquot of this extract was extended to 25 ml using methanol, producing solution E1. For solution E2, 1.0 ml of the original tea waste samples was combined with 9.0 ml of distilled water and then volumetrically adjusted to 25 ml with methanol. Solution E3 was formulated by combining 1.0 ml of tea infusion with 1.0 ml of a saturated 10% oxalic acid aqueous solution and 8.0 ml of distilled water, followed by dilution to 25 ml with methanol. The optical densities of solutions E1, E2, and E3 were ascertained at wavelengths of 380 nm and 460 nm using a Shimadzu UV-1800 series spectrophotometer, calibrated against distilled water. The percentage Thearubigins content was determined using equation 2.

$$\%\,TR = (7.06 \times \frac{4E3 - E1}{DM}) \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots Eqn.2$$

➢ *Determination of the Concentration of Various Catechins in the Tea Samples*

The concentration of catechins in the various tea waste samples from various factories were quantitatively assessed using High-Performance Liquid Chromatography (HPLC) based on protocol described by Ahmad et al. [26], and in accordance with the ISO 14502-2-2005E standards. The analysis employed a Knaer Hitachi HPLC chromatograph from Darmstadt, Germany, featuring a L-7100 pump, a Rheodyne 7725I injection valve with a 20 µL loop, and a diode array detector (DAD) L-7455 set at 278 nm. The system was controlled via a personal computer with a Knaer Hitachi D-7000 interface and Merck Hitachi HPLC manager software. Chromatographic separation was achieved using a 25 cm x 4 mm Lichro CART RP-18 5 µm column under conditions of a two-solvent gradient: mobile phase A (acetonitrile/acetic acid/distilled water at 8:2:90 v/v/v) and phase B (80:2:18 v/v/v). With a flow rate of 1 µL/min, a 20 µL injection volume, and a column operating temperature of 35 °C, the catechins were detected at 278 nm. For analysis, 1.0 ml of the sample was diluted to 5.0 ml, filtered, and vial loaded. Individual catechin identification was based on retention time comparison with known standards (+C, EC, EGC, ECG, EGCG). Quantification utilized external calibration curves at 278 nm (R^2=0.9984), incorporating consensus relative response factors related to caffeine on a dry matter basis. The method's precision was verified through multiple analyses of a standard solution across various days.

III. RESULTS

➢ *Free Radical Scavenging Activity of the Tea Waste Samples*

This study assessed the free radical scavenging activity of tea samples collected from various factories to evaluate their antioxidant effectiveness (Table 1). The results indicated that both the tested tea samples and the reference antioxidant, butylated hydroxytoluene (BHT), exhibited a significant increase (P<0.05) in percentage free radical scavenging activity as the concentration increased (Table 1).

At a concentration of 3.125 µg/ml, the cyclone fluff samples obtained from Boito and Chelal factories, cyclone samples from Gitambo factory, and fluff from Gitambo factory displayed significantly lower percentage free radical scavenging activities than all the other samples. Conversely, the fluff and cyclone samples from Ogembo and Tombe factories had significantly higher percentage free radical scavenging activities at a concentration of 3.125 µg/ml than all the other samples (P<0.05; Table 1). Notably, the percentage free radical scavenging activities at this concentration ranged from about 12 % to 51 %, depending on the sample type and source (Table 1).

The cyclone sample Tombe factory demonstrated a significantly higher percentage free radical scavenging activity than all the other samples at a concentration of 6.25 µg/ml (P<0.05; Table 1), while samples from Boito, Chelal, and Gitambo factories (both cyclone and fluff) displayed significantly lower activities (P<0.05; Table 1). Most samples at this concentration (6.25 µg/ml) had activities exceeding 40 %, ranging from approximately 20 % to 66 %, depending on the sample type and source (Table 1).

Besides, the cyclone sample obtained from the Tombe factory exhibited a significantly higher percentage free radical scavenging activity (P<0.05). In contrast, both the cyclone and fluff samples from the Gitambo factory had significantly lower scavenging activity compared to the other samples (P<0.05) at a concentration of 12.5 µg/ml (Table 1). It was observed that the percentage of radical scavenging activities at a concentration of 12.5 µg/ml varied from about 50% to 75%, depending on the sample type and its source, as shown in Table 1.

The results also showed that the cyclone sample obtained from the Tombe factory had a significantly higher percentage free radical scavenging activity than all the other samples at a concentration of 25 µg/ml (P<0.05; Table 1), while the cyclone and fluff samples from Gitambo factory showed significantly lower activities compared to other samples (P<0.05; Table 1). Overall, the percentage free radical scavenging activities ranged from about 55% to 85% at a concentration of 25 µg/ml, depending on the sample type and factory of origin (Table 1).

Finally, at the highest tested concentration of 50 µg/ml, the cyclone fluff sample from Momul factory and the cyclone sample from the Tombe factory exhibited comparable percentage free radical scavenging activity to BHT (P>0.05) and significantly higher activity than all other samples (P<0.05; Table 1). Additionally, the fluff dry mouth and fluff samples from Tombe, and Gitambo factories, as well as the cyclone sample from Gitambo factory, displayed significantly lower scavenging activities than the other samples (P<0.05; Table 1). Notably, the percentage free radical scavenging activities of the tested samples at a concentration of 50 µg/ml ranged from about 69% to 90%,

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depending on the sample type and factory of origin (Table 1).

