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Phytochemical and Antioxidant Profiling of Ivorian Cocoa Powders: A Comparative Study across Cultivation Zones

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Abstract

Cocoa (*Theobroma cacao L.*) is a major economic crop in Côte d'Ivoire, the world's leading producer, yet the influence of growing region on the phytochemical quality of exported cocoa powder remains insufficiently documented. This study compared the total polyphenol, flavonoid, and tannin contents, together with the antioxidant activity, of cocoa powders sourced from six major Ivorian production zones (Dabou, Séguéla, Man, Abengourou, Yamoussoukro, and San-Pédro). Bioactive compounds were extracted by ethanol/water maceration and quantified by UV-visible spectrophotometry using the Folin-Ciocalteu, aluminium chloride, and vanillin-type colorimetric assays; antioxidant activity was assessed by ABTS·+ radical scavenging. Total polyphenol content ranged from 10.87 ± 0.35 mg GAE/g (San-Pédro) to 33.06 ± 0.55 mg GAE/g (Dabou), with cultivation zone exerting a statistically significant effect (one-way ANOVA, $F(5, 12) = 5.73$, $p = 0.006$). Flavonoid content (3.03 - 5.95 mg CE/g) and tannin content (1.76 - 3.45 mg TAE/g) also varied across zones, although these differences did not reach statistical significance. Antioxidant activity, expressed as ABTS·+ inhibition, ranged from 29.82 ± 0.48% (San-Pédro) to 67.98 ± 0.46% (Dabou) and differed significantly among zones ($p < 0.05$), closely tracking polyphenol and flavonoid levels. Cocoa powders from Dabou and Séguéla consistently displayed the richest phytochemical profiles and the strongest antioxidant capacity, whereas San-Pédro cocoa was consistently the least concentrated. These findings indicate that cultivation zone is an important determinant of the functional quality of Ivorian cocoa powder and support the development of origin-based sourcing strategies for antioxidant-enriched cocoa products.

Keywords

Theobroma cacao, Polyphenols, Flavonoids, Tannins, Antioxidant Activity

1. Introduction

Cocoa remains a cornerstone of the Ivorian economy: Côte d'Ivoire is the world's leading producer, accounting for close to 40% of global output during the 2023/2024 season, according to the International Cocoa Organization (ICCO, 2025). The cocoa tree (*Theobroma cacao*), a member of the Malvaceae family native to South America, is now cultivated throughout the equatorial belt. Its seeds, commonly known as cocoa beans, form the raw material for chocolate manufacture [1] [2] and are also processed into cocoa powder and cocoa butter, two widely consumed products with applications in the food, pharmaceutical, and cosmetic industries [3]. In recent years, a growing body of research has highlighted the potential health and nutritional benefits of cocoa by-products [4]-[6]. The predominant bioactive compounds in cocoa are polyphenols, secondary metabolites responsible for the bitterness and astringency of the beans [7] and a major contributor to their sensory qualities [8]. Cocoa polyphenols comprise several classes, including flavonoids, tannins, phenolic acids, and various polymerised derivatives [9], dominated by procyanidins (58%), flavan-3-ols such as catechins (37%), and anthocyanins (4%) [10]. These compounds have been associated with antidiabetic and anti-obesity effects [11] [12] and may support gut health by favouring beneficial bacterial taxa over pathogenic species, suggesting a prebiotic potential [13]. On this basis, cocoa is increasingly regarded as a functional food owing to its content of bioactive compounds with putative benefits for human health [14]. Building on this foundation, the present study assessed the total polyphenol, flavonoid, and tannin contents, together with the antioxidant activity, of cocoa powders exported from Côte d'Ivoire, using spectrophotometric methods, and examined how these parameters vary with cultivation zone. This work aims to clarify the extent to which geographic origin shapes the phytochemical composition and functional quality of Ivorian cocoa.

2. Materials and Methods

2.1. Plant Material

The plant material used in this study consisted of dried cocoa beans (marketable cocoa) collected from six cocoa-growing zones of Côte d'Ivoire: Abengourou (east), Dabou (south), Niablé (central-west), Man (west), San-Pédro (south-west), and Yamoussoukro (centre).

