Journal of Agricultural Chemistry and Environment, 2026, 15(2), 216-240
https://www.scirp.org/journal/jacen
ISSN Online: 2325-744X
ISSN Print: 2325-7458

Influence of Soil Physicochemical Properties on the Polyphenolic Composition, Nutritional Profile and Antioxidant Activity of *Hibiscus sabdariffa* (R72-1 Variety) Calyces Cultivated in the Oubri and Guiriko Regions of Burkina Faso

Abdoul Rasmane Sawadogo[1]*, Arouna Ganame[1], Constantin Manienou Dabire[1], Bily Nebie[1], Abdoulaye Yougoubo[2], Mohammad Ouedraogo[1], Siaka Sosso[1], Remy Bationo[2], Tatiafa Da[1], Momouni Ouedraogo[1], Kalifa Coulibaly[3], Aboubacar Toguyeni[4]

[1]Laboratory of Chemistry and Renewable Energies, Nazi BONI University, Bobo-Dioulasso, Burkina Faso
[2]Department of Natural Substances, IRSAT/CNRST, Ouagadougou, Burkina Faso
[3]Laboratory for Soil Fertility Research and Studies, Nazi BONI University, Bobo-Dioulasso, Burkina Faso
[4]Laboratory for Natural Resources and Environmental Sciences Research and Studies, Nazi BONI University, Bobo-Dioulasso, Burkina Faso
Email: *abdouls40@yahoo.com

How to cite this paper: Sawadogo, A.R., Ganame, A., Dabire, C.M., Nebie, B., Yougoubo, A., Ouedraogo, M., Sosso, S., Bationo, R., Da, T., Ouedraogo, M., Coulibaly, K. and Toguyeni, A. (2026) Influence of Soil Physicochemical Properties on the Polyphenolic Composition, Nutritional Profile and Antioxidant Activity of *Hibiscus sabdariffa* (R72-1 Variety) Calyces Cultivated in the Oubri and Guiriko Regions of Burkina Faso. *Journal of Agricultural Chemistry and Environment*, **15**, 216-240.
https://doi.org/10.4236/jacen.2026.152012

Received: January 30, 2026
Accepted: May 11, 2026
Published: May 14, 2026

Abstract

The calyces of *Hibiscus sabdariffa*, predominantly produced in the Guiriko region of Burkina Faso, constitute a potential source of micronutrients owing to their high content of phenolic compounds. This study aimed to evaluate the influence of soil physicochemical characteristics on the polyphenolic composition and nutritional potential of calyx extracts from the R72-1 variety of *Hibiscus sabdariffa* cultivated in the Guiriko and Oubri regions of Burkina Faso. Soil samples collected from the Guiriko and Oubri regions were analyzed following the standardized BUNASOL procedures. Calyces' extracts were prepared using acetone/water/acetic acid (70:29.5:0.5), methanol-HCl (99:1), and distilled water as solvents. Total phenolic compounds, flavonoids, anthocyanins, and tannins were quantified by spectrophotometric methods, whereas total proteins and sugars were determined by the Kjeldahl and phenol-sulfuric methods, respectively. Antioxidant activity was assessed using DPPH, ABTS, and FRAP methods. Chromatographic profiles of the extracts were established by HPTLC. The results suggest that calyces harvested from Guiriko soils, which showed higher measured nutrient contents and more favorable soil chemical properties (score 37.5 ± 0.5 versus 25.75 ± 0.0 for Oubri), tended to exhibit greater contents of total phenolics (72.69 ± 1.89 mg GAE/g), flavonoids

(32.16 ± 1.95 mg QE/g), anthocyanins (25.48 ± 2.51 mg Cyd-3-glc eq/g), proteins (1.556% ± 0.312%), and sugars (40.82 ± 1.05 mg GE/g), the lowest levels of condensed tannins (0.198 ± 0.092 mg CE/g) and hydrolyzable tannins (0.278 ± 0.105 mg GAE/g), as well as stronger antioxidant activity (IC_{50} = 30.85 µg/ml), compared to calyces collected from Oubri soils. Conversely, six similar chromatographic bands were observed in calyces from both soil types, indicating that soil characteristics may influence anthocyanin content quantitatively rather than qualitatively. These findings highlight the nutritional and antioxidant potential of *Hibiscus sabdariffa* calyces and suggest that soil characteristics may help optimize their chemical and nutritional quality.

Keywords

Hibiscus sabdariffa, Anthocyanins, Polyphenolic Constituents, Nutritional Potential

1. Introduction

Hibiscus sabdariffa L., an herbaceous plant belonging to the family Malvaceae, is widely cultivated in tropical and subtropical regions, particularly in West Africa [1]. It is primarily valued for its fleshy calyces, which are used in the preparation of beverages, jams, natural colorants, and in traditional medicine [2]. The refreshing and tonic beverages derived from the calyces are rich in bioactive compounds such as phenolic compounds (anthocyanins, flavonoids, and tannins) [3], known for their antioxidant activities [2] [4]-[6] and notable therapeutic properties, including anti-hypoglycemic, anti-hyperinsulinemic, and anti-insulin-resistant effects [7]-[9]. They also represent an important source of sugars and proteins [3] [10].

However, several factors may influence the polyphenolic compositions and nutritional potential of calyx extracts, particularly the plant variety and the physicochemical characteristics of the soils [11]. Indeed, previous studies have documented significant variations in the polyphenolic composition and nutritional potential of calyces of the Korr, Vimto, Thai, and CLT92 cultivated in different regions of Senegal [12].

In Burkina Faso, the Guiriko region is one of the main production areas of the R72-1 variety of *Hibiscus sabdariffa*, where it is generally cultivated without fertilizer input [13]. Conversely, the Oubri region is characterized by low productivity of this crop [14] [15].

Understanding the influence of the physicochemical characteristics of soils in these regions on the polyphenolic compositions and nutritional potential of calyx extracts from the R72-1 variety of *Hibiscus sabdariffa* would be highly valuable and would contribute to the valorization of this variety. Therefore, the objective of this study was to assess how soil properties in the Guiriko and Oubri regions influence the phenolic composition, nutritional profile and antioxidant activities of calyces from the R72-1 variety of *Hibiscus sabdariffa*.

2. Methodology

2.1. Soil Sampling

Soil samples were collected from the 0-20 cm horizon using an auger, following the method described by Saba *et al.* [16], from soils in the Oubri region (SO) (Latitude: N 12˚39'20.67", Longitude: W 1˚14'50.226") and the Guiriko region (SG) (Latitude: N 11˚21'00.00", Longitude: W 4˚41'90.00"). The Guiriko site corresponds to a cultivated experimental soil, whereas the Oubri site corresponds to a degraded and previously abandoned soil with low agricultural productivity. At each site, five experimental plots established for this study were selected and five subsamples were collected from each plot and combined to form one composite soil sample. Sampling was performed along two diagonals with a central point. The 0 - 20 cm depth was chosen because it corresponds to the main root zone of *Hibiscus sabdariffa* and represents the soil layer most influenced by agricultural practices and nutrient availability. The collected samples were air-dried at room temperature for 7 days, gently crushed, and sieved before being packed in plastic bags and transported to the Laboratory for Soil Fertility Studies and Research for chemical analyses. Soil analyses were carried out over two consecutive years. In the first year, soils were characterized before sowing to determine their physicochemical properties and chemical fertility classes. *Hibiscus sabdariffa* was then cultivated, and the harvested calyces were analyzed for their phenolic and nutritional composition. In the second year, only soil analysis was performed after the rainy season to assess the evolution of soil physicochemical characteristics and chemical fertility.

2.2. Seed Sowing and Calyx Harvesting

The R72-1 variety of *Hibiscus sabdariffa* was selected for this study. The seeds were obtained from the Institute of Environmental and Agricultural Research (INERA) in Farako-Bâ (Bobo-Dioulasso). Manual sowing was carried out from July 14 to 16, 2024. Four to five seeds were placed per hole, with spacing of 0.40 m between holes and 0.80 m between rows [13].

The harvesting of calyces was carried out in successive stages from November 15 to December 20, 2024. After harvest, the calyces were air-dried on racks for seven days, protected from direct sunlight and humidity. The dried calyces were then crushed, packed in zip-lock bags, and stored for subsequent analyses.

2.3. Soil Sample Analysis

Soil pH was determined by direct reading using an electronic pH meter in a 1:2.5 soil-to-distilled water suspension for pH-H_2O and in a 1 M potassium chloride (KCl) solution for pH-KCl. Organic carbon (C-org) and total nitrogen (N-total) were measured by dry combustion after decarbonation using the Dumas method [17]. Total phosphorus (P-total) was quantified by automated colorimetry of the mineralized product after reaction with ammonium molybdate and ascorbic acid [18]. Available phosphorus (P-avail) was determined at 720 nm using a spectrophotometer after extraction with a mixed solution of ammonium chloride and hydrochloric acid followed by reaction with ammonium molybdate [19]. Total potassium (K-total) was measured using a flame photometer on the filtrate obtained after mineralization [20]. Available potassium (K-avail) and exchangeable bases (K^+, Ca^{2+}, Mg^{2+}, and Na^+) were extracted with 1 M ammonium acetate at pH 7. Calcium (Ca^{2+}) and magnesium (Mg^{2+}) were quantified by atomic absorption spectrophotometry, while K^+, Na^+, and K-avail were determined by flame photometry. Cation exchange capacity (CEC) was measured by percolating soil columns with 1 M ammonium acetate buffered at pH 7.

The assessment of soil fertility was based on the complete soil fertility scale described by Jongschaap, 1995 [21]. The classification was performed according to predefined threshold ranges for each parameter, allowing the soils to be categorized into different fertility classes.

2.4. Preparation of Calyx Extracts

Three types of extracts were prepared: a total phenolic-rich extract, an anthocyanin-rich extract, and an aqueous extract.

The total phenolic-rich extract (TPE) was prepared by macerating 100 g of plant material in 300 mL of an acetone/water/acetic acid mixture (70:29.5:0.5, v/v/v) for 72 h at 4˚C [22]. The resulting extract was then subjected to liquid-liquid partitioning with two 50 mL portions of chloroform to remove non-phenolic compounds [23].

For the anthocyanin-rich extract (ARE) and the aqueous extract (AE), 100 g of crushed dried calyx were macerated respectively in 300 mL of acidified methanol (1% HCl) and 300 mL of distilled water for 72 h at 4˚C, following one hour of ultrasonic sonication [24].