Moreover, we calculated the median effective concentration (EC_{50}) for each tested sample to assess its efficacy. As indicated in Table 1, the Tombe factory tea sample had the lowest EC_{50} (7.41 µg/ml), while the cyclone and fluff samples from the Gitambo factory had the highest EC_{50} values (29.28 µg/ml and 29.16 µg/ml, respectively). Based on the obtained EC_{50} values, the anti-radical scavenging efficacy of the tested tea samples followed this trend (from highest to lowest): Cyclone (Gitambo factory) > Fluff (Gitambo factory) > > Cyclone (Itumbe factory) > Cyclone fluff (Chelal factory) > Fluff dry mouth (Tombe factory) > Fluff (Kaptumo factory) > Cyclone fluff (Boito factory) > Fluff (Tombe factory) > Cyclone fluff (Kinoro factory) > Fluff (Chelal factory) > Cyclone (Mudete factory) > Fluff (Mudete factory) > Cyclone fluff (Kaptumo factory) > Fluff (Boito factory) > Cyclone (Mogogosiek factory) > BHT (Reference antioxidant) > Fluff (Itumbe factory) > Fluff (Kionyo factory) > Fluff (Momul factory) > Drier fly-off (Kinoro factory) > Fly-off (Kionyo factory) > Fluff (Mogogosiek factory) > Cyclone fluff (Mogogosiek factory) > Next to fly-off (Kionyo factory) > Fluff (Ogembo factory) > Cyclone (Tombe factory) (Table 1).

Table 1 Free Radical Scavenging Effects of the Studied Tea Samples

Sample	Percentage free radical scavenging activity at different sample concentrations					EC_{50} (µg/ml)
	3.125 µg/ml	6.25 µg/ml	12.5 µg/ml	25 µg/ml	50 µg/ml	
BCF	$15.50\pm0.60^{e}_{kl}$	$24.97\pm1.75^{d}_{lm}$	$49.35\pm1.57^{c}_{hij}$	$68.69\pm1.31^{b}_{ef}$	$85.34\pm0.51^{a}_{bcd}$	21.91
BF	$22.00\pm1.00^{e}_{hi}$	$32.68\pm2.54^{d}_{jk}$	$53.79\pm1.69^{c}_{g}$	$73.53\pm1.52^{b}_{cd}$	$85.78\pm0.70^{a}_{bcd}$	19.60
CCF	$14.51\pm1.02^{e}_{kl}$	$24.03\pm2.00^{d}_{m}$	$45.03\pm1.96^{c}_{k}$	$60.16\pm2.02^{b}_{jk}$	$77.03\pm1.24^{a}_{ijk}$	25.29
CF	$20.24\pm1.61^{e}_{ij}$	$40.73\pm1.49^{d}_{fgh}$	$52.96\pm1.06^{c}_{gh}$	$67.92\pm1.67^{b}_{efg}$	$79.77\pm1.96^{a}_{ghi}$	20.77
GC	$12.77\pm1.28^{e}_{l}$	$20.14\pm1.12^{d}_{m}$	$35.22\pm1.07^{c}_{l}$	$57.21\pm1.46^{b}_{kl}$	$69.33\pm1.35^{a}_{n}$	29.28
GF	$15.25\pm0.86^{e}_{kl}$	$20.75\pm2.54^{d}_{m}$	$33.14\pm2.11^{c}_{l}$	$55.37\pm1.24^{b}_{l}$	$70.88\pm1.30^{a}_{lmn}$	29.16
IC	$17.22\pm0.69^{e}_{jk}$	$29.00\pm2.65^{d}_{kl}$	$46.00\pm1.00^{c}_{jk}$	$63.00\pm1.00^{b}_{hij}$	$71.21\pm1.33^{a}_{lmn}$	25.60
IF	$29.67\pm1.53^{e}_{g}$	$45.33\pm1.53^{d}_{ef}$	$59.81\pm0.33^{c}_{ef}$	$71.00\pm1.00^{b}_{de}$	$82.00\pm1.00^{a}_{defg}$	17.67
KCF	$33.93\pm1.68^{e}_{ef}$	$42.33\pm1.53^{d}_{efgh}$	$52.33\pm2.08^{c}_{gh}$	$66.00\pm1.00^{b}_{fgh}$	$79.10\pm1.02^{a}_{ghi}$	19.65
KF	$25.22\pm2.11^{e}_{h}$	$39.67\pm1.53^{d}_{hi}$	$50.67\pm0.58^{c}_{ghi}$	$63.33\pm1.16^{b}_{hij}$	$74.67\pm2.52^{a}_{jkl}$	22.41
KNCF	$25.00\pm1.00^{e}_{h}$	$40.00\pm2.00^{d}_{ghi}$	$53.67\pm1.53^{c}_{g}$	$62.67\pm2.31^{b}_{hij}$	$79.00\pm1.00^{a}_{ghi}$	21.19
KDFo	$42.67\pm0.58^{e}_{bc}$	$51.67\pm1.53^{d}_{c}$	$61.00\pm1.00^{c}_{def}$	$74.33\pm1.16^{b}_{bcd}$	$81.00\pm1.00^{a}_{fghi}$	14.66
KOF	$39.00\pm1.00^{e}_{cd}$	$53.00\pm1.00^{d}_{c}$	$65.00\pm1.00^{c}_{bcd}$	$71.00\pm1.00^{b}_{de}$	$80.17\pm1.26^{a}_{ghi}$	14.99
KOFo	$45.00\pm1.00^{e}_{b}$	$52.00\pm2.00^{d}_{c}$	$63.33\pm1.16^{c}_{bcde}$	$75.67\pm0.58^{b}_{bc}$	$85.22\pm1.35^{a}_{cde}$	14.33
KNFo	$46.67\pm0.58^{e}_{b}$	$55.00\pm1.00^{d}_{bc}$	$62.67\pm1.16^{c}_{cdef}$	$76.33\pm1.53^{b}_{bc}$	$84.68\pm0.59^{a}_{cdef}$	12.70
MGC	$23.67\pm1.16^{e}_{hi}$	$39.00\pm1.00^{d}_{hi}$	$59.00\pm1.00^{c}_{f}$	$74.33\pm0.58^{b}_{bcd}$	$82.85\pm0.79^{a}_{defg}$	18.58
MGF	$43.33\pm1.16^{e}_{b}$	$51.33\pm0.58^{d}_{cd}$	$64.00\pm1.00^{c}_{bcd}$	$75.33\pm0.58^{b}_{bc}$	$81.33\pm1.52^{a}_{efgh}$	14.01
MCF	$43.33\pm0.58^{e}_{b}$	$52.33\pm1.16^{d}_{c}$	$67.33\pm1.16^{c}_{b}$	$75.00\pm1.00^{b}_{bc}$	$89.22\pm0.69^{a}_{ab}$	12.75
MMF	$35.67\pm1.16^{e}_{de}$	$50.33\pm1.16^{d}_{cd}$	$62.67\pm1.16^{c}_{cdef}$	$77.67\pm0.58^{b}_{b}$	$85.26\pm1.11^{a}_{bcde}$	14.71
MDC	$30.33\pm2.52^{e}_{gh}$	$46.67\pm0.58^{d}_{de}$	$53.33\pm1.16^{c}_{gh}$	$63.67\pm0.58^{b}_{hij}$	$77.37\pm0.55^{a}_{hijk}$	20.10
MDF	$35.00\pm1.00^{e}_{de}$	$43.00\pm1.00^{d}_{efgh}$	$54.00\pm1.73^{c}_{g}$	$62.33\pm1.16^{b}_{hij}$	$78.00\pm1.00^{a}_{hij}$	20.00
OGF	$51.00\pm1.00^{e}_{a}$	$59.33\pm1.53^{d}_{b}$	$66.67\pm1.53^{c}_{bc}$	$75.33\pm0.58^{b}_{bc}$	$88.26\pm1.10^{a}_{abc}$	10.62
TBC	$51.33\pm1.53^{e}_{a}$	$65.67\pm1.16^{d}_{a}$	$74.58\pm1.01^{c}_{a}$	$83.67\pm1.53^{b}_{a}$	$90.33\pm1.53^{a}_{a}$	7.41
FTDM	$22.33\pm1.53^{e}_{hi}$	$33.67\pm1.16^{d}_{jk}$	$48.00\pm1.73^{c}_{ijk}$	$61.00\pm1.00^{b}_{ijk}$	$70.70\pm1.22^{a}_{lmn}$	25.01
TBF	$30.00\pm2.00^{e}_{gh}$	$44.67\pm0.58^{d}_{efg}$	$51.67\pm0.58^{c}_{ghi}$	$64.67\pm1.16^{b}_{ghi}$	$73.67\pm1.53^{a}_{klm}$	21.23
BHT	$25.51\pm1.15^{e}_{h}$	$38.13\pm0.57^{d}_{hj}$	$55.21\pm0.21^{c}_{g}$	$70.00\pm1.52^{b}_{de}$	$89.21\pm0.37^{a}_{ab}$	18.51