In each zone, three samples were collected from three producers located in different localities. In total, 18 samples were collected. Each sample was ground separately using a multifunctional grinder (QE-100, Zhejiang Yili Tool Co., Ltd.,

China) and then sieved through a 125 μm mesh. The resulting powder was stored under refrigeration until subsequent analysis. Cocoa powder was prepared following the method of [15].

2.2. Extraction Procedure

Secondary metabolites were extracted according to [16]. Dried extracts were obtained by macerating 5 g of cocoa powder in 100 mL of ethanol/water (70:30, v/v) for 24 h in the dark under continuous magnetic stirring (1 g powder per 10 mL solvent). Extraction was repeated three times on the same plant residue (marc). The combined extracts were filtered and the solvent removed by oven evaporation at 40˚C. Extraction yield was calculated as follows:

$$\text{Yield}(\%) = \frac{M_1}{M_0} * 100$$

where M_1 is the mass of the plant extract (g) and M_0 is the mass of the plant material used (g).

2.3. Determination of Total Polyphenol Content

Total polyphenol content was determined by the Folin-Ciocalteu method [17]. Dried extracts, dissolved in ethanol/water (70:30, v/v) to a final concentration of 5 mg/mL, were mixed (30 μL) with 2.5 mL of Folin-Ciocalteu reagent (diluted 1:10) and incubated in the dark at room temperature (30˚C ± 2˚C) for 2 min. Sodium carbonate solution (2 mL, 7.5% Na_2CO_3) was then added, and the mixture incubated in a water bath at 50˚C for 15 min before rapid cooling. Absorbance was read at 765 nm against a distilled-water blank. Gallic acid was used as the reference standard, selected for its stability, well-characterized pharmacological activity, and representativeness of phenolic compounds generally [18] [19]. Content was calculated from a calibration curve constructed with gallic acid solutions (0 - 1.4 mg/mL $Y = 0.2X + 0.0012$; $R^2 = 1$) and expressed, from triplicate measurements, as milligrams of gallic acid equivalents per gram of cocoa powder (mg GAE/g).

2.4. Determination of Total Flavonoid Content

Total flavonoid content was determined colorimetrically [16] [20]. Dried extracts (5 mg/mL in ethanol/water, 70:30, v/v; 2.5 mL) were mixed with 0.75 mL of 5% sodium nitrite ($NaNO_2$) for 5 min, followed by 0.75 mL of 10% aluminium chloride ($AlCl_3$) and a 6 min dark incubation. Sodium hydroxide solution (5 mL, 1 N) was then added and the final volume adjusted to 25 mL with distilled water. Absorbance was read at 510 nm against a distilled-water blank. Catechin was used as the reference standard, and content was calculated from a calibration curve (0 - 0.9 g/L; $Y = 0.543X - 0.0379$, $R^2 = 0.992$) and expressed, from $Y = 2X + 0.0012$ triplicate measurements, as milligrams of catechin equivalents per gram of cocoa powder (mg CE/g).

2.5. Determination of Total Tannin Content

Total tannin content was measured by a colorimetric assay adapted from [21]. Extract (100 µL, 5 mg/mL) was combined with 7.5 mL of distilled water and 0.5 mL of Folin-Ciocalteu reagent, followed by 1 mL of 35% sodium carbonate (Na_2CO_3) solution and dilution to 10 mL with distilled water. The mixture was left to react at room temperature (25˚C - 30˚C) for 30 min. Absorbance was read at 734 nm against a distilled-water blank. Tannic acid was used as the standard (calibration range 0 - 1000 µg/mL; $Y = 2X - 0.0012$, $R^2 = 1$); results are expressed as milligrams of tannic acid equivalents per gram of cocoa powder (mg TAE/g).

