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ORIGINAL PAPER

Phenolic compounds characterisation and antioxidant activity of black plum (*Vitex doniana)* fruit pulp and peel from Côte d'Ivoire

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Abstract

This study was conducted to first determine the phenolic compounds and then the antioxidant activity of black plum fruit pulp and peel. For these characterisations, classic methods were used. Moreover, the ability of extracts to scavenge free radicals and their reducing power were measured according to 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) methods, respectively. The total polyphenol, flavonoid and anthocyanin contents of pulp and peel ranged from 202.51 ± 4.19 to 463.45 ± 6.85 mg gallic acid equivalent (GAE)/100 g of Dry Weight (DW), 75.71 ± 1.03 to 145.55 ± 1.03 mg quercetin equivalent (QE)/100 g DW, and from 1.91 ± 0.08 to 8.28 ± 0.83 mg cyanidin 3-O-β-D-glucoside equivalent (C3GE)/100 g DW respectively. However, these compounds were higher in peel extracts than in pulp extracts. In addition, peel extract showed the strongest antioxidant capacities. Significant correlations were found between methods applied to determine antioxidant activity (DPPH and FRAP) in black plum pulp and peel extracts and their total phenols and flavonoids contents. Cinnamic acid and gallic acid were mains phenols in pulp and peel extracts respectively, except fruits peel from Ferke where the main phenol was cinnamic acid. Thus, peel of black plum fruit could be used as an inexpensive and natural source of antioxidants and contribute to the prevention of degenerative diseases.

Keywords Black plum (*vitex doniana*) · Pulp · Peel · Phenolic compounds · Antioxidant activity

Introduction

In recent years, the biological world and medical science have registered a new concept, that of 'oxidative stress', the main initial cause of many diseases: cancer, diabetes, cardiovascular diseases, cataracts, pulmonary oedema, rheumatism, atherosclerosis, etc. [1–4]. Oxidative stress is defined as a disturbance in the balance between the production of reactive oxygen species (ROS), free radicals and antioxidant defences [5–7]. Reactive oxygen species are by-products of oxygen. These ROS include all free radicals and their precursors. Examples include superoxide anion ($O_2^{\cdot-}$), hydroxyl radical($OH^{\cdot}$), nitrogen monoxide($NO^{\cdot}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and nitroperoxide (ONOOH) [8, 9]. However, the imbalance between antioxidants and prooxidants is usually caused by lifestyle (smoking, alcoholism, obesity, intense physical exercise) but also through a food deficient in one or more antioxidants [10–14].

On the basis of these data, scientists are placing increasing importance on a diet rich in fruit and vegetables and/or antioxidants to prevent these diseases.

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Vitex doniana is a tropical plant species of the family Lamiaceae, highly prized in parts of Côte d'Ivoire including Gontougo region (East) [15, 16] and Poro [17] for its fruit and edible leaves. In Côte d'Ivoire, in nutritional therms, Soro et al. [18] reported that black plum fruit pulp is a good source of sugar, vitamin C, phenolic compounds including flavonoids, and minerals such as magnesium, potassium and calcium. Traditionally, black plum fruits are not eaten with the peel, which is usually discarded when eating fruit or making beverages. Many studies have shown that fruit peel, including those of papaya, mango, jujube and apple, has high content of phenolic compounds and antioxidant activities [19–22]. However, no information is available on the antioxidant activity of black plum fruit peel or on the phenolic compound composition of its pulp and peel. To achieve the complete utilization of such fruits, it would be beneficial if the peels could be used as a source of natural food additives and ingredients. The purpose of this study is to determine the total phenolic compounds, total flavonoids and total anthocyanins, and to identify and quantify the phenolic compounds present in the fruit pulp and peel of *V. doniana*.

Material and methods

Sample preparation

Ripe *V. doniana* fruit were harvested in three Côte d'Ivoire localities: Ferkessedougou (Ferke), Tiébissou and Yamoussoukro, which are parts of high production. Fruits were randomly collected, at the optimum ripening stage recommended for consumption (based on the maturity index) from five trees (about 3 kg of fruit by tree), in August for Ferké, September for Tiebissou and October for Yamoussoukro. The fresh fruit was harvested and washed with tap and distilled water. After drying with paper towel, the fruit pulp and peel were separated by hand with a stainless-steel knife. Each resulting part was dried in an oven (Memmert U30, Gemini BV GmbH, Germany) at 50 °C, 70% of ventilation and air velocity 2.7 m/s for 24 h, then milled (ZBK220077-88 LW74d(B) A (China). The milled pulp and peel were packed in polyethylene bags with a vacuum packager and frozen at – 19 °C until analysis. All tests were performed in triplicate.