The results are presented as $\bar{x} \pm SD$ for three replicate experiments. Means with different subscript letters within the same column and different superscript letters within the same row are significantly different ($P<0.05$; One-Way ANOVA with Tukey's *post hoc* test); BCF: Boito cyclone fluff; BF: Boito fluff; CCF: Chelal cyclone fluff; CF: Chelal fluff; GC: Gitambo cyclone; GF: Gitambo fluff; IC: Itumbe cyclone; IF: Itumbe fluff; KCF: Kaptumo cyclone fluff; KF: Kaptumo fluff; KNCF: Kinoro cyclone fluff; KDFo: Kinoro Drier fly-off; KOF: Kionyo fluff; KOFo: Kionyo fly-off; KNFo: Kionyo next to fly-off; MGC: Mogogosiek cyclone; MGF: Mogogosiek fluff; MCF: Momul cyclone fluff; MMF: Momul fluff; MDC: Mudete cyclone; MDF: Mudete fluff; OGF: Ogembo fluff; TBC: Tombe cyclone; TFDM: Tombe fluff dry mouth; TBF: Tombe fluff; BHT (Butylated Hydroxytoluene).

- *Concentration of Polyphenols of the Studied Tea Samples*

The current study investigated the polyphenolic content of various tea samples (Table 2). The tea samples collected as cyclone fluff and fluff from the Chelal and Mudete factories showed significantly higher concentrations of Theaflavin than the rest ($P<0.05$; Table 2). In contrast, the fly-off sample from the Kionyo factory exhibited a significantly lower Theaflavin concentration than all the other samples ($P<0.05$; Table 2). However, samples from different factories, such as Boito, Kinoro, Mogogosiek, and others, displayed no significant differences in their Theaflavin levels ($P>0.05$; Table 2).

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We also assessed differences in Theaflavin percentage abundance across samples, whereby the fluff sample from the Momul factory was distinct, presenting a significantly higher Theaflavin percentage than all the other samples (P<0.05; Table 2). Conversely, the fluff and fly-off samples from the Gitambo and Kionyo factories showed considerably lower percentages of Theaflavin (P<0.05) than other samples (Table 2). Nevertheless, many samples revealed no significant differences in their Theaflavin percentages (P>0.05), including those from Chelal, Ogembo, Gitambo, Itumbe, Mudete, and others (Table 2).

A pivotal aspect of the study also revolved around analysing Thearubigins, another polyphenolic compound. It was discerned that samples such as the cyclone fluff from the Chelal, Kaptumo, and Momul factories, among others, possessed comparable Thearubigins percentages (P>0.05); however, these percentages were significantly higher than other samples (P<0.05; Table 2). Besides, samples from places like Gitambo, Tombe, Chelal, and Kionyo displayed insignificant differences in their Thearubigins content (P>0.05; Table 2).

The investigation extended its purview to gauge the abundance of total polyphenols across the studied tea samples. Remarkably, cyclone samples from the Gitambo and Magogosiek factories and the cyclone fluff sample from Momul significantly exhibited higher percentages of total polyphenols than their counterparts (P<0.05; Table 2). In addition, various samples, including those from Boito, Chelal, Gitambo, Itumbe, Tombe, and others, demonstrated no meaningful differences in their total polyphenolic content (P>0.05; Table 2).