2.6. Antioxidant Activity Assay

Antioxidant activity was assessed using the ABTS·+ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical-scavenging assay, following [22] with minor modifications. The ABTS·+ stock solution was prepared by combining 8 mmol/L ABTS solution (87.7 mg in 20 mL distilled water) with 3 mmol/L potassium persulfate solution (16.2 mg $K_2S_2O_8$ in 20 mL distilled water) and incubating in the dark at room temperature (30˚C ± 2˚C) for 16 h. A working solution was freshly prepared before each assay by diluting the stock solution in methanol to an absorbance of 0.70 ± 0.02 at 734 nm. Cocoa extract (100 µL, 2.5 mg/mL) was added to 3.9 mL of the ABTS·+ working solution, vortexed, and incubated in the dark at room temperature for 6 min. Absorbance was read at 734 nm against a methanol blank. Antioxidant activity was expressed as the percentage inhibition of the ABTS·+ radical relative to a Trolox calibration curve, with results additionally convertible to micromoles of Trolox equivalents (µmol TE) per gram of sample.

2.7. Statistical Analysis

Differences in total polyphenol, flavonoid, and tannin content, and in antioxidant activity, among production zones were tested by one-way ANOVA (factor "production zone", six levels, n = 3 replicates per level, N = 18 observations), with statistical significance set at $p < 0.05$.

3. Results

One-way ANOVA showed that production zone had a statistically significant effect on total polyphenol content ($F(5, 12) = 5.73$, $p = 0.006$, $\eta_p^2 = 0.70$) and on antioxidant activity, with San-Pédro cocoa consistently displaying markedly lower polyphenol levels than Dabou, Séguéla, and Man. No significant differences among zones were detected for total flavonoid or total tannin content, although the trend observed for flavonoid content ($p = 0.085$) suggests that a larger sample size might reveal a significant effect.

3.1. Total Polyphenol Content

Mean total polyphenol concentrations in cocoa powder extracts from the six production zones, determined from the gallic acid calibration curve, are presented in

Table 1 and Figure 1, Figure 2. Dabou cocoa contained the highest total polyphenol content (33.06 ± 0.55 mg GAE/g), followed by intermediate levels in Séguéla (27.78 ± 0.75 mg GAE/g), Man (25.69 ± 0.85 mg GAE/g), Yamoussoukro (23.20 ± 0.45 mg GAE/g), and Abengourou (21.61 ± 0.11 mg GAE/g); San-Pédro recorded the lowest content (10.87 ± 0.35 mg GAE/g). Zones ranked by increasing polyphenol concentration were therefore: San-Pédro < Abengourou < Yamoussoukro < Man < Séguéla < Dabou. One-way ANOVA ($p < 0.05$) confirmed that these inter-zone differences were statistically significant, identifying Dabou beans as the richest in total polyphenols and San-Pédro beans as the poorest.

Table 1. Total polyphenol content of Ivorian cocoa powder by production zone.

Production zone	Dabou	Séguéla	Abengourou	San-Pédro	Man	Yamoussoukro
Sample 1 (mg/g)	31.33	21.55	16.33	14.67	20.89	21.34
Sample 2 (mg/g)	31.33	23.83	28.62	09.32	24.25	23.90
Sample 3 (mg/g)	36.50	37.96	19.89	08.63	31.94	24.34
Mean ± SD (mg GAE/g)	33.06 ± 0.55	27.78 ± 0.75	21.61 ± 0.11	10.87 ± 0.35	25.69 ± 0.85	23.20 ± 0.45

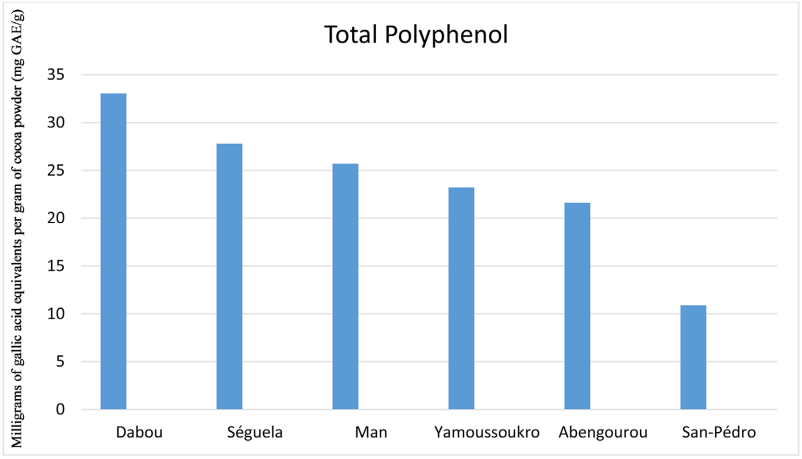


Figure 1. Mean total polyphenol content of exported cocoa by production zone.