All procedures were performed in triplicate. The filtrates and fractions (TPE, ARE, and AE) were pooled and concentrated to dryness under reduced pressure (40 mbar) using a rotary evaporator and stored at 4˚C. The aqueous extract was subsequently lyophilized.

The different extraction yields were calculated using the following formula:

$$y(\%) = \frac{\text{mass of dry extract}}{\text{mass of macerated plant material}} \times 100$$

2.5. Purification of Anthocyanin Extracts

The anthocyanin extract was purified following the method described by Giusti *et al.* [25]. The crude extract was dissolved in acidified water (pH = 1), filtered, and loaded onto an Amberlite XAD-7 column (24 cm × 3 cm) packed with 200 g of resin. The column was first washed with 1000 mL of distilled water to remove sugars and other polar non-anthocyanin constituents. Elution was then carried out with 600 mL of methanol acidified with 1% HCl, and fractions of 200 mL were collected. The anthocyanin-enriched fractions were pooled, concentrated to a minimal volume, precipitated with ethyl acetate, and dried under vacuum in a desiccator.

2.6. Determination of the Contents of Selected Groups of Phenolic Compounds and Nutrients

The total phenolic content (TPC) of the extracts was determined using the Folin-Ciocalteu reagent method as described by Boizot *et al.* [26]. For each measurement, 60 µL of Folin-Ciocalteu reagent was added to 60 µL of diluted extract, followed by the addition of 120 µL of 7.5% sodium carbonate after 8 minutes. The mixture was then incubated at 37˚C for 30 minutes, and absorbance was measured at 760 nm. Total phenolic contents were calculated using a gallic acid calibration curve and expressed as mg GAE/g of plant material.

Total flavonoid content was assessed using the aluminum trichloride method described by Lebreton *et al.* [27]. A total of 50 µL of diluted extract was mixed with 150 µL of distilled water and 15 µL of 5% $NaNO_2$, followed by incubation for 5 min at room temperature. Then, 15 µL of 10% aluminum chloride was added and the mixture was incubated for 6 min, followed by the addition of 50 µL of 1 N NaOH. Absorbance was measured at 510 nm, and flavonoid contents were calculated using a quercetin calibration curve and expressed as mg QE/g of plant material.

Total anthocyanin content was measured using the pH-differential method according to Wrolstad *et al.* [24]. Two buffer systems (KCl, pH 1.0 and sodium acetate, pH 4.5) were used, and 0.5 mL of extract was mixed with 9.5 mL of the corresponding buffer and incubated for 15 min in the dark. Absorbance was measured at 510 nm and 700 nm, and the absorbance difference (A) was calculated between the two pH conditions. Monomeric anthocyanin content was determined using the following standard formula and expressed as mg/g:

$$[\text{Anthocyanes}]\left(\frac{\text{mg}}{\text{g}}\right)=\frac{A\times MW\times DF\times V\times 1000}{\varepsilon\times l\times m}$$

where A is the absorbance difference, MW the molecular weight, DF the dilution factor, V the volume, ε the molar extinction coefficient, l the path length (cm), and m the sample mass (g).

Hydrolyzable tannins were quantified using the method of Mole and Waterman [28]. For each analysis, 40 µL of diluted extract was mixed with 200 µL of 2.5% KIO_3 solution and incubated for 2 min (extracts) or 4 min (tannic acid standard). Absorbance was then measured at 550 nm against a reagent blank. Hydrolyzable tannin content was determined using a tannic acid calibration curve and expressed as mg TAE/g of dry material.

Condensed tannins were determined according to the method proposed by Swain and Hillis [28]. For each analysis, 23 µL of diluted extract was mixed with 171 µL of 4% vanillin solution and 86 µL of concentrated HCl, then incubated for 20 min. Absorbance was measured at 500 nm, and results were calculated using a catechin calibration curve and expressed as mg CE/g of dry material.

Total protein content was measured by the Kjeldahl method as described by Saez-Plaza *et al.* [29], based on nitrogen quantification and subsequent conversion into protein content. The distillation step was carried out using a UDK 149 distillation unit.

Total sugars were determined using the phenol-sulfuric acid method described by R'Zina *et al.* [30]. To 50 µL of extract were added 50 µL of 5% phenol solution and 250 µL of concentrated sulfuric acid. After mixing, the reaction mixture was incubated for 20 min at room temperature, and the absorbance was measured at 490 nm. Results were expressed as mg of glucose equivalents per gram, based on a calibration curve established using glucose as the standard.

All absorbance readings were carried out using a Multiskan SkyHigh microplate spectrophotometer (Thermo Scientific, USA). All calibration curves used for quantification are provided in the Supplementary Materials (**Figure S1 to S5**).

2.7. Determination of the Chromatographic Profiles of the Purified Anthocyanin Extracts

High-Performance Thin-Layer Chromatography (HPTLC) was performed using a CAMAG system. Solutions of the dry extracts were prepared at a concentration of 10 mg/mL and filtered through 0.22 µm syringe-compatible filters. An aliquot of 10 µL of each solution was automatically applied onto glass HPTLC plates (20 × 5 cm) coated with silica gel 60 F254 (MERCK KGaA, Germany) using a CAMAG applicator. Chromatographic development was carried out using a solvent system composed of ethyl acetate/formic acid/glacial acetic acid/water (100:11:11:26, v/v). Plates were visualized under visible light. No reference standards were used, as the objective of this analysis was not the formal identification of individual compounds but rather the assessment of the chromatographic profile. The number of detected bands and their corresponding Rf values were used to estimate the diversity of compounds present in the extracts [31].

2.8. Evaluation of Antioxidant Activities of the Extracts

Antioxidant activity of the extracts was evaluated using the DPPH, ABTS, and FRAP methods, as described by Lamien-Meda *et al.* [32], Miller *et al.* [33] and Pulido *et al.* [34], respectively. In these assays, Trolox was used as a reference antioxidant to establish calibration curves and express the antioxidant activity of the samples. Standard solutions of Trolox were prepared at concentrations ranging from 0 to 0.1 $mg \cdot mL^{-1}$.

In addition, the radical-scavenging activity of the extracts was further assessed using the DPPH method described by Dosseh *et al.* [35]. For this purpose, Trolox and ascorbic acid were used as reference antioxidants, and their standard solutions were prepared over a concentration range of 0 - 25 $\mu g \cdot mL^{-1}$. Similarly, extract solutions were prepared over concentration ranges of 0 - 50, 0 - 75, 0 - 100, or 0 - 150 $\mu g \cdot mL^{-1}$, depending on the extract. These ranges were used to establish regression curves of absorbance versus concentration for both reference antioxidants and sample extracts. The reaction mixtures were incubated for 15 min at room temperature in the dark before measuring the absorbance.

IC_{50} values were determined from the dose-response curves as the concentrations required to achieve 50% inhibition of the radical signal. The antioxidant activity index (AAI) was subsequently calculated to compare the radical-scavenging efficiency of the extracts. All measurements were performed in triplicate, and results were expressed as mean ± standard deviation. Statistical analyses were conducted to assess significant differences between samples ($p < 0.05$).

$$\text{AAI} = \frac{\text{Concentration of DDPH}^{\bullet}\left(\mu\text{g}\cdot\text{ml}^{-1}\right)}{\text{IC}_{50}\left(\mu\text{g}\cdot\text{ml}^{-1}\right)}$$

Based on the AAI values, classification was performed according to the scale proposed by Scherer *et al.* [36]:

- $\text{AAI} < 0.5$: low antioxidant activity
- $0.5 < \text{AAI} < 1$: moderate antioxidant activity
- $1 < \text{AAI} < 2$: strong antioxidant activity
- $\text{AAI} > 2$: very strong antioxidant activity

The calibration curves of Trolox and ascorbic acid for the DPPH, ABTS, and FRAP assays, as well as the curves used for the determination of IC_{50} values of Trolox, ascorbic acid, and the different extracts in the DPPH assay, are presented in the Supplementary Materials (**Figure S6 to S18**).

2.9. Data Analysis

Statistical analyses were performed using Statistica software version 12.3. Results are expressed as mean ± standard deviation (SD). Data were analyzed using one-way analysis of variance (ANOVA), followed by pairwise comparisons between groups. For soil parameters, comparisons were performed between soil types (Guiriko vs Oubri) within the same year, and between years for each soil type (year 1 vs year 2). For extract analyses, comparisons were systematically performed between samples from Guiriko and Oubri for each type of extract. Differences were considered statistically significant at $p < 0.05$. p-values were reported in the tables to facilitate interpretation of the observed differences.

3. Results

3.1. Physicochemical Characteristics of the Different Soils

Soil physicochemical parameters, as well as nutrient-related chemical properties, measured before sowing (year 1) and after the rainy season (year 2), are presented in Table 1 and Table 2.

Table 1 highlights the differences between the two soil types (Guiriko and Oubri) for each year. Overall, regardless of the year considered, Guiriko soils exhibited significantly higher nutrient contents than Oubri soils.

Table 2, on the other hand, illustrates the variations in physicochemical properties within the same soil type across the two years. The results indicate that, depending on the parameter considered, some variables showed significant differences between year 1 and year 2, whereas others did not vary significantly.

Table 1. Comparison of soil physicochemical parameters and nutrient-related properties between Guiriko and Oubri soils before sowing (year 1) and after the rainy season (year 2).