Table 2 Concentration of Polyphenols the Tested Samples

Sample	Theaflavin (µmole/g)	Theaflavin (%)	Thearubigins (%)	Total polyphenols (%)
BCF	9.18±0.67^{jk}	0.48±0.01^{jk}	13.55±0.15^{i}	12.91±0.20^{hij}
BF	10.35±0.22^{hi}	0.51±0.03^{ij}	15.43±0.04^{gh}	13.79±0.76^{gh}
CCF	22.86±1.19^{a}	0.95±0.04^{de}	22.48±0.24^{a}	18.70±0.50^{abc}
CF	15.10±0.32^{f}	0.86±0.04^{fgh}	17.48±0.59^{ef}	14.58±0.14^{fg}
GC	19.27±0.33^{b}	0.86±0.03^{fgh}	20.34±1.10^{c}	19.34±0.71^{a}
GF	17.66±0.42^{de}	0.21±0.01^{m}	10.76±0.41^{j}	15.03±0.68^{ef}
IC	15.27±0.38^{f}	0.77±0.03^{h}	20.05±1.22^{cd}	15.33±0.40^{ef}
IF	15.38±0.47^{f}	0.94±0.12^{ef}	15.20±0.23^{h}	15.46±0.26^{ef}
KCF	18.94±0.37^{bc}	1.22±0.05^{b}	21.52±1.25^{abc}	18.13±0.95^{cd}
KF	15.26±0.09^{f}	1.05±0.04^{c}	15.25±0.30^{gh}	17.98±0.12^{cd}
KNCF	7.98±0.54^{l}	0.41±0.01^{kl}	13.99±0.64^{hi}	9.96±0.12^{l}
KDFo	14.90±0.18^{f}	0.84±0.01^{gh}	16.82±0.35^{fg}	12.63±0.20^{ijk}
KOF	9.98±0.47^{ij}	0.52±0.01^{ij}	13.56±0.65^{i}	9.00±0.18^{l}
KOFo	4.34±0.21^{m}	0.22±0.02^{m}	18.26±0.64^{ef}	4.32±0.50^{m}
KNFo	15.23±0.50^{f}	0.79±0.01^{h}	21.04±1.23^{abc}	13.66±0.59^{ghi}
MGC	9.51±0.11^{ij}	0.39±0.04^{l}	14.63±0.36^{hi}	11.73±0.83^{k}
MGF	12.89±0.35^{g}	0.58±0.01^{i}	20.16±0.38^{cd}	19.24±0.33^{ab}
MCF	17.24±0.31^{e}	0.89±0.01^{efg}	20.77±0.64^{bc}	18.78±0.37^{abc}
MMF	18.28±0.61^{cd}	1.33±0.04^{a}	22.11±0.62^{ab}	17.29±0.38^{d}
MDC	10.95±0.26^{h}	0.54±0.04^{ij}	13.30±0.28^{i}	18.20±0.17^{bcd}
MDF	22.12±0.74^{a}	1.21±0.06^{b}	21.49±1.60^{abc}	12.18±0.52^{jk}
OGF	15.22±0.33^{f}	0.78±0.01^{h}	21.04±1.24^{abc}	12.12±1.06^{jk}
TBC	14.91±0.18^{f}	0.94±0.04^{ef}	18.68±0.47^{de}	15.90±0.58^{e}
TFDM	17.92±0.60^{de}	1.04±0.08^{c}	20.72±1.04^{bc}	14.90±0.13^{ef}
TBF	17.36±0.52^{de}	1.03±0.06^{cd}	20.62±1.19^{bc}	15.46±0.31^{ef}

The results are presented as $\bar{x} \pm SD$ for two replicate experiments. Means with different superscript letters within the same column are significantly different (P<0.05; One-Way ANOVA with Fisher's LSD *post hoc* test); BCF: Boito cyclone fluff; BF: Boito fluff; CCF: Chelal cyclone fluff; CF: Chelal fluff; GC: Gitambo cyclone; GF: Gitambo fluff; IC: Itumbe cyclone; IF: Itumbe fluff; KCF: Kaptumo cyclone fluff; KF: Kaptumo fluff; KNCF: Kinoro cyclone fluff; KDFo: Kinoro Drier fly-off; KOF: Kionyo fluff; KOFo: Kionyo fly-off; KNFo: Kionyo next to fly-off; MGC: Mogogosiek cyclone; MGF: Mogogosiek fluff; MCF: Momul cyclone fluff; MMF: Momul fluff; MDC: Mudete cyclone; MDF: Mudete fluff; OGF: Ogembo fluff; TBC: Tombe cyclone; TFDM: Tombe fluff dry mouth; TBF: Tombe fluff.

➢ *Concentration of Catechins in the Studied Tea Samples*

The study focused on determining the relative abundance of compounds such as Gallic acid (GA), Epigallocatechin (EGC), Catechin (C), Caffeine (CAFF),

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Epicatechin (EC), Epigallocatechin gallate (EGCG), and Epicatechin gallate (ECG) across various tea samples (Table 3). For Gallic acid (GA), the Kionyo factory's fly-off sample showed that significantly higher GA percentage than all other samples ($P<0.05$; Table 3). However, fluff samples from Mudete and Itumbe factories, cyclone fluff from Kaptumo and Kionyo factories, drier fly-off from Kinoro factory, fluff drier from Kionyo factory, and cyclone from Mogogosiek factory had significantly lower % GA than the rest of the samples ($P<0.05$; Table 3). In contrast, the % GA of the cyclone fluff and fluff samples obtained from Chelal factory, and cyclone from Ogembo factory, and among the samples drawn from Boito (cyclone and fluff), Gitambo (cyclone and fluff), Mogosiek (fluff), Momul (cyclone and cyclone fluff), Kinoro (fluff), Mudete (cyclone), Ogembo (fluff), and Tombe (fluff drier) were not significantly different ($P>0.05$; Table 3).