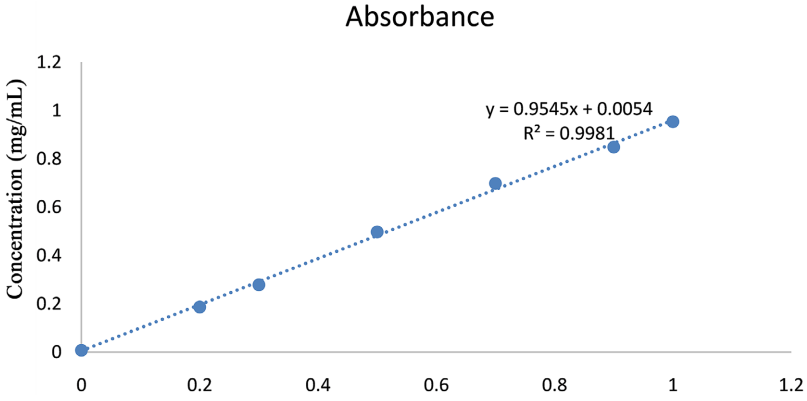


Figure 2. Calibration curve for total polyphenols (gallic acid standard).

3.2. Total Flavonoid Content

Total flavonoid content, determined from the catechin calibration curve, is presented in Table 2 and Figure 3, Figure 4. Content was highest in cocoa from Dabou (5.96 ± 0.12 mg CE/g) and Séguéla (4.58 ± 0.10 mg CE/g), with intermediate levels in Man (3.97 ± 0.04 mg CE/g), Abengourou (3.73 ± 0.08 mg CE/g), Yamoussoukro (3.07 ± 0.03 mg CE/g), and San-Pédro (3.03 ± 0.02 mg CE/g). Zones ranked by decreasing flavonoid content were: Dabou > Séguéla > Man > Abengourou > Yamoussoukro ≈ San-Pédro, indicating that Dabou and Séguéla beans were the richest in

Table 2. Total flavonoid content of Ivorian cocoa powder by production zone.

Production zone	Dabou	Séguéla	Abengourou	San-Pédro	Man	Yamoussoukro
Sample 1 (mg/g)	4.93	2.49	2.92	2.58	2.23	2.20
Sample 2 (mg/g)	6.41	5.40	4.49	3.95	4.49	3.11
Sample 3 (mg/g)	6.53	5.86	3.78	2.57	5.19	3.89
Mean ± SD (mg CE/g)	5.96 ± 0.12	4.58 ± 0.10	3.73 ± 0.08	3.03 ± 0.02	3.97 ± 0.04	3.07 ± 0.03

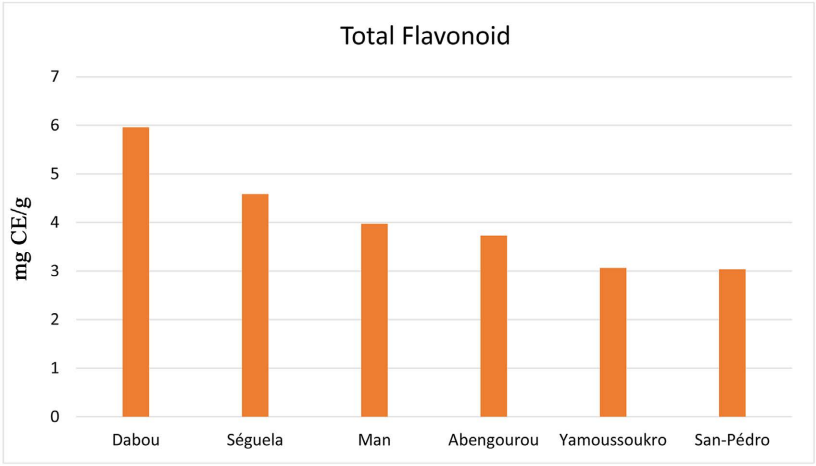


Figure 3. Mean total flavonoid content of exported cocoa by production zone.