Parameters	Soil types (Year 1)				p-values	Soil types (Year 2)				p-values
	SG		SO			SG		SO		
	Mean	Rat	Mean	Rat		Mean	Rat	Mean	Rat	
OM (%)	17.93 ± 0.03	**5.00**	2.05 ± 0.03	**4.00**	0.000000*	18.76 ± 0.24	**5.00**	2.79 ± 0.08	**4.00**	0.000000*
N total (%)	0.92 ± 0.02	**4.00**	0.07 ± 0.02	**3.00**	0.000002*	1.02 ± 0.20	**4.00**	0.09 ± 0.03	**3.00**	0.001316*
P avail (mg/kg)	40.74 ± 0.31	**4.00**	18.67 ± 0.23	**3.00**	0.000005*	41.09 ± 026	**4.00**	19.12 ± 0.45	**3.00**	0.000000*
P total (mg/kg)	125.42 ± 4.34	**2.75**	67.37 ± 0.47	**2.50**	0.000077*	127.02 ± 1.14	**2.75**	72.08 ± 0.54	**2.50**	0.000001*
K avail (mg/kg)	63.42 ± 0.42	**3.00**	41.92 ± 0.20	**2.50**	0.000007*	59.69 ± 0.18	**3.00**	43.28 ± 0.26	**2.50**	0.000000*
K total (mg/kg)	2878.94 ± 17.35	**3.25**	965.15 ± 12.31	**2.75**	0.000001*	2865.94 ± 12.83	**3.25**	987.15 ± 16.83	**2.75**	0.000000*
CEC (cmol$^+$/kg)	12.96 ± 0.09	**3.00**	3.04 ± 0.03	**2.00**	0.000001*	12.37 ± 0.13	**3.00**	3.88 ± 0.28	**2.00**	0.000001*
BSR (%)	93.01 ± 0.20	**4.00**	46.71 ± 0.39	**3.00**	0.000000*	97.00 ± 0.15	**4.00**	45.10 ± 0.20	**3.00**	0.000000*
SEB (cmol$^+$/kg)	12.03 ± 0.11	**4.00**	1.42 ± 0.04	**2.00**	0.000001*	12.00 ± 0.11	**4.00**	1.75 ± 0.09	**2.00**	0.000001*
Ca^{2+} (cmol$^+$/kg)	6.42 ± 0.31	-	0.65 ± 0.16	-	0.000159*	6.96 ± 0.45	-	0.73 ± 0.19	-	0.000019*
Mg^{2+} (cmol$^+$/kg)	4.14 ± 0.47	-	0.28 ± 0.08	-	0.001668*	3.98 ± 0.68	-	0.33 ± 0.09	-	0.000000*
K^+ (cmol$^+$/kg)	0.97 ± 0.05	-	0.12 ± 0.05	-	0.000282*	1.02 ± 0.11	-	0.15 ± 0.06	-	0.000001*
Na^+ (cmol$^+$/kg)	0.02 ± 0.006	-	0.42 ± 0.08	-	0.000698*	0.04 ± 0.01	-	0.46 ± 0.05	-	0.000009*
pH (H_2O)	6.20 ± 0.02	**5.00**	4.15 ± 0.03	**1.00**	0.000007*	6.03 ± 0.05	**4.00**	4.18 ± 0.09	**1.00**	0.000001*
pH (KCl)	4.98 ± 0.28	-	2.81 ± 0.16	-	0.003591*	5.02 ± 0.34	-	2.92 ± 0.18	-	0.000492*
Final rating	**38.00**		**25.75**			**37.00**		**25.75**		
Chemical fertility class	High		Low			High		Low		

Rat: Rating; **SG:** soil from Guiriko region; **SO:** soil from Oubri region, **OM:** organic matter (1.724 × organic carbon), *$p < 0.05$: significant.

Table 2. Changes in soil physicochemical parameters and nutrient-related properties within each soil type (Guiriko and Oubri) between year 1 (before sowing) and year 2 (after the rainy season).

Parameters	SG				p-values	SO				p-values
	Year 1		Year 2			Year 1		Year 2		
	Mean	Rat	Mean	Rat		Mean	Rat	Mean	Rat	
OM (%)	17.93 ± 0.03	**5.00**	18.76 ± 0.24	**5.00**	0.004019*	2.05 ± 0.03	**4.00**	2.79 ± 0.08	**4.00**	0.007662*
N total (%)	0.92 ± 0.02	**4.00**	1.02 ± 0.20	**4.00**	0.408603	0.07 ± 0.02	**3.00**	0.09 ± 0.03	**3.00**	0.043572*
P avail (mg/kg)	40.74 ± 0.31	**4.00**	41.09 ± 026	**4.00**	0.208413	18.67 ± 0.23	**3.00**	19.12 ± 0.45	**3.00**	0.003131*
P total (mg/kg)	125.42 ± 4.34	**2.75**	127.02 ± 1.14	**2.75**	0.879010	67.37 ± 0.47	**2.50**	72.08 ± 0.54	**2.50**	0.032289*
K avail (mg/kg)	63.42 ± 0.42	**3.00**	59.69 ± 0.18	**3.00**	0.000145*	41.92 ± 0.20	**2.50**	43.28 ± 0.26	**2.50**	0.00299*
K total (mg/kg)	2878.94 ± 17.35	**3.25**	2865.94 ± 12.83	**3.25**	0.355639	965.15 ± 12.31	**2.75**	987.15 ± 16.83	**2.75**	0.555044
CEC (cmol$^+$/kg)	12.96 ± 0.09	**3.00**	12.37 ± 0.13	**3.00**	0.018246*	3.04 ± 0.03	**2.00**	3.88 ± 0.28	**2.00**	0.001215*
BSR (%)	93.01 ± 0.20	**4.00**	97.00 ± 0.15	**4.00**	0.001048*	46.71 ± 0.39	**3.00**	45.10 ± 0.20	**3.00**	0.979706
SEB (cmol$^+$/kg)	12.03 ± 0.11	**4.00**	12.00 ± 0.11	**4.00**	0.667738	1.42 ± 0.04	**2.00**	1.75 ± 0.09	**2.00**	0.321046

Continued

Ca^{2+} (cmol$^+$/kg)	6.42 ± 0.31	-	6.96 ± 0.45	-	0.162131	0.65 ± 0.16	-	0.73 ± 0.19	-	0.728171
Mg^{2+} (cmol$^+$/kg)	4.14 ± 0.47	-	3.98 ± 0.68	-	0.564556	0.28 ± 0.08	-	0.33 ± 0.09	-	0.061944
K^+ (cmol$^+$/kg)	0.97 ± 0.05	-	1.02 ± 0.11	-	0.222236	0.12 ± 0.05	-	0.15 ± 0.06	-	0.078979
Na^+ (cmol$^+$/kg)	0.02 ± 0.006	-	0.04 ± 0.01	-	0.041126*	0.42 ± 0.08	-	0.46 ± 0.05	-	0.023200*
pH (H_2O)	6.20 ± 0.02	**5.00**	6.03 ±0.05	**4.00**	0.005442*	4.15 ± 0.03	**1.00**	4.18 ± 0.09	**1.00**	0.961892
pH (KCl)	4.98 ± 0.28	-	5.02 ± 0.34	-	0.882632	2.81 ± 0.16	-	2.92 ± 0.18	-	0.130048
Final rating	38.00		37.00			25.75		25.75		
Chemical fertility class	High		High			Low		Low		

Rat: Rating; **SG:** soil from Guiriko region; **SO:** soil from Oubri region, **OM:** organic matter (1.724 × organic carbon), *p < 0.05: significant.

3.2. Extraction Yield

The extraction yields of the different extracts are presented in Figure 1. For all extracts, calyces harvested from Guiriko soils generally exhibited higher extraction yields than those obtained from Oubri soils.

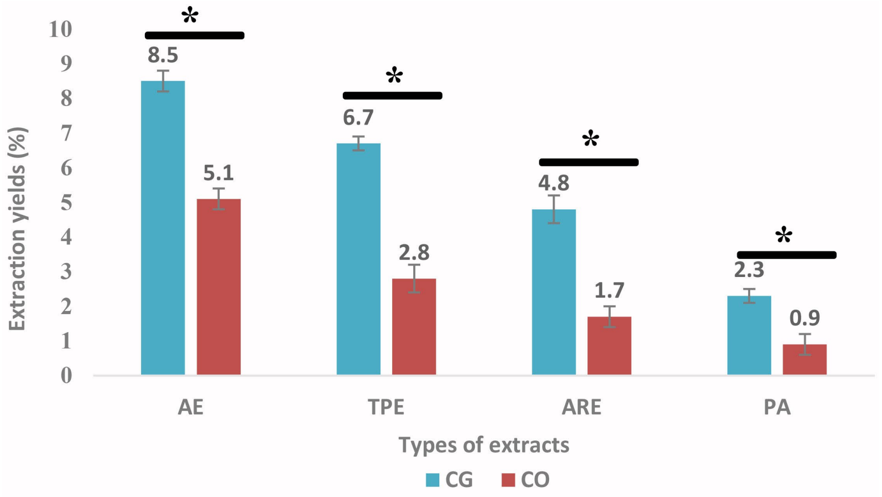


Figure 1. Extraction yields of calyx extracts. (**CG:** calyces extract from the Guiriko region; **CO:** calyces extract from the Oubri region; **AE**: aqueous extract; **TPE**: total phenolic-rich extract; **ARE**: anthocyanin-rich extract; **PA**: purified anthocyanins. Horizontal lines indicate comparisons between groups, and asterisks (*) denote statistically significant differences between CG and CO for each extract ($p < 0.05$)).

3.3. Contents of Selected Groups of Phenolic Compounds and Nutrients

The contents of phenolic compounds and nutrients in calyx extracts from Guiriko and Oubri soils are presented in Table 2. Guiriko samples had significantly higher levels of total phenolics, flavonoids, anthocyanins, proteins, and sugars than Oubri samples ($p < 0.05$). In contrast, condensed tannins and hydrolyzable tannins were significantly higher in Oubri samples.

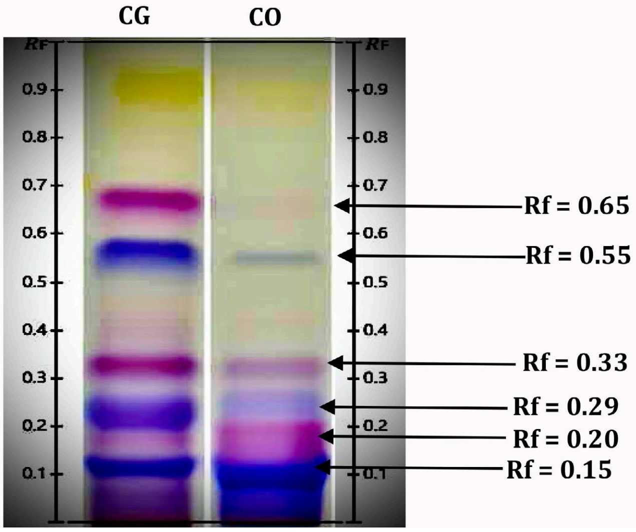
Table 3. Phenolic compounds groups and nutrients contents of calyx extracts from Guiriko and Oubri soils.