The fly-off sample from Kionyo factory had a significantly lower % (-) EGC than all the other samples ($P<0.05$; Table 3). Notably, the differences among the % (-) EGC of Boito (cyclone and cyclone fluff), Chelal factory (cyclone and cyclone fluff), Itumbe factory (cyclone), Kaptumo factory (fluff and cyclone fluff), Kinoro factory (drier fly-off), Kionyo factory (next to fly-off), Mogogosiek factory (Fluff), Mudete factory (cyclone), Ogembo factory (cyclone and fluff), and Tombe factory (fluff drier and fluff) ($P>0.05$; Table 3). Additionally, the % (-) EGC in the cyclone sample from Gitambo factory, cyclone fluff from Kaptumo factory, fluff drier from Kionyo, and cyclone fluff from Momul factory, and among sampled from Gitambo (fluff), Mogogosiek (cyclone), Momul (fluff), and Mudete (fluff) were not significantly different ($P>0.05$).

The results revealed that the cyclone samples from Itumbe and Ogembo factories, and fluff samples from Ogembo and Tombe factories had comparable ($P>0.05$) % (+) C levels, which were significantly ($P<0.05$) higher than those of all the other samples (Table 3). The % (+) C amounts measured in all the other analysed samples were not significantly different ($P>0.05$) as shown in Table 3).

Our findings also showed that the fly-off sample from Kionyo factory had significantly higher caffein content (% CAFF) than all the other samples ($P<0.05$; Table 3). Conversely, the fluff sample derived from Itumbe factory contained a significantly lower % CAFF than the rest of the sampled analysed int this study ($P<0.05$; Table 3). Comparatively, the % CAFF content in the samples from Boito factory (cyclone and fluff), Chelal factory (cyclone), Mogogosiek factory (cyclone), and Mudete factory (fluff) was not significantly different ($P>0.05$), as those from Chelal factory (cyclone fluff), Gitambo factory (cyclone), Kaptumo factory (fluff), Mogogosiek factory (fluff), Mudete factory (cyclone), and Ogembo factory (fluff) ($P>0.05$), and those from Kaptumo factory (cyclone fluff) and Kionyo factory (drier fly-off and fluff drier) ($P>0.05$; Table 3). Moreover, the samples sourced from Itumbe factory (cyclone), Kinoro factory (cyclone fluff), Momul factory (fluff and cyclone fluff), Ogembo factory (fluff), and Tombe factory (fluff drier and fluff) had comparable % CAFF content ($P>0.05$; Table 3).

As shown in Table 3, the % (-) EC content was significantly higher in the cyclone sample drawn from Boito factory than all the other samples ($P<0.05$). Besides, the % (-) EC levels in the samples from Gitambo factory (cyclone), Itumbe factory (cyclone and fluff), Kaptumo factory (fluff), Kinoro (drier fly-off), and Mudete factory (cyclone) were not significantly different ($P>0.05$); however, these levels were significantly lower than those in all the other samples ($P<0.05$; Table 3). In addition, the differences observed among the % (-) EC levels in the samples obtained from Chelal factory (cyclone fluff), Kaptumo factory (cyclone fluff), Momul factory (cyclone fluff and fluff), Mudete factory (cyclone), Ogembo factory (cyclone and fluff), and Tombe factory (fluff drier and fluff) were insignificant ($P>0.05$; Table 3).

In this study, the fluff sample from Itumbe factory had significantly lower % (-) EGCG content, while the cyclone fluff sample from Kaptumo factory and cyclone from Mudete factory were significantly higher than those of all the other tested samples ($P<0.05$; Table 3). However, no significant differences were observed among the % (-) EGCG contents of the samples from Boito factory (cyclone), Chelal factory (cyclone fluff), Kionyo factory (next to fly-off), Ogembo factory (fluff), and Itumbe factory (fluff and fluff drier) ($P>0.05$; Table 3). Also, the differences % (-) EGCG contents observed in the tea samples obtained from Boito factory (fluff), Chelal factory (cyclone), Kaptumo factory (cyclone fluff), Kinoro factory (drier fly-off), Kionyo factory (fluff drier and fly-off), Mogogosiek factory (cyclone), Momul factory (fluff), and Mudete factory (fluff), and among those obtained from Gitambo factory (cyclone and fluff), Itumbe factory (fluff and cyclone), Kaptumo factory (cyclone fluff), Momul factory (fluff and cyclone fluff), Mudete factory (cyclone) and Ogembo factory (cyclone) were not significantly ($P>0.05$; Table 3).

We observed that the epicatechin gallate content (% (-) ECG) in the tea samples from Boito factory, Chelal factory (fluff), and Mogogosiek factory (fluff and cyclone) were significantly higher than those of all the other tested samples ($P<0.05$; Table 3). Contrariwise, the tea samples from Itumbe factory (fluff and cyclone), and Kaptumo factory (cyclone fluff) had significantly lower % (-) ECG content than all the other evaluated samples ($P<0.05$; Table 3). Nevertheless, no significant differences were observed among the % (-) ECG measured in the tea samples from Gitambo factory (cyclone and fluff), Kaptumo factory (fluff), Momul factory (fluff), and Mudete factory (cyclone) ($P>0.05$; Table 3). Also, this study did not show any significant differences ($P>0.05$) among the % (-) ECG content obtained in the tea samples drawn from Chelal factory (cyclone fluff), Kaptumo factory (cyclone fluff), Kinoro factory (drier fly-off), Kionyo factory (fluff drier, next to fly off, and fly-off), Momul factory (cyclone fluff), Mudete factory (fluff), Ogembo factory (cyclone and fluff), and Tombe factory (fluff drier and fluff) ($P>0.05$; Table 3).