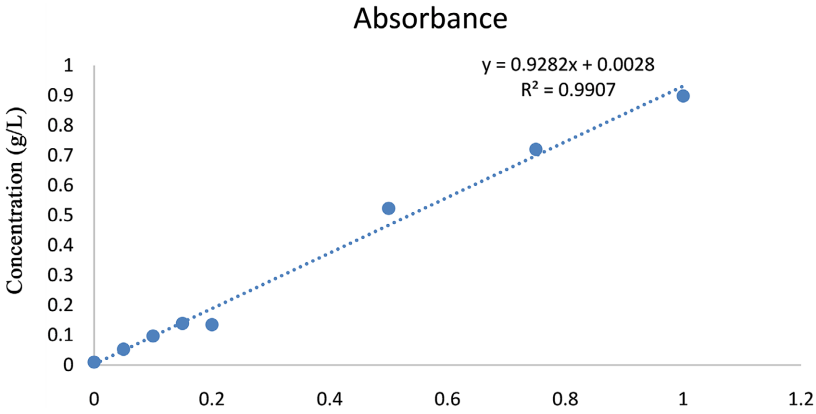


Figure 4. Calibration curve for total flavonoids (catechin standard).

total flavonoids while San-Pédro beans contained the lowest amounts. One-way ANOVA did not detect statistically significant differences among zones ($p > 0.05$), although the trend approached significance ($p = 0.085$).

3.3. Total Tannin Content

Mean total tannin concentrations, determined from the tannic acid calibration curve, are presented in Table 3 and Figure 5, Figure 6. Content was highest in Dabou cocoa (3.45 ± 0.15 mg TAE/g), with intermediate levels recorded in Séguéla (2.66 ± 0.10 mg TAE/g), Yamoussoukro (2.53 ± 0.05 mg TAE/g), Abengourou

Table 3. Total tannin content of Ivorian cocoa powder by production zone.

Production zone	Dabou	Séguéla	Abengourou	San-Pédro	Man	Yamoussoukro
Sample 1 (mg/g)	2.17	2.04	1.04	1.68	2.01*	1.57
Sample 2 (mg/g)	4.34	1.77	3.30	2.26	2.73*	2.67
Sample 3 (mg/g)	3.85	4.16	1.98	1.35	3.91*	3.34
Mean ± SD (mg TAE/g)	3.45 ± 0.15	2.66 ± 0.10	2.11 ± 0.09	1.76 ± 0.15	2.03 ± 0.07	2.53 ± 0.05

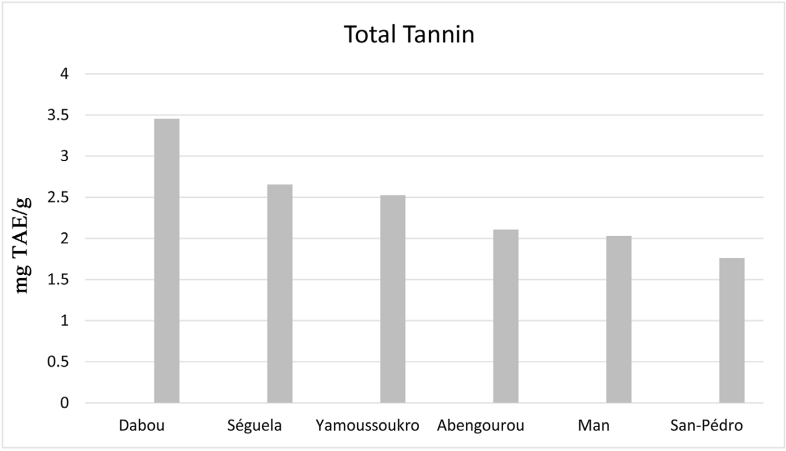


Figure 5. Mean total tannin content of exported cocoa by production zone.