Phenolic compound and nutrients	Contents		p-values
	CG	CO	
TPC (mg GAE/g)	72.69 ± 1.89	28.65 ± 1.73	0.000003*
TFC (mg QE/g)	32.16 ± 1.95	15.92 ± 2.98	0.000002*
TAC (mg ECy/g)	25.48 ± 2.51	09.72 ± 1.98	0.000043*
CT (mg CAT/g)	0.198 ± 0.092	0.964 ± 0.124	0.000011*
HT (mg GAE/g)	0.278 ± 0.105	1.122 ± 0.098	0.000004*
TP (%)	1.556 ± 0.312	0.312 ± 0.068	0.001270*
TS (mg GE/g)	40.82 ± 1.05	25.43 ± 1.85	0.000006*

CG: calyces extract from the Guiriko region; **CO**: calyces extract from the Oubri region; **TPC (mg GAE/g)**: Total phenolics content (mg of gallic acid equivalents per g); **TFC (mg QE/g)**: Total flavonoids content (mg of quercetin equivalents per g); **TAC (mg ECy/g)**: Total anthocyanins content (mg of cyanidin equivalents per g); **CT (mg CAT/g)**: Condensed tannins (mg of catechin equivalents per g); **HT (mg GAE/g)**: Hydrolyzable tannins (mg of gallic acid equivalents per g); **TP**: Total proteins (%); **TS (mg GE/g)**: Total sugars (mg of glucose equivalents per g); Values are mean ± SD. * indicates significant differences between CG and CO ($p < 0.05$).

3.4. Chromatographic Profile of Anthocyanin Extracts

The chromatographic profiles obtained after purification of anthocyanin extracts from *Hibiscus sabdariffa* calyces grown on Guiriko and Oubri soils are illustrated in Figure 2.



Eluent: Ethyl acetate/formic acid/glacial acetic acid/water (100 :11 :11 :26, v/v)

Figure 2. Chromatographic profiles of anthocyanin extracts from *Hibiscus sabdariffa* calyces according to the soil. **CG:** calyces extract from the Guiriko region; **CO:** calyces extract from the Oubri region; **Rf**: retention factor.

Six similar chromatographic bands were observed in the purified anthocyanin extracts from *Hibiscus sabdariffa* calyces. These bands appeared in samples from both Guiriko and Oubri soils, although the bands from the Oubri soil showed lower intensities.

3.5. Total Antioxidant Capacity

The total antioxidant capacity of the calyx extracts, obtained from Guiriko and Oubri soils and evaluated using the DPPH, ABTS, and FRAP methods, is presented in Table 3.

Regardless of the method used, extracts from Guiriko soils consistently exhibited significantly higher antioxidant contents than those obtained from Oubri soils ($p < 0.05$).

Table 4. Total antioxidant capacity of calyx extracts from Guiriko and Oubri soils, determined by DPPH, ABTS, and FRAP methods.

Methods	Extracts	Total antioxidant capacity (mg TE/g of extract)		p-values
		CG	CO	
DPPH	**AE**	342.24 ± 8.02	185.32 ± 4.74	0.000008*
	TPE	286.62 ± 5.85	145.54 ± 5.18	0.000006*
	ARE	188.56 ± 4.96	95.82 ± 4.32	0.000016*
	PA	136.58 ± 6.68	67.98 ± 4.09	0.000106*
ABTS	**AE**	428.52 ± 5.66	287.72 ± 5.36	0.000005*
	TPE	372.56 ± 4.76	201.56 ± 4.88	0.000001*
	ARE	246.83 ± 5.12	142.42 ± 4.13	0.000009*
	PA	214.76 ± 4.92	96.58 ± 5.02	0.000009*
FRAP	**AE**	396.63 ± 4.89	257.54 ± 6.33	0.000007*
	TPE	321.08 ± 6.07	169.87 ± 2.62	0.000002*
	ARE	298.02 ± 3.72	105.78 ± 3.56	0.000000*
	PA	245.96 ± 5.42	83.92 ± 4.64	0.000002*

CG: calyces extract from the Guiriko region; **CO:** calyces extract from the Oubri region; **AE**: aqueous extract; **TPE**: total phenolic-rich extract; **ARE**: anthocyanin-rich extract; **PA**: purified anthocyanins, **TE**: trolox equivalent; * indicates statistically significant differences between CG and CO samples ($p < 0.05$).

3.6. Antiradical Activities

Figure 3 shows the antioxidant activities of calyx extracts from Guiriko and Oubri soils, along with standard antioxidants, expressed both as IC_{50} values and as antioxidant activity indices (AAI).

A statistically significant difference was observed between the IC_{50} and AAI values of CG and CO samples ($p < 0.05$), with CG samples exhibiting lower IC_{50} values and consequently higher AAI values.

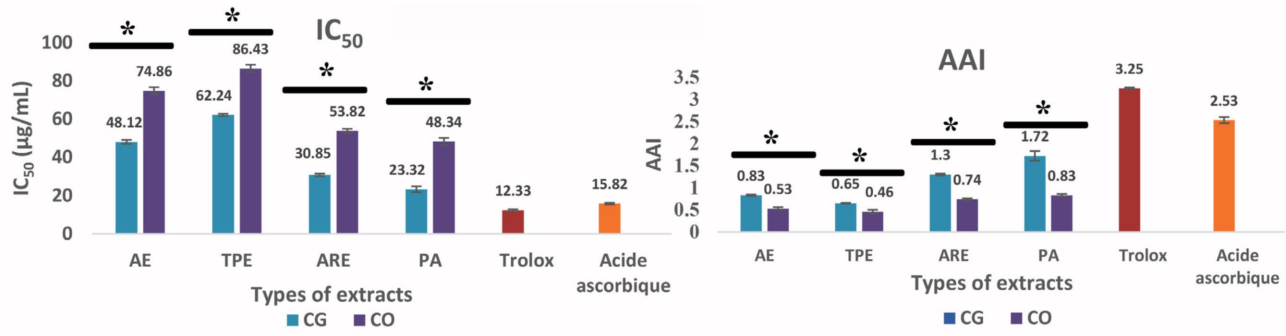


Figure 3. IC$_{50}$ and AAI of *Hibiscus sabdariffa* calyx extracts and standards. **CG:** calyces extract from the Guiriko region; **CO:** calyces extract from the Oubri region; **AE**: aqueous extract; **TPE**: total phenolic-rich extract; **ARE**: anthocyanin-rich extract; **PA**: purified anthocyanins. Horizontal lines indicate comparisons between groups, and asterisks (*) denote statistically significant differences between CG and CO for each extract ($p < 0.05$).

4. Discussion

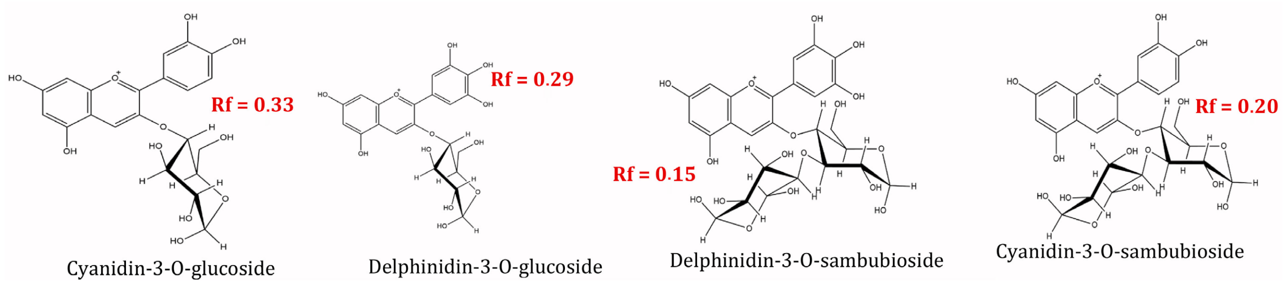
The Guiriko soil exhibited C/N ratios of 11.30 and 10.66 in the first and second years, respectively, which may reflect good organic matter stability and favorable soil chemical conditions [37]. In contrast, the Oubri soil, with ratios of 16.98 and 17.98, showed lower nutrient availability and less favorable soil chemical properties. Overall, some physicochemical parameters did not differ significantly between the two consecutive years at either site, suggesting relative stability that may be associated with the regularity of the rainfall regime. However, significant differences were observed in other parameters, which could be related to the presence and irregular decomposition of organic matter, thereby contributing to variations in the chemical properties of the soils [38]. The overall soil scores confirm these trends: Guiriko soils (scores 38 and 37) fall within the high soil fertility class according to the BUNASOL scale, whereas Oubri soils (25.75) correspond to the low chemical fertility class in the same classification system [21].

This edaphic disparity may contribute to the differences observed in extraction yields and in the phenolic and nutritional composition of the calyces. Samples from SG show the highest extraction yields and contents of phenolic compounds, flavonoids, anthocyanins, sugars, and proteins, which may reflect an intensification of secondary metabolism associated with higher measured soil nutrient contents (organic matter, nitrogen, phosphorus, and exchangeable bases). Conversely, calyces from SO exhibit lower levels of these groups of compounds, except for condensed and hydrolyzable tannins, whose higher accumulation may result from oxidative stress induced by the salinity of sodium-rich soils [39].

These observations contrast with some studies reporting a negative correlation between soil nutrient availability and secondary metabolite production [40] [41], but are consistent with other works highlighting the stimulatory effect of chemical fertilization on phenolic biosynthesis [42] [43]. These discrepancies may be explained by the specificity of the plant species, variety, and organ studied [44]. Indeed, Coly *et al.* [45] reported anthocyanin extraction yields of 1.48% and 0.77% from the dried calyces of the Vimto and Thai varieties of *Hibiscus sabdariffa*, respectively. Yields of 0.5% and 0.96% anthocyanins were also obtained from the Koor and CLT 92 varieties of the same plant, according to the studies of Cisse *et al.* and Coly *et al.* [3] [45].

Chromatographic analysis revealed six distinct bands in the calyces from both soils, indicating that both samples contain the same anthocyanin compounds. These bands exhibited retention factor (Rf) values of 0.15, 0.20, 0.29, 0.33, 0.55, and 0.65. This observation indicates that the effect of the soil physicochemical characteristics on anthocyanin composition is quantitative rather than qualitative. However, the chromatographic profile observed in the present study differs from those reported in the literature, where four bands corresponding to four distinct anthocyanins [3] [46] or five bands corresponding to five different anthocyanin compounds [47] have been described. This variation could be attributed to the specificity of the *Hibiscus sabdariffa* variety studied. The higher anthocyanin content observed in calyces from Guiriko soils suggests that nutrient availability may stimulate the phenylpropanoid, mevalonate, and shikimate pathways involved in anthocyanin biosynthesis [48]. By comparing the chromatographic profile obtained in the present study with the Rf values and chromatographic patterns previously reported for anthocyanins from *Hibiscus sabdariffa* analyzed under similar HPTLC conditions, four major bands were tentatively associated with delphinidin-3-sambubioside, cyanidin-3-sambubioside, delphinidin-3-glucoside, and cyanidin-3-glucoside. These assignments were based on the similarity between the observed Rf values of the major bands and those reported in the literature for these anthocyanins [49]. The chemical structures of these anthocyanins, reported in the literature for *Hibiscus sabdariffa*, are presented in **Figure 4**. However, these assignments remain provisional because HPTLC analysis alone does not allow unambiguous structural identification. Structural confirmation would require further purification and analysis using high-resolution chromatographic and spectroscopic techniques such as HPLC-DAD-MS/MS or NMR.