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Table 3 Concentration of Catechins in the Tea Samples from Various Factories

Sample	% GA	% (-) EGC	% (+) C	% CAFF	% (-) EC	% (-) EGCG	% (-) ECG
BC	0.15±0.00^{fghi}	1.28±0.13^{f}	0.54±0.02^{bcde}	3.43±0.16^{jkl}	1.62±0.07^{a}	0.57±0.03^{ghi}	0.83±0.03^{a}
BF	0.15±0.01^{fghi}	1.39±0.02^{cdef}	0.42±0.05^{bcde}	3.60±0.21^{ijk}	1.20±0.14^{cd}	0.48±0.01^{jkl}	0.83±0.01^{a}
CCF	0.19±0.01^{bcd}	1.48±0.11^{abcd}	0.58±0.04^{bcde}	4.67±0.21^{c}	0.78±0.05^{f}	0.65±0.03^{efg}	0.47±0.02^{efg}
CF	0.19±0.02^{bcd}	1.33±0.05^{def}	0.56±0.01^{bcde}	3.79±0.19^{hij}	1.20±0.05^{cd}	0.47±0.02^{kl}	0.83±0.01^{a}
GC	0.16±0.01^{defg}	0.70±0.07^{k}	0.48±0.03^{bcde}	4.37±0.19^{cdef}	0.53±0.04^{ijk}	0.78±0.07^{cd}	0.68±0.01^{bc}
GF	0.18±0.01^{cde}	1.03±0.11^{gh}	0.34±0.36^{cde}	0.42±0.02^{o}	0.76±0.06^{f}	0.80±0.01^{c}	0.70±0.02^{bc}
IC	0.16±0.01^{defg}	1.51±0.06^{abc}	0.84±0.05^{abc}	4.03±0.25^{fgh}	0.40±0.03^{k}	0.78±0.01^{cd}	0.19±0.01^{h}
IF	0.11±0.01^{k}	0.92±0.02^{ghi}	0.32±0.04^{cde}	0.37±0.07^{p}	0.49±0.02^{jk}	0.29±0.07^{m}	0.19±0.02^{h}
KPCF	0.14±0.01^{ijk}	1.39±0.02^{cdef}	0.60±0.07^{bcde}	4.14±0.19^{efgh}	0.65±0.10^{fghi}	1.07±0.05^{ab}	0.42±0.02^{gh}
KP	0.16±0.01^{defg}	1.41±0.03^{bcdef}	0.53±0.01^{bcde}	4.54±0.02^{cd}	0.50±0.02^{jk}	0.99±0.08^{b}	0.62±0.04^{cd}
KCF	0.14±00^{ijk}	0.72±0.12^{jk}	0.37±0.11^{cde}	2.85±0.50^{mn}	1.37±0.03^{b}	0.52±0.03^{ijkl}	0.57±0.05^{de}
KNDFo	0.13±0.01^{ijk}	1.61±0.01^{a}	0.30±0.07^{de}	5.61±0.16^{b}	0.54±0.11^{hijk}	0.52±0.01^{ijkl}	0.40±0.04^{g}
KFD	0.11±0.01^{k}	0.75±0.14^{ijk}	0.32±0.04^{cde}	2.23±0.10^{n}	1.21±0.04^{cd}	0.45±0.04^{l}	0.55±0.03^{de}
KFO	0.26±0.01^{a}	0.42±0.01^{l}	0.17±0.04^{e}	2.98±0.18^{lmn}	0.94±0.08^{e}	0.45±0.04^{l}	0.37±0.01^{g}
KNF	0.17±0.01^{cdef}	1.45±0.15^{abcdef}	0.62±0.03^{bcde}	6.08±0.11^{a}	0.96±0.07^{e}	0.60±0.02^{ghi}	0.52±0.21^{ef}
MC	0.13±00^{ijk}	1.09±0.01^{g}	0.37±0.02^{cde}	3.14±0.23^{klm}	1.42±0.04^{b}	0.47±0.02^{kl}	0.77±0.02^{ab}
MF	0.20±0.01^{bc}	1.45±0.14^{abcdef}	0.57±0.03^{bcde}	4.64±0.17^{c}	1.34±0.01^{bc}	0.85±0.04^{c}	0.82±0.01^{a}
MCF	0.17±0.01^{cdef}	0.89±0.02^{hij}	0.44±0.02^{bcde}	4.38±0.26^{cdef}	0.61±0.04^{ghij}	0.71±0.02^{de}	0.45±0.02^{fg}
MLF	0.17±0.03^{cdef}	1.04±0.09^{gh}	0.44±0.02^{bcde}	4.23±0.04^{defg}	0.69±0.06^{fgh}	0.54±0.04^{hij}	0.62±0.06^{cd}
MTC	0.17±0.02^{cdef}	1.51±0.03^{abc}	0.50±0.00^{bcde}	4.45±00^{cde}	0.48±0.01^{jk}	1.13±0.01^{a}	0.65±0.05^{cd}
MTF	0.12±0.02^{jk}	1.09±0.13^{g}	1.16±1.19^{a}	3.29±0.02^{jkl}	0.66±0.01^{fghi}	0.55±0.07^{hij}	0.40±0.04^{g}
OC	0.19±0.01^{bcd}	1.47±0.05^{abcdef}	0.91±0.01^{ab}	4.31±0.06^{cdefg}	0.71±0.03^{fg}	0.70±0.04^{ef}	0.42±0.02^{fg}
OF	0.17±0.03^{cdef}	1.58±0.12^{ab}	0.74±0.01^{abcd}	4.69±0.33^{c}	0.68±0.19^{fgh}	0.59±0.02^{ghi}	0.40±0.02^{g}
TFD	0.16±00^{defg}	1.32±0.07^{def}	0.45±0.01^{bcde}	3.94±0.05^{ghi}	0.66±0.04^{fghi}	0.65±0.04^{efg}	0.43±0.03^{fg}
TTF	0.21±0.01^{b}	1.30±0.11^{ef}	0.76±0.14^{abc}	4.12±0.18^{efgh}	0.61±0.06^{ghij}	0.62±0.03^{fgh}	0.44±0.01^{fg}