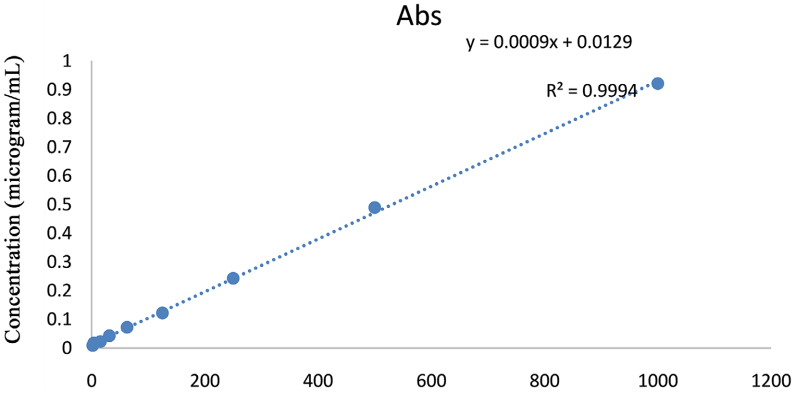


Figure 6. Calibration curve for total tannins (tannic acid standard).

(2.11 ± 0.09 mg TAE/g), and Man (2.03 ± 0.07 mg TAE/g); San-Pédro contained the lowest tannin content (1.76 ± 0.15 mg TAE/g). Zones ranked by decreasing tannin content were: Dabou > Séguéla > Yamoussoukro > Abengourou > Man > San-Pédro, confirming that Dabou beans possessed the highest tannin concentration and San-Pédro beans the lowest. Despite this apparent variability, one-way ANOVA revealed no statistically significant difference in tannin content among the six production zones ($p > 0.05$).

3.4. Antioxidant Activity

Antioxidant activity, measured as ABTS·+ inhibition, is presented in Table 4 and Figure 7, Figure 8. Activity was highest in cocoa from Dabou (67.98 ± 0.46%) and Séguéla (57.92 ± 0.59%), followed by comparable but slightly lower activity in Man (52.52 ± 0.51%), Yamoussoukro (50.60 ± 0.60%), and Abengourou (49.62 ± 0.42%); San-Pédro showed the lowest activity (29.82 ± 0.48%). Zones ranked by increasing antioxidant activity were: San-Pédro < Abengourou < Yamoussoukro < Man < Séguéla < Dabou, mirroring the ranking observed for total polyphenol content. Inhibition percentages ranged overall from 29.82% to 67.98%, indicating substantial variability in antioxidant potential according to bean origin. One-way ANOVA ($p < 0.05$) confirmed that these inter-zone differences were statistically significant.

Table 4. Antioxidant activity (ABTS·+ inhibition) of Ivorian cocoa powder by production zone.

Production zone	Dabou	Séguéla	Abengourou	San-Pédro	Man	Yamoussoukro
Sample 1 (%)	56.85	58.25	20.75	29.92	20.56	29.26
Sample 2 (%)	79.46	42.91	72.61	33.33	57.44	57.87
Sample 3 (%)	67.62	72.61	55.49	26.20	79.55	64.67
Mean ± SD (mg GAE/g)	67.98 ± 0.46	57.92 ± 0.59	49.62 ± 0.42	29.82 ± 0.48	52.52 ± 0.51	50.60 ± 0.60

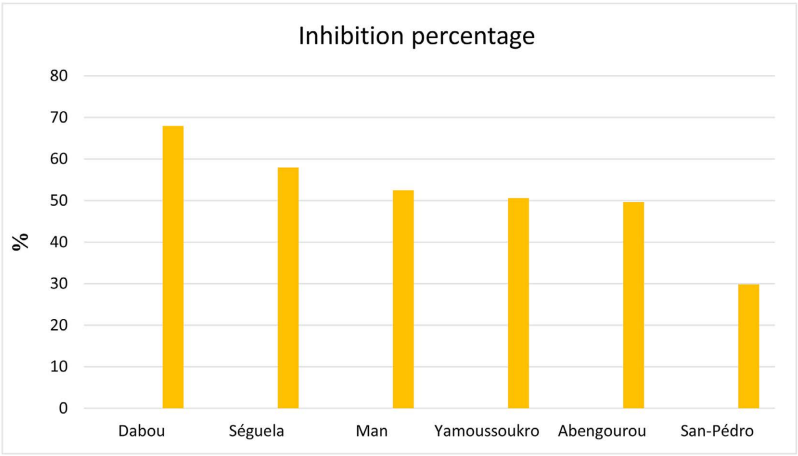


Figure 7. Inhibition percentage of exported cocoa powder by production zone.