Furthermore, the CG extracts exhibited the strongest antioxidant activity, as indicated by their low IC_{50} values and high antiradical activity indices. The purified anthocyanin extract from CG showed an IC_{50} of 23.32 ± 1.49 μg/mL, close to that of ascorbic acid (15.82 ± 0.46 μg/mL), with a strong antioxidant activity (AAI = 1.72 ± 0.11). This variation in antioxidant activities could be explained by differences in phenolic compound and nutrient contents. These results support the important role of phenolic compounds, particularly anthocyanins, in the antioxidant activity of *Hibiscus sabdariffa* calyces, which is consistent with the higher contents of total phenolics, flavonoids, and anthocyanins observed in the samples from Guiriko soils. [4]. They also support the findings of Arena *et al.* [50], who reported an increase in the antioxidant activity of *Berberis microphylla* extracts in relation to differences in soil nutrient availability.



Cyanidin-3-O-glucoside Delphinidin-3-O-glucoside Delphinidin-3-O-sambubioside Cyanidin-3-O-sambubioside

Figure 4. Chemical structures of anthocyanins reported in the literature [46] [49].

5. Conclusions

This study evaluated the effect of soil physicochemical properties from the Guiriko and Oubri regions on the phenolic composition and nutritional potential of calyx extracts from the R72-1 variety of *Hibiscus sabdariffa*.

The soils from the Guiriko region, characterized by higher measured nutrient contents and more favorable chemical properties, could contribute to the accumulation of secondary metabolites and enhance the antioxidant activity of calyces from the R72-1 variety of *Hibiscus sabdariffa*. In contrast, the soils from the Oubri region, characterized by lower nutrient contents and higher sodium levels, may limit the biosynthesis of these compounds, while inducing an increase in tannins, likely as a response to salt stress. These results suggest an important influence of soil physicochemical properties on the polyphenolic constituents and nutritional characteristics, as well as the antioxidant potential, of the calyces. These findings also open new perspectives for the targeted improvement of calyx quality through optimized soil nutrient management in Oubri, highlighting the importance of appropriate agronomic practices to maximize the bioactive value of this plant resource.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Cisse, M., Dornier, M., Sakho, M., MarDiop, C., Reynes, M. and Sock, O. (2009) La production du bissap (*Hibiscus sabdariffa* L.) au Sénégal. *Fruits*, **64**, 111-124. https://doi.org/10.1051/fruits/2009006

[2] Da-Costa-Rocha, I., Bonnlaender, B., Sievers, H., Pischel, I. and Heinrich, M. (2014) *Hibiscus sabdariffa* L.—A Phytochemical and Pharmacological Review. *Food Chemistry*, **165**, 424-443. https://doi.org/10.1016/j.foodchem.2014.05.002

[3] Cisse, M., Dornier, M., Sakho, M., Ndiaye, A., Reynes, M. and Sock, O. (2009) Le bissap (*Hibiscus sabdariffa* L.): Composition et principales utilisations. *Fruits*, **64**, 179-193. https://doi.org/10.1051/fruits/2009013

[4] Palé, É., Kouda-Bonafos, M. and Nacro, M. (2004) Caractérisation et mesure des activités anti-radicalaires d'anthocyanes de plantes du Burkina Faso. *Comptes Rendus. Chimie*, **7**, 973-980. https://doi.org/10.1016/j.crci.2003.12.019

[5] Mohd-Esa, N., Hern, F.S., Ismail, A. and Yee, C.L. (2010) Antioxidant Activity in

Different Parts of Roselle (*Hibiscus sabdariffa* L.) Extracts and Potential Exploitation of the Seeds. *Food Chemistry*, **122**, 1055-1060. https://doi.org/10.1016/j.foodchem.2010.03.074

[6] Serrano-Cruz, M.R., Villanueva-Carvajal, A., Morales Rosales, E.J., Ramírez Dávila, J.F. and Dominguez-Lopez, A. (2013) Controlled Release and Antioxidant Activity of Roselle (*Hibiscus sabdariffa* L.) Extract Encapsulated in Mixtures of Carboxymethyl Cellulose, Whey Protein, and Pectin. *LWT—Food Science and Technology*, **50**, 554-561. https://doi.org/10.1016/j.lwt.2012.08.013

[7] Kuo, C., Kao, E., Chan, K., Lee, H., Huang, T. and Wang, C. (2012) *Hibiscus sabdariffa* L. Extracts Reduce Serum Uric Acid Levels in Oxonate-Induced Rats. *Journal of Functional Foods*, **4**, 375-381. https://doi.org/10.1016/j.jff.2012.01.007

[8] Lin, H., Chan, K., Sheu, J., Hsuan, S., Wang, C. and Chen, J. (2012) *Hibiscus sabdariffa* Leaf Induces Apoptosis of Human Prostate Cancer Cells *in Vitro* and *in Vivo*. *Food Chemistry*, **132**, 880-891. https://doi.org/10.1016/j.foodchem.2011.11.057

[9] Peng, C., Chyau, C., Chan, K., Chan, T., Wang, C. and Huang, C. (2011) *Hibiscus sabdariffa* Polyphenolic Extract Inhibits Hyperglycemia, Hyperlipidemia, and Glycation-Oxidative Stress While Improving Insulin Resistance. *Journal of Agricultural and Food Chemistry*, **59**, 9901-9909. https://doi.org/10.1021/jf2022379

[10] Ismail, A., Ikram, E.H.K. and Nazri, H.S.M. (2008) Roselle (*Hibiscus sabdariffa* L.) Seeds Nutritional Composition Protein Quality and Health Benefits. *Food*, **2**, 1-16.

[11] Mahunu, G.K. (2021) Breeding, Genetic Diversity, and Safe Production of *Hibiscus sabdariffa* under Climate Change. In: Mariod, A.A., Tahir, H.E. and Mahunu G.K.,, Eds., *Roselle (Hibiscus sabdariffa)*, Elsevier, 1-14. https://doi.org/10.1016/b978-0-12-822100-6.00005-7

[12] Alé, K. (2016) Valorisation de *Hibiscus sabdariffa* L.: Amélioration des procédés traditionnels de transformation, développement de nouveaux produits fonctionnels (extraits anthocyaniques). Ph.D. Thesis, Université Cheikh Anta Diop de Dakar.

[13] Hien, N. (2012) Etude comparée de deux systèmes de culture (pluvial et irrigue) de la roselle (*Hibiscus sabdariffa* L.) dans la province du IOBA: Évaluation du potentiel de production en calices. Ph.D. Thesis, Université Polytechnique, Bobo-Dioulasso, 62 p.

[14] Nébié, O. (1996) Dégradation du milieu et aménagement dans le plateau central, Burkina Faso. *Berichte des Sonderforschungsbereichs*, **268**, 149-176.

[15] Toure, H.A. and Zerbo, R. (2022) Perceptions du changement climatique et adaptation aux risques naturels au Centre-Nord et au Plateau-Central du Burkina Faso. *Espaces Africains* (*Revue des Sciences Sociales*), **1**, 93-108.

[16] Saba, F. (2023) Etude des effets de l'utilisation combinée du biochar et de la fertilisation microdose sur les propriétés agro-pédologiques des zones ouest et centre-nord du Burkina Faso. Ph.D. Thesis, Université Nazi Boni.

[17] Caria, G., Proix, N. and Ciesielski, H. (2007) Dosage du carbone organique par combustion sèche après décarbonatation automatisée des sols. Institut National de la Recherche Agronomique, Les 8èmes rencontres de la fertilisation raisonnée et de l'analyse de terre du COMIFER et du GEMAS.

[18] Novozamsky, I., Houba, V.J.G., van Eck, R. and van Vark, W. (1983) A Novel Digestion Technique for Multi-Element Plant Analysis. *Communications in Soil Science and Plant Analysis*, **14**, 239-248. https://doi.org/10.1080/00103628309367359

[19] Bray, R.H. and Kurtz, L.T. (1945) Determination of Total, Organic, and Available Forms of Phosphorus in Soils. *Soil Science*, **59**, 39-46. https://doi.org/10.1097/00010694-194501000-00006

[20] Walinga, I. (1989) Plant Analysis Procedures. Syllabus Part 7. 1989: Wageningen Agricultural University.

[21] Jongschaap, R. (1995) BAOBAB-La Base des donnees des Analyses Chimiques et des OBservations Agro-pédologiques du Bunasols. Version française. AB-DLO.

[22] Kaboré, D.S., Hema, A., Koala, M., Somé, K., Palé, E., Somé, I.T., Duez, P. and Nacro, M. (2021) Evaluation des teneurs en antioxydants et micronutriments des feuilles de onze variétés de Ipomoea batatas à chairs orange et pourpre produites au Burkina Faso. *Journal de la Société Ouest-Africaine de Chimie*, **50**, 1-10. https://hdl.handle.net/20.500.12907/35813

[23] Asami, D.K., Hong, Y., Barrett, D.M. and Mitchell, A.E. (2003) Comparison of the Total Phenolic and Ascorbic Acid Content of Freeze-Dried and Air-Dried Marionberry, Strawberry, and Corn Grown Using Conventional, Organic, and Sustainable Agricultural Practices. *Journal of Agricultural and Food Chemistry*, **51**, 1237-1241. https://doi.org/10.1021/jf020635c

[24] Wrolstad, R.E., Acree, T.E., Decker, E.A., Penner, M.H., Reid, D.S., Schwartz, S.J., Shoemaker, C.F., Smith, D.M. and Sporns, P. (2005) Handbook of Food Analytical Chemistry, Volume 1: Water, Proteins, Enzymes, Lipids, and Carbohydrates. John Wiley & Sons.

[25] Giusti, M.M. and Wrolstad, R.E. (2001) Characterization and Measurement of Anthocyanins by UV-Visible Spectroscopy. *Current Protocols in Food Analytical Chemistry*. https://doi.org/10.1002/0471142913.faf0102s00

[26] Boizot, N. and Charpentier, J.P. (2006) Méthode rapide d'évaluation du contenu en composés phénoliques des organes d'un arbre forestier. Le Cahier des Techniques de l'INRA, In: Numéro spécial, 79-82.