The results are presented as $\bar{x} \pm SD$ for two replicate experiments. Means with different superscript letters within the same column are significantly different (P<0.05; One-Way ANOVA with Fisher's LSD *post hoc* test); BC: Boito cyclone; BF: Boito fluff; CCF: Chelal Cyclone Fluff; CF: Chelal fluff; GC: Gitambo Cyclone; GF: Gitambo Fluff; IC: Itumbe Cyclone; IF: Itumbe Fluff; KCF: Kaptumo Cyclone Fluff; KP: Kaptumo Fluff; KPCF: Kinoro Cyclone fluff; KNDFo: Kinoro drier Fly off; KFD: Kionyo Fluff drier; KFO: Kionyo Fly off; KNF: Kionyo next to Fly-off; MC: Mogogosiek cyclone; MF: Mogogosiek Fluff; MCF: Momul; Cyclone Fluff; MLF: Momul Fluff; MTC: Mudete cyclone; MTF: Mudete Fluff; OC: Ogembo Cyclone; OF: Ogembo Fluff; TFD: Tombe Fluff drier; TTF: Tombe fluff; GA: Gallic acid; (-) EGC: Epigallocatechin; (+)C: Catechin, CAFF: Caffeine; (-) EC: Epicatechin; (-) EGCG: Epigallocatechin gallate; (-) ECG: Epicatechin gallate.

IV. DISCUSSION

This current study elucidates the antioxidant potential of tea waste samples harvested from diverse factories by evaluating their free radical scavenging activity at various concentrations. The findings underscore a notable trend whereby the free-radical-scavenging activity of the tea waste samples and the reference antioxidant-butylated hydroxytoluene (BHT) significantly increased, exhibiting an enhanced overall efficacy in antioxidation. Therefore, supporting an earlier report [27,28], there appears to be a dose-dependent relationship between concentration and phytochemical-derived-antioxidant activity.

Our findings highlight a significant variability in the percentage of free radical scavenging activity based on the sample's factory of origin. For instance, at a concentration of 3.125 µg/ml, cyclone fluff samples from Boito and Chelal factories demonstrated significantly lower free radical scavenging activities than their counterparts. Conversely, Ogembo and Tombe factories produced cyclone and fluff samples that exhibited markedly higher antioxidant activities when compared to those from other origins. Multiple factors, such as soil quality, cultivation practices, and harvesting techniques, may contribute to these inter-factory variations in the antioxidant efficacy of tea wastes [29,30]. This suggests that the geographic location possibly plays a critical role in tea's bioactive composition and antioxidant potential [31,32]. Furthermore, across various concentrations (6.25 µg/ml, 12.5 µg/ml, and 25 µg/ml), the cyclone sample from Tombe factory consistently outperformed other samples; this implies that specific processing or cultivating conditions at Tombe ultimately enhanced its free radical scavenging capability. Future research stands to gain significant benefits by delving into the specific factors responsible for this observed efficacy [11–13,33]. When we examined the highest concentration (50 µg/ml), cyclone fluff from the Momul factory and Tombe factory's cyclone surprisingly demonstrated a comparable efficacy to BHT. We found these results noteworthy, considering that BHT is

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an artificial antioxidant, and encourages us to explore further using natural alternatives because their performance could match specific pure tea samples and synthetically available antioxidants [34–36].

Regarding EC_{50} values, it was evident that Tombe Factory samples exhibited the lowest value – implying superior efficiency as antioxidants by scavenging the free radicals. Conversely, Gitambo's cyclone and fluff samples exhibited lower efficiency with the highest EC_{50} values - indicating reduced effectiveness. These findings align consistently across observations of these two distinct materials. Our findings corroborate those reported earlier [37–39] where variances in antioxidant activities within tea samples from diverse regions were also observed. However, some of our samples exhibit a higher degree of activity than what prior research [40] has reported. This discrepancy prompts further investigation: is it due to methodological differences, or could this be a groundbreaking discovery in natural antioxidants? Additionally, identifying samples with elevated antioxidant activities paves the way for more effective development of natural antioxidants. Further research suggesting that incorporating these potent tea samples in dietary regimens may provide prophylactic benefits as diseases related to oxidative stress - including cancer and cardiovascular disorders - are becoming more prevalent; furthermore, their use could mitigate the risks associated with such conditions [8,10,16,17].

The health benefits of Theaflavin and Thearubigins, essential polyphenols in tea are renowned for their antioxidant and anti-inflammatory effects [12,27]. Determining the concentration of these vital compounds in tea samples provides insightful data on variations among different factory processes. Factors such as tea type, processing methods, and storage conditions significantly influence their concentrations [38,41]. The current study noted that the cyclone fluff and fluff samples from Chelal and Mudete factories had significantly higher concentrations of Theaflavin, thus corroborating an earlier study [42]. Further investigation is necessary to determine the exact reasons for these elevated levels. Potential factors may include variations in the maturation levels of tea leaves, processing techniques, or even climatic conditions within the factory [43,44]. On the contrary, the Kionyo factory's fly-off sample exhibited a lower Theaflavin concentration; such diminished levels could affect the quality and flavour of tea and its health benefits - potentially rendering it less desirable among health-conscious consumers. Previous research has associated suboptimal processing conditions with decreased Theaflavin levels in teas [33].

Upon observation, several samples from different factories exhibited a Theaflavin concentration with no significant differences. This suggests potential uniformity in certain processing stages or raw material quality; an industry standard can be derived from this parity [2,45]. These findings align perfectly with those of other scholars who noted minimal variations of polyphenol levels within teas produced under similar processing standards at various factories and ecological zones [42,44]. Further emphasising the variations in absolute concentrations were the results of percentage abundance. Notably, the Theaflavin percentage in the fluff sample from the Momul factory stood higher, this hints at a superior tea quality with enhanced health benefits. Conversely, lower percentages appeared within both fluff and fly-off samples from Gitambo to Kionyo - an indicator once more towards possible suboptimal processing or inferior raw materials used [30,45].

Most samples had comparable levels of Thearubigins; however, some samples exhibited significantly higher quantities. The similarity in Thearubigins content of the studied samples across multiple factories potentially suggests an industry-wide practice finely tuned to retain Thearubigins at high concentrations [25,41,46]. This underlines the significance of thearubigins regarding temperature's impact on chemical reactions.