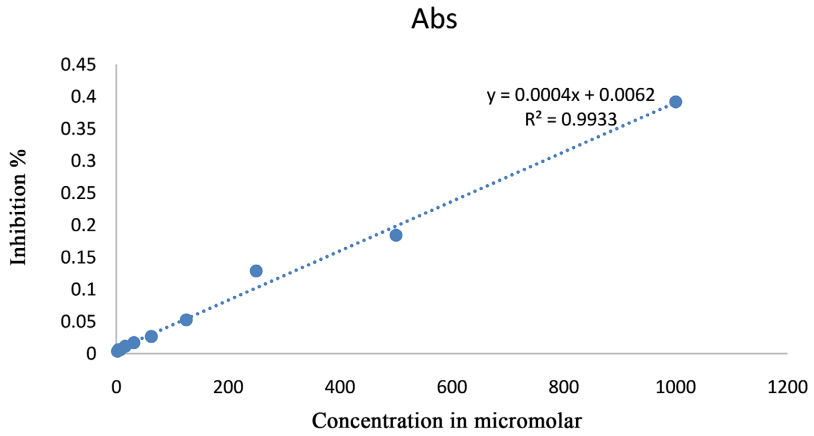


Figure 8. Trolox calibration curve for the ABTS·+ assay.

4. Discussion

4.1. Total Polyphenol Content

The total polyphenol contents recorded in this study are lower than several previously reported values for cocoa beans. Borja Fajardo *et al*. [23] found concentrations of 44.51 - 95.41 mg GAE/g in Colombian cocoa, Samaniego *et al*. [24] reported 42.75 - 71.66 mg GAE/g in Ecuadorian beans, and Sanchez-Capa *et al*. [25] recorded 57.23 - 79.18 mg GAE/g for National Arriba cocoa and 48.46 - 55.54 mg GAE/g for Trinitario cocoa. Several factors may account for the comparatively lower values observed here. Drying is a critical post-harvest operation that strongly influences the final quality of cocoa beans and their derived products [26]; five drying techniques are commonly used in the cocoa sector - sun, solar, oven, microwave, and freeze-drying [27]—each of which alters the physical structure of the beans and promotes the degradation of polyphenols [28] [29]. Oxidative degradation converts these compounds first into quinones and subsequently into melanins under the action of polyphenol oxidase [28] [30], an effect compounded by fermentation [25]. Regional differences in vegetation and agroecological conditions may further influence polyphenol biosynthesis. Conversely, several other studies have reported markedly lower polyphenol contents than those obtained here: Febrianto and Zhu [31] reported 0.501 - 0.917 mg GAE/g, Mihai *et al*. [32] 2.29 - 2.97 mg GAE/g, and Cortez *et al*. [33] values as low as 0.19 mg GAE/g, while de Barros Kobi *et al*. [34] reported 0.257 mg GAE/g and Deus *et al*. [29] 0.65 - 1.074 mg GAE/g. By contrast, Nieto-Figueroa *et al*. [35] (24.8 - 27.3 mg GAE/g) and Alean *et al*. [28] (33.30 mg GAE/g) reported values close to those of the present study, as did Akafou *et al*. [36] who, although working on cocoa pod husk rather than beans, measured higher polyphenol contents in yellow pods (35.50 ± 0.50 mg GAE/g dry extract) than in green pods (25.02 ± 0.01 mg GAE/g dry extract) - a range broadly comparable to our own results.