[27] Lebreton, P., Jay, M., Voirin, B. and Bouchez, M. (1967) Sur l'analyse qualitative et quantitative des flavonoïdes. *Chimie Analytique en France*, **49**, 375-383.

[28] Héma, A. (2010) Etude de molécules bioactives isolées de plantes du Burkina Faso. Ph.D. Thesis, Université de Ouagadougou, 170 p.

[29] Sáez-Plaza, P., Navas, M.J., Wybraniec, S., Michałowski, T. and Asuero, A.G. (2013) An Overview of the Kjeldahl Method of Nitrogen Determination. Part II. Sample Preparation, Working Scale, Instrumental Finish, and Quality Control. *Critical Reviews in Analytical Chemistry*, **43**, 224-272. https://doi.org/10.1080/10408347.2012.751787

[30] R'Zina, Q., Cheggour, M. and Fares, K. (2000) Dosage des polysaccharides totaux de la racine de betterave sucriere: effets du choix de la variete et de quelques pratiques culturales. *Industries Alimentaires et Agricoles* (*France*), **117**, 23-26.

[31] Sherma, J. and Fried, B. (2003) Handbook of Thin-Layer Chromatography. CRC Press. https://doi.org/10.1201/9780203912430

[32] Lamien-Meda, A., Lamien, C.E., Compaoré, M.M.Y., Meda, R.N.T., Kiendrebeogo, M., Zeba, B., *et al.* (2008) Polyphenol Content and Antioxidant Activity of Fourteen Wild Edible Fruits from Burkina Faso. *Molecules*, **13**, 581-594. https://doi.org/10.3390/molecules13030581

[33] Miller, N.J., Rice-Evans, C., Davies, M.J., Gopinathan, V. and Milner, A. (1993) A Novel Method for Measuring Antioxidant Capacity and Its Application to Monitoring the Antioxidant Status in Premature Neonates. *Clinical Science*, **84**, 407-412. https://doi.org/10.1042/cs0840407

[34] Pulido, R., Bravo, L. and Saura-Calixto, F. (2000) Antioxidant Activity of Dietary Polyphenols as Determined by a Modified Ferric Reducing/antioxidant Power Assay.

Journal of Agricultural and Food Chemistry, **48**, 3396-3402. https://doi.org/10.1021/jf9913458

[35] Dosseh, K., Kpatcha, T., Adjrah, Y., Idoh, K., Agbonon, A. and Gbéassor, M. (2014) Anti-Inflammatory Effect of *Byrsocarpus coccineus* Schum. and Thonn. (*Connaraceae*) Root. *World Journal of Pharmaceutical Research*, **3**, 3585-3598

[36] Scherer, R. and Godoy, H.T. (2009) Antioxidant Activity Index (AAI) by the 2,2-Diphenyl-1-Picrylhydrazyl Method. *Food Chemistry*, **112**, 654-658. https://doi.org/10.1016/j.foodchem.2008.06.026

[37] Yang, W., Pan, R., Nong, T., Zhao, Y., Liu, X., Zhang, H., *et al.* (2025) Shifts and Drivers in Soil Carbon and Nitrogen Sequestration and Stabilization along a 2100-M Altitudinal Gradient from Temperate Forests to Alpine Shrub Meadow. *Plant and Soil*, **519**, 687-713. https://doi.org/10.1007/s11104-025-08150-7

[38] Rajak, P., Afreen, T., Raghubanshi, A.S. and Singh, H. (2025) The Impact of Rainfall Variability on Selected Soil Properties and Ecophysiological Traits in *Prosopis juliflora* Invaded Plots. *Scientific Reports*, **15**, Article No. 31473. https://doi.org/10.1038/s41598-025-97750-5

[39] El-Lamey, T. (2012) Effect of Salinity on Tannins Content of *Leucaena leucocephala* (Lam.) de Wit. and *Prosopis chilensis* (Molina) Stuntz and Techniques for Their Reduction. *Egyptian Journal of Botany*, **52**, 51-63.

[40] Bustamante, M.Á., Michelozzi, M., Barra Caracciolo, A., Grenni, P., Verbokkem, J., Geerdink, P., *et al.* (2020) Effects of Soil Fertilization on Terpenoids and Other Carbon-Based Secondary Metabolites in Rosmarinus Officinalis Plants: A Comparative Study. *Plants*, **9**, Article 830. https://doi.org/10.3390/plants9070830

[41] Stewart, A.J., Chapman, W., Jenkins, G.I., Graham, I., Martin, T. and Crozier, A. (2001) The Effect of Nitrogen and Phosphorus Deficiency on Flavonol Accumulation in Plant Tissues. *Plant, Cell & Environment*, **24**, 1189-1197. https://doi.org/10.1046/j.1365-3040.2001.00768.x

[42] Coria-Cayupán, Y.S., Sánchez de Pinto, M.I. and Nazareno, M.A. (2009) Variations in Bioactive Substance Contents and Crop Yields of Lettuce (*Lactuca sativa* L.) Cultivated in Soils with Different Fertilization Treatments. *Journal of Agricultural and Food Chemistry*, **57**, 10122-10129. https://doi.org/10.1021/jf903019d

[43] Girodon, F., *et al.* (1999) Impact of Trace Elements and Vitamin Supplementation on Immunity and Infections in Institutionalized Elderly Patients: A Randomized Controlled Trial. *Archives of Internal Medicine*, **159**, 748-754. https://doi.org/10.1001/archinte.159.7.748

[44] Bourgaud, F., Gravot, A., Milesi, S. and Gontier, E. (2001) Production of Plant Secondary Metabolites: A Historical Perspective. *Plant Science*, **161**, 839-851. https://doi.org/10.1016/s0168-9452(01)00490-3

[45] Coly, E.V., Seck, P.A. and Mbaye, A.A. (2004) Les productions horticoles. Bilan de la recherche agricole et agroalimentaire au Sénégal.

[46] Palé, É. (2002) Étude des anthocyanes de plantes du Burkina Faso: Structures et activités antioxydantes. Ph.D. Thesis, Université de Ouagadougou.

[47] Adjé, F., Lozano, Y., Adima, A.A., Meudec, E., Gaydou, E. and Agbo N'zi, G. (2007) Structures et composition en anthocyanes d'extraits aqueux de plantes de Côte d'Ivoire Delonix regia, *Hibiscus sabdariffa* et *Carapa procera*. Cirad.

[48] Steyn, W.J. (2008) Prevalence and Functions of Anthocyanins in Fruits. In: Winefield, C., Davies, K. and Gould, K., Eds., *Anthocyanins*, Springer New York, 86-105. https://doi.org/10.1007/978-0-387-77335-3_4

[49] Chongwilaikasem, N., Sithisarn, P., Rojsanga, P., Ruenraroengsak, P. and Sithisarn, P. (2024) Development of Roselle Ointment with Antibacterial Effects. *Pharmaceutical Sciences Asia*, **51**, 369-380.
https://doi.org/10.29090/psa.2024.04.24.ap0750

[50] Arena, M.E., Postemsky, P.D. and Curvetto, N.R. (2017) Changes in the Phenolic Compounds and Antioxidant Capacity of *Berberis microphylla* G. Forst. Berries in Relation to Light Intensity and Fertilization. *Scientia Horticulturae*, **218**, 63-71.
https://doi.org/10.1016/j.scienta.2017.02.004

Appendix: Supplementary Material

Supplementary tables and figures presenting the classification table of soil chemical fertility, followed by calibration curves and regression analyses for the quantification of phenolic compounds, tannins, and antioxidant activity.

Table S1. Threshold ranges used for soil chemical fertility classification according to Jongschaap, 1995.

Parameters		Interpretation class for each parameter				
		Very low	**Low**	**Medium**	**High**	**Very high**
OM	%	<0.5	0.5 - 1.0	1.0 – 2.0	2.0 - 3.0	>3.0
	Score	1	2	3	4	5
Total N	%	<0.02	0.02 - 0.06	0.06 - 0.10	0.10 - 0.14	>0.14
	Score	2	2.5	3	3.5	4
P avail	ppm	<5	5 - 10	10 - 20	20 - 30	>30
	Score	2	2.5	3	3.5	4
Total P	ppm	<100	100 - 200	200 - 400	400 - 600	>600
	Score	2.5	2.75	3	3.25	3.5
K avail	ppm	<25	25 - 50	50 - 100	100 - 200	>200
	Score	2	2.5	3	3.5	4
Total K	ppm	<500	500 - 1000	1000 - 2000	2000 - 4000	>4000
	Score	2.5	2.75	3.0	3.25	3.5
CEC	meq/100g	<5	5 - 10	10 - 15	15 - 20	>20
	Score	2	2.5	3	3.5	4
BSR	%	<20	20 - 40	40 - 60	60 - 80	>80
	Score	2	2.5	3	3.5	4
SEB	meq/100g	<1	1 - 6	6 - 11	11 - 16	>16
	Score	1	2	3	4	5
pH (H_2O)	**Value**	<4.5	4.6 - 5.0	5.1 - 5.5	5.6 - 6.0	6.1 - 7.3
	Score	1	2	3	4	5
Sum of scores		<20.9	21.0 - 26.9	27.0 - 32.9	33.0 - 38.9	>39
Chemical fertility class		**Very low**	**Low**	**Medium**	**High**	**Very high**

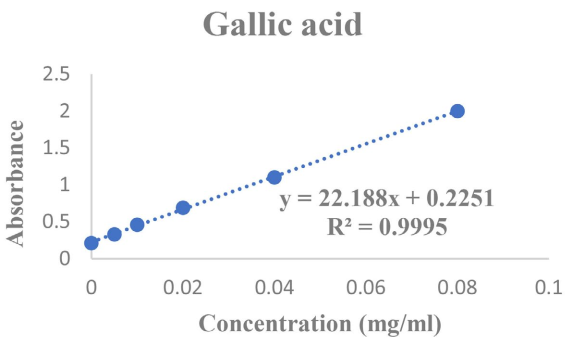


Figure S1. Calibration curve of gallic acid used for the determination of total phenolic content.