This study explored another pivotal aspect: the total polyphenols—a broad metric that constitutes all beneficial compounds in tea. The highest percentages were recorded in cyclone samples from Gitambo and Magogosiek factories, as well as a cyclone fluff sample from Momul. These high concentrations yield richer flavours and deliver superior health benefits [10,42]. It remains imperative to acknowledge- with precision and caution-the limitations of basing our judgments entirely on factory-based differentiations for polyphenol concentrations; other factors such as tea variety, harvest time, processing conditions, waste type, and storage conditions also exert significant influence [38,39].

The necessity of unravelling the chemical composition of various tea-derived products, particularly concerning catechins: these compounds are globally recognised for their health benefits, which include antioxidant, anticancer effects, antiinflammation, and cardioprotection [33,43]. Understanding this is imperative not only due to its potential industrial applications, but also because global consumption underscores a need for heightened consumer awareness regarding what they consume. This study determined the concentration of Gallic acid (GA), a pivotal polyphenolic compound; it unveils variations in the abundance of GA based on its factory of origin. Notably, compared to other samples: the fly-off sample from Kionyo factory presents an alarmingly higher percentage of GA. Available evidence attribute GA with three essential properties: anti-inflammatory action; antioxidation capability – crucial for neutralising free radicals that induce cellular damage–and anticancer potential–a promising defence against malignancy [47,48]. Consumers seeking these benefits might preferentially select products with a high GA content. Conversely, some factories may produce samples featuring significantly reduced GA percentages; this suggests variations in processing methods, the maturity of tea leaves, or environmental factors impact catechin synthesis and preservation [30].

Considering Epigallocatechin (% (-) EGC), a renowned antioxidant in tea [49], this study uncovered intriguing trends: notably lower % EGC within Kionyo factory's fly-off sample compared to others. This suggests potential loss

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during processing or sourcing from tea varieties with inherently low (-) EGC levels. The uniform lack of significant differences across various samples from multiple factories might suggest some degree of standardisation in these catechin-related processing or sourcing methods. Catechin (+C) - a well-known compound for its antioxidant activities. The results further revealed that factories that produce samples with notably higher levels of (+C), may confer intriguing health-benefits to consumers [49]. Interestingly, most samples showed no significant variation in % C+-, thus suggesting an industry-wide consistency in preserving it during tea processing.

Previous research shows that caffeine (% CAFF) not only holds prominence for its characteristic stimulatory effect but also for potential health benefits such as enhanced cognitive function [50]. The Kionyo factory fly-off sample had elevated caffeine content and can be beneficial to consumers who desire pronounced stimulation from their brew. The significantly lower levels of caffeine exhibited in specific samples - like the fluff from the Itumbe factory - suggest intriguing nuances in processing or sourcing; these warrant further exploration.

Boito factory's cyclone sample had significantly higher Epicatechin (% (-) EC) composition, compared to other samples. Research has shown that high levels of epicatechin can offer cardiovascular health benefits; thus, understanding the mechanisms or practices that elevate these levels could prove vital for consumers and industries alike [51]. Given its prominence in scientific discourse concerning the health benefits of tea - which span antioxidant, neuroprotective and anticancer properties[52] - we must accord special attention to Epigallocatechin gallate (% (-) EGCG). Certain samples notably from Kaptumo and Mudete factories boasted higher levels of EGCG—thus potentially positioning them as superior choices for those seeking health-centric consumption [53,54]. The study further highlights certain factories that prominently provided samples with significantly higher or lower levels of Epicatechin gallate (% (-) ECG). Like other catechins, ECG potentially confers health benefits— notably in terms of its antioxidant and anti-inflammatory capacities [52–55]. The observed variation among these samples from various tea processing factories emphasises the critical need for deeper investigations into the practices causing such disparities.

Thus, based on the study findings, tea wastes are potential sources of efficacious antioxidant amalgams that be used to avert oxidative stress and associated conditions. Furthermore, the utility of these wastes in nutraceutical and medical fields will significantly help mitigate environmental pollution through tea processing [4] by fostering their recycling into useful materials. Moreover, this initial report lays a firm framework valorizing the potential and sustainable utilization of tea waste, a previously neglected resource with diverse applications.

V. CONCLUSIONS AND RECOMMENDATIONS

We observed variability in free radical scavenging activity according to each sample's origin, suggesting significant roles of geographic location, soil quality, cultivation techniques, and harvesting and processing methods in forming bioactive composition within these tea waste materials. Notably, some tea waste samples' antioxidant activity was at par with the synthetic antioxidant BHT, prompting a more rigorous investigation into natural substitutes, especially from tea wastes. By showcasing superior antioxidant efficiency under specific processing conditions, the Tombe factory samples have advanced our understanding of tea waste's bioactive potential. This underscores the necessity for standardized processing practices to optimize polyphenol retention at optimum levels. Further investigations are encouraged to unearth the specific factors responsible for the observed efficacy, potential health benefits of tea waste samples in dietary regimens, and methodological differences that may lead to higher antioxidant activity compared to prior research. Besides, studies on this line should focus on understanding variations in polyphenol concentrations among various factory processes to demystify their impacts on quality and associated health benefits.

- *Availability of Data and Materials*

All data is presented within the manuscript; however, the authors may provide any additional information upon reasonable request.

- *Competing Interests*

We, the authors, declare that we do not have any competing interests/conflicts of interest regarding this publication.

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- *Authors' Contributions*

Thaddeus Mangenya conceived the research idea which was fostered by Daniel Kariuki, Johnson Kinyua, Martin Obanda, and Simon Ochanda. Thaddeus Mangenya performed the experiments, analysed the data, and wrote the draft manuscript. Gervason Moriasi donated reagents and optimised the design and experimental methods. Daniel Kariuki, Johnson Kinyua, Martin Obanda, and Simon Ochanda supervised the entire study. All authors reviewed and approved the final draft for submission and publication.

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