4.2. Total Flavonoid Content

Flavonoid content varied by production zone and was markedly lower than in

several previous reports. Loureiro *et al*. [37] recorded an epicatechin content of 43.27 mg/g in Forastero cocoa from the Amazon, close to the catechin content of 43.6 mg/g described by Febrianto and Zhu [38]. Schlüter *et al*. [39] found unfermented Trinitario cocoa to contain 38.9 mg/g (defatted dry matter) of epicatechin and a lower but still substantial 1.67 mg/g of catechin. Akafou *et al*. [36] similarly reported high total flavonoid contents in cocoa pod husk, both yellow (65.33 ± 0.33 mg epicatechin equivalents/g dry extract) and green (65.06 ± 0.04 mg epicatechin equivalents/g dry extract). De Taeye *et al*. [40], working with two Trinitario clones, detected the anthocyanins cyanidin-3-arabinoside and cyanidin-3-galactoside at 2.850 - 3.112 mg/g - higher than the levels found in fermented beans - while N'goran Haddad *et al*. [41] reported values ranging from 4.37 to 6.47 mg/g dry matter, closer to those obtained in the present study. This variability likely reflects differences in geographic origin, post-harvest processing, and analytical methodology; farming system, notably shading conditions, has also been shown to influence epicatechin and catechin content [34].

4.3. Total Tannin Content

Mean total tannin content in the present study ranged from 2.03 ± 0.07 to 3.45 ± 0.15 mg TAE/g dry extract. These values are substantially lower than those reported by Akafou *et al*. [36] for unfermented green and yellow cocoa pod husks (23.98 ± 0.02 and 12.23 ± 0.35 mg TAE/g dry extract, respectively) and by Ibero-Baraibar *et al*. [42], who reported a total tannin content of 240 mg TAE/g dry matter, but higher than the 0.908 mg/g dry matter reported by Gomez *et al*. [43]. This discrepancy may reflect differences in sample type and processing, particularly the absence of fermentation in some of the comparator studies.

4.4. Antioxidant Activity

The antioxidant activity values obtained here indicate that cocoa beans from Dabou and Séguéla possess strong antioxidant properties, consistent with the 47.2% - 85.8% inhibition range reported by Ali Faisal *et al*. [44] and the 68.09% inhibition reported by Racine *et al*. [45]. The strong correlation observed between antioxidant activity and both polyphenol and flavonoid content in the present dataset supports the established contribution of these compounds to the free-radical-scavenging capacity of cocoa [7], and underlines total polyphenol content as the principal driver of regional differences in antioxidant potential among the cocoa powders studied.

5. Conclusions

This study examined how geographic origin shapes the phytochemical composition and antioxidant potential of cocoa powders exported from six major Ivorian production zones—Dabou, Séguéla, Man, Abengourou, Yamoussoukro, and San-Pédro. Total polyphenol, flavonoid, and tannin contents, together with antioxidant activity, were quantified as key quality indicators and revealed substantial

regional variability. Cocoa powders from Dabou and Séguéla consistently showed the highest concentrations of bioactive compounds and the strongest antioxidant activity; Man, Yamoussoukro, and Abengourou produced cocoa of intermediate quality, while San-Pédro recorded the lowest values for all parameters measured.

The strong positive correlation observed between antioxidant activity and the levels of total polyphenols and flavonoids confirms the established role of these compounds in free-radical scavenging and in the nutritional quality of cocoa. These findings underscore the importance of cultivation zone in determining cocoa quality and suggest that cocoa from Dabou and Séguéla may offer enhanced nutraceutical and functional value. This regional profiling can support targeted sourcing strategies and inform the development of high-value, antioxidant-enriched cocoa products. These differences were statistically significant for total polyphenol content and antioxidant activity, and appear to be influenced by environmental factors such as soil moisture, organic matter content, pH, and mineral composition. A study of the soil chemical composition will be considered. Future work should examine the bioavailability of these phytochemicals and further investigate the agronomic and environmental determinants of cocoa quality at the regional level.

Author Contributions

K.A.B.B. and F.K. conceived the study. F.K. developed the methodology, and F.K. and C. S performed the computational analyses. D.S. and K.A.B. carried out validation and formal analysis. F.K. and K.A.B.B. conducted the investigation. S.C., D.S., K.A.B.B. and N.C.M.A.L. curated the data. S.C., F.K., and A.K.B. wrote the original manuscript. S.C. and A.K.B. contributed to writing, review, and editing. N.C.M.A.L. prepared the visualizations. K.A.B.B., D.S., and A.B. supervised the study. All authors discussed the results, contributed to the final manuscript, and approved the final version.

Conflicts of Interest

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

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