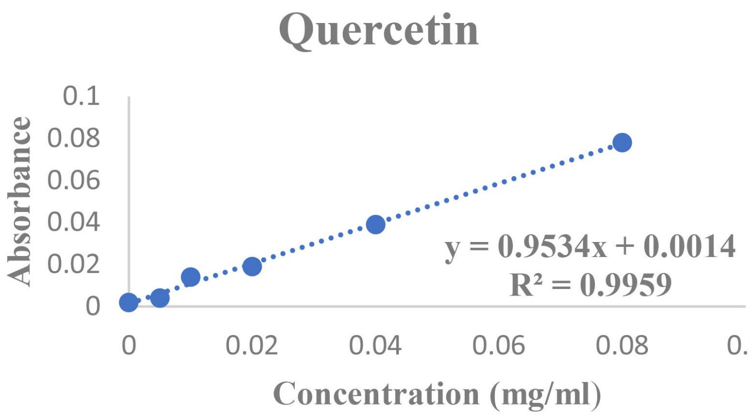


Figure S2. Calibration curve of quercetin used for the determination of total flavonoid content.

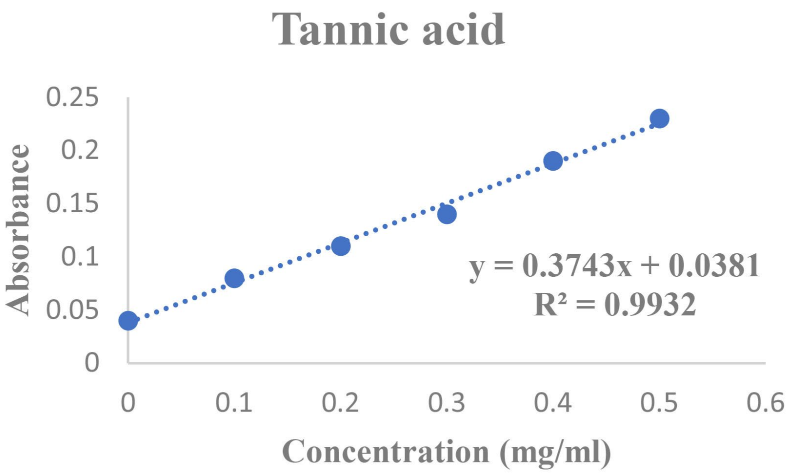


Figure S3. Calibration curve of tannic acid used for the determination of condensed tannins.

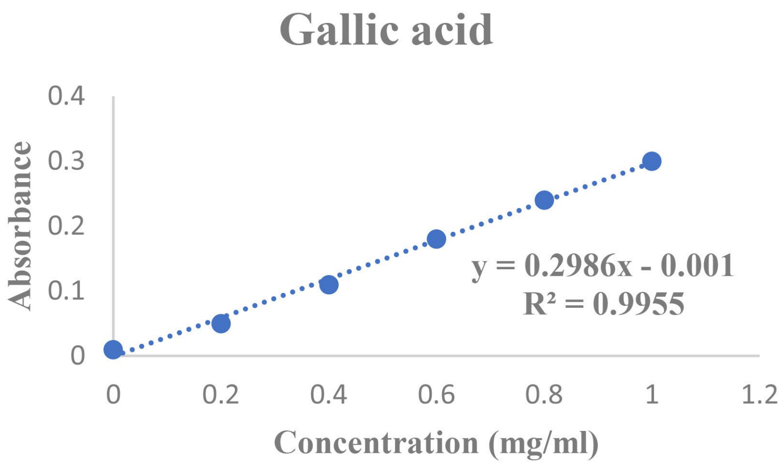


Figure S4. Calibration curve of gallic acid used for the determination of hydrolyzable tannins.

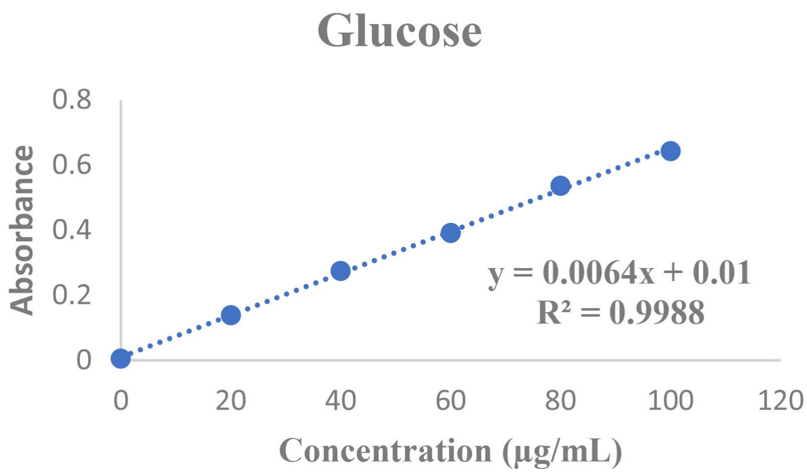


Figure S5. Calibration curve of glucose using the phenol-sulfuric acid method.

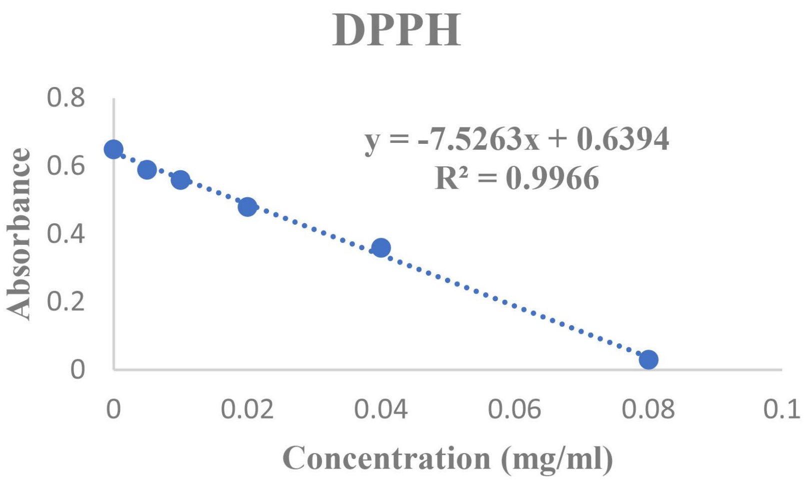


Figure S6. Regression curve of absorbance versus concentration used for the DPPH assay.

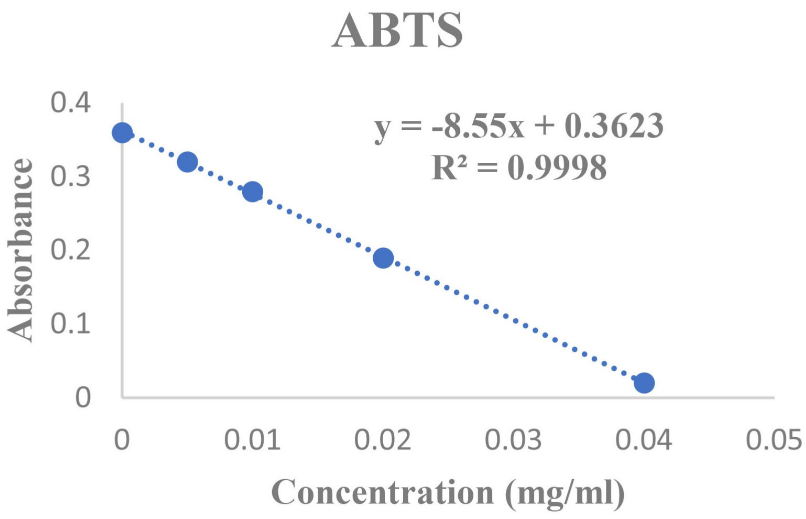


Figure S7. Regression curve of absorbance versus concentration used for the ABTS assay.

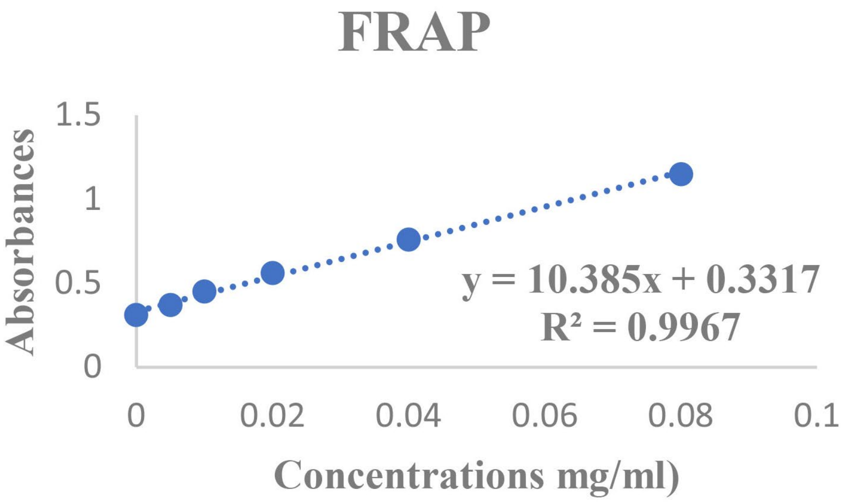


Figure S8. Regression curve of absorbance versus concentration used for the FRAP assay.

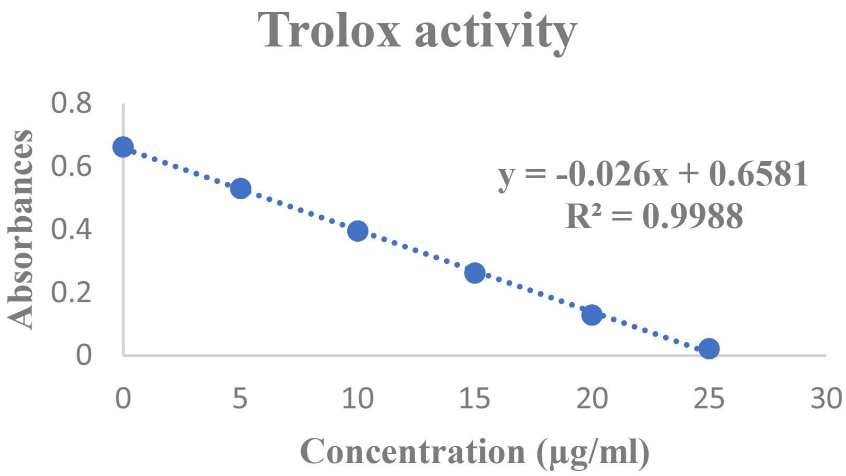


Figure S9. Regression curve for Trolox used as a standard antioxidant.

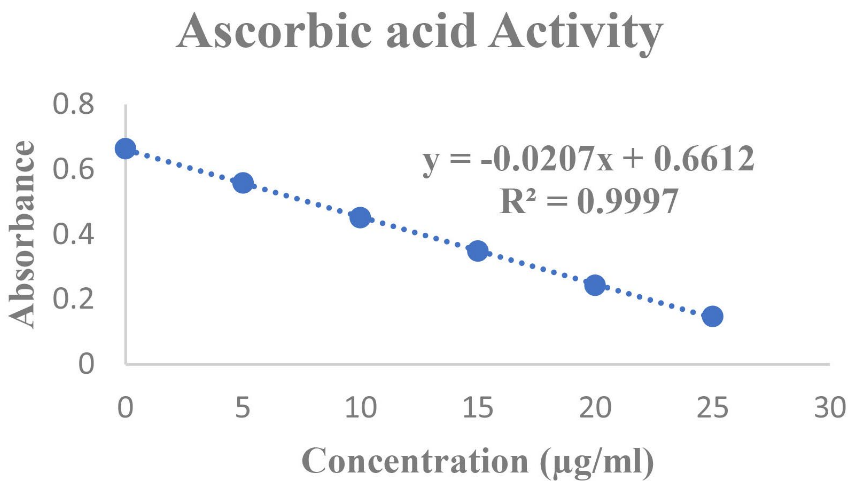


Figure S10. Regression curve for ascorbic acid used as a standard antioxidant.

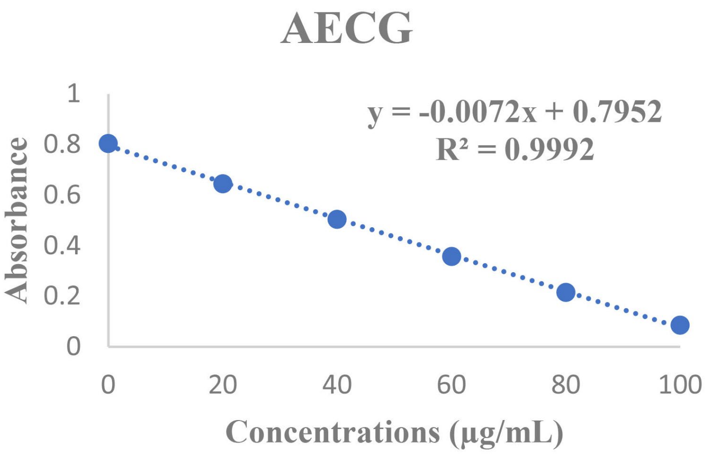


Figure S11. Regression curve of the aqueous extract from Guiriko calyces (AECG).

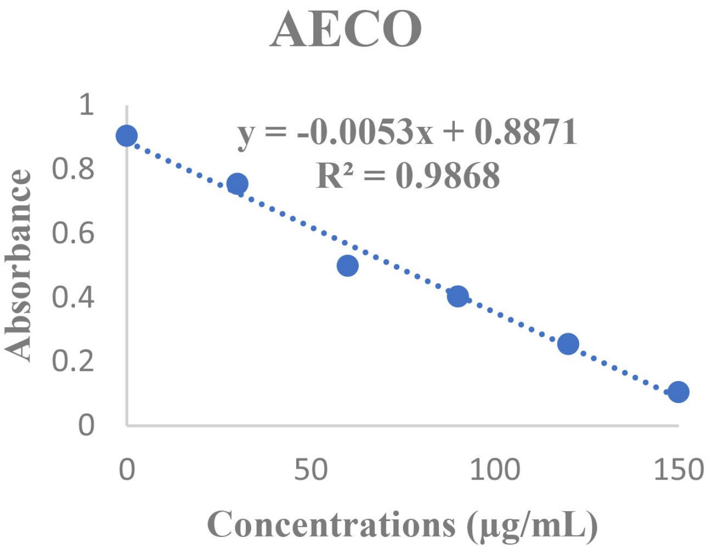


Figure S12. Regression curve of the aqueous extract of calyces from Oubri soils (AECO).

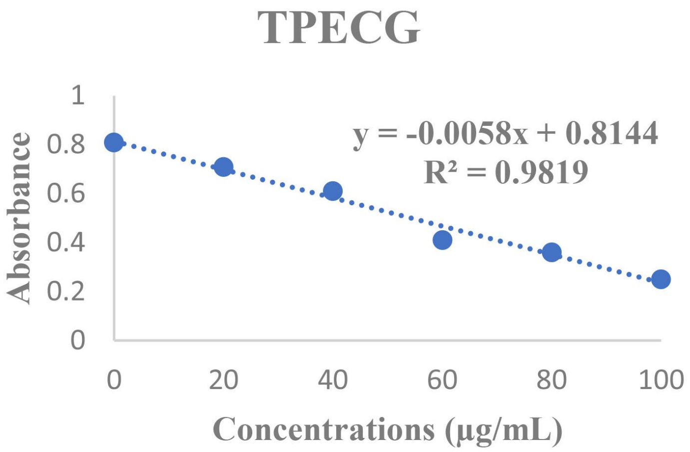


Figure S13. Regression curve of the total phenolic extract of calyces from Guiriko soils (TPECG).

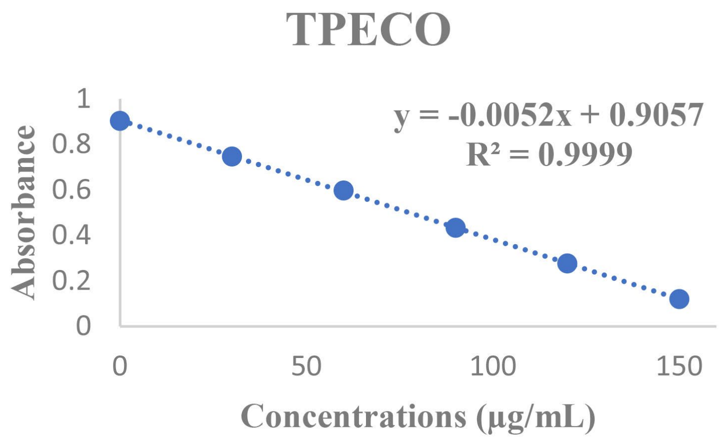


Figure S14. Regression curve of the total phenolic extract from Oubri calyces (TPECO).

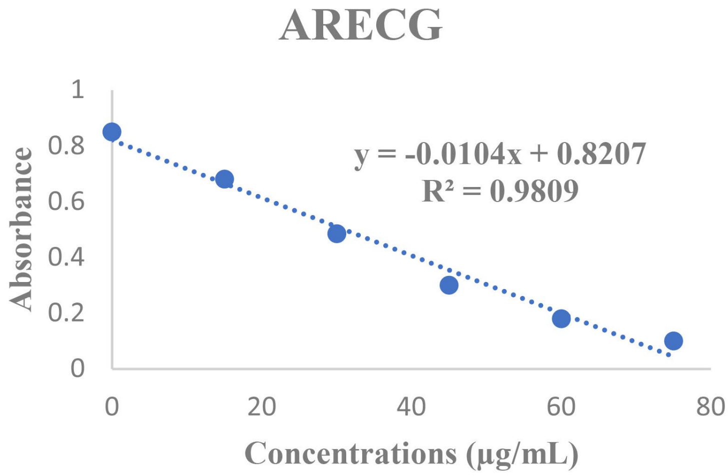


Figure S15. Regression curve of the anthocyanin-rich extract of calyces from Guiriko soils (ARECG).

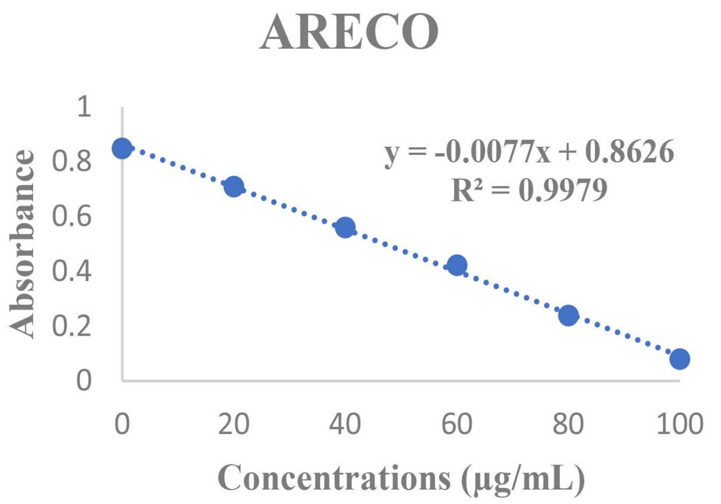


Figure S16. Regression curve of the anthocyanin-rich extract from Oubri calyces (ARECO).

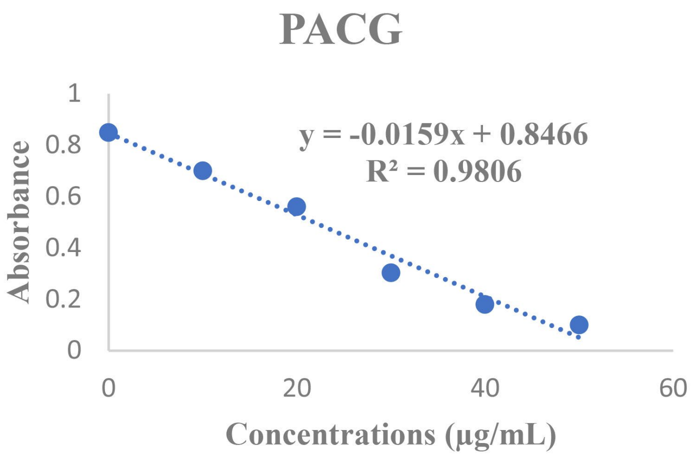


Figure S17. Regression curve of the purified anthocyanin extract of calyces from Guiriko soils (PACG).

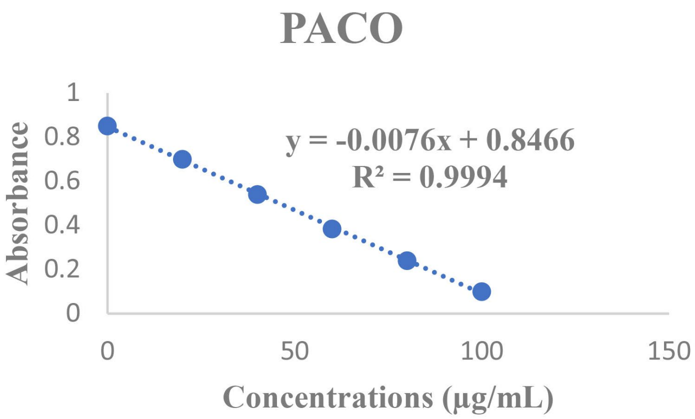


Figure S18. Regression curve of the purified anthocyanin extract from Oubri calyces (PACO).