Determination of ascorbic acid

Ascorbic acid content was measured by titration with 2,6-dichlorophenolindophenol (2,6-DCPIP) according Mau et al. [23] with slight modifications. To 5 g (m_e) of sample was added 50 mL of distilled water. After 5 min homogenisation with a magnetic stirrer and 20 min centrifugation at 1400 g (TDL-4 Centrifuge, China), the supernatant was collected in a beaker. To 10 mL of supernatant, 10 mL of metaphosphoric acid was added to stabilise the vitamin C. The sample to be analysed was obtained by taking 2 mL of the stabilised solution which was then titrated with 2,6-DCPIP solution at a concentration of 0.5 g/L. V (mL) represents the 2,6-DCPIP volume delivered at equivalence. Calibration of the 2,6-DCPIP solution was previously done with ascorbic acid solution at a concentration of 0.5 g/L. Another solution prepared from metaphosphoric acid/acetic acid was also titrated with a volume of 2,6-DCPIP solution. Trials were repeated three times for all samples.

$$Vitamin\ C(\%) = \frac{0.5 \times V \times 5 \times 100}{m_e} \quad (1)$$

Phenols extraction

Phenols were extracted by the method of Soro et al. [18]. Dried *V. doniana* pulp and peel samples (1 g) were homogenised in 10 mL solution of 80% methanol and 2% formic acid, using an Ultra TurraxT25 basic homogeniser (Heldoph Instruments D-91126, Schabach, Germany) at room temperature. The homogenate was sonicated for 30 min in a Bandelin electronic RK 541 H sonicator (Heinrichstrasse 3-4 D-12207, Germany) and then centrifuged at 9400×*g* for 25 min in a DBS centrifuger (PCB 1500, Italy). The supernatant was collected and the precipitate extracted again with 10 mL of 80% methanol, under the conditions previously described. The two supernatants were mixed and filtered using Whatman filter paper No. 1. The final methanolic extract was stored at 25 °C to be used in determination of total phenols, flavonoids and anthocyanin contents.

Determination of total phenols

Total phenolic compounds (TPC) determination was performed as described by Gao et al. [24] Phenolic extract (100 μL) was mixed with 0.2 mL Folin-Ciocalteu reagent (Sigma), 2 mL of H_2O and 1 mL of 15% Na_2CO_3 and the absorbance measured at 765 nm in a spectrophotometer (Thermo Scientific™ 75003631, ThermoFisher Scientific SAS, Strasbourg, France) after 2 h incubation at room temperature. Gallic acid was used for the calibration curve with a concentration range from 0 to 200 mg/L. Total phenols were expressed as mg gallic acid equivalent (GAE)/100 g DW.

Determination of total flavonoids

Total flavonoid (TF) contents were determined according method used by Meda et al. [25], but slightly modified. A

volume of 0.5 mL of sample methanolic extract was diluted in 0.5 mL of distilled water. Then, 0.5 mL of aluminium chloride 10% (w/v) and the same volume of 1 M sodium acetate was added. Finally, 2 mL of distilled water was added and absorption reading at 415 nm was taken after 30 min against a blank sample consisting of a 4 mL methanolic extract without aluminium chloride. Quercetin was used for the calibration curve with a concentration range from 0 to 3.125 mg/mL. Results were expressed as mg quercetin equivalent (QE)/100 g DW.

Determination of total anthocyanin content

Total anthocyanin (TA) content was determined by pH-differential method [26]. Absorbance was measured at 510 and 700 nm in buffers at pH 1.0 and 4.5. Anthocyanin concentration was calculated and expressed as mg cyanidin 3-glucoside equivalent (C3GE)/100 g DW, using the formula:

$$TA = \frac{A \times MW \times DF}{\varepsilon \times 1} \quad (2)$$

where A = (A510–A700 nm) pH 1.0—(A510–A700 nm) pH 4.5; MW (molecular weight) = 449.2 g/mol; DF = dilution factor; 1 = cuvette pathlength in cm; ε = 26,900 L/mol cm (molar extinction coefficient for cyanidin 3-*O*-β-D-glucoside).

Antioxidant activity determination

Sample extraction

Approximately 5 g of each dried plant part was homogenised in a blender with 50 mL MeOH/formic acid (99:1, v/v) and extracted for 1 h three times at room temperature. The fractions were collected and the solution filtered and concentrated under reduced pressure to evaporate the solvent.

All extracts obtained as described above were tested for their antioxidant activity using the following two methods:

DPPH radical-scavenging

Free radical-scavenging of black plum extracts were determined by using the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical [27].

Briefly, the assay consisted of 1 mL of 0.1 mM DPPH$^{\bullet}$ in methanol and varying concentrations of extracts (50–1000 µg/mL) in methanol or standards in the same solvent made up to 3.5 mL with methanol. The contents were mixed well immediately and then incubated for 30 min at room temperature in the dark. The absorbance was recorded by UV–vis spectrophotometer (Ultrospec 7000, ThermoFisher Scientific Labofuge 200, Thermo Electron LED Gmbl D-37520 Osterode, Germany) at 517 nm.

The percentage of scavenging activity was calculated as:

$$I(\%) = \left(\frac{A_b - A_e}{A_b}\right) \times 100 \quad (3)$$

where A_b = control absorbance (without sample) and A_e = sample absorbance.

Percentage radical scavenging activity was plotted against corresponding extract concentration to obtain the half maximal inhibitory concentration (IC_{50}) values, which are defined as the amount of antioxidant material required to scavenge 50% of free radicals in the assay system. IC_{50} values are inversely proportional to antioxidant activity.

Ferric reducing antioxidant power (FRAP)

The extracts reducing power was determined using the method of Hseu et al. [28]. Extract solution (1 mL) in different concentrations (50–1000 µg/mL) were mixed with phosphate buffer (1 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (1 mL, 1%). The mixture was then incubated at 50 °C for 20 min. A portion of trichloroacetic acid (1 mL, 10%) was added to the mixture, which was then centrifuged for 10 min at 3000 rpm (1000 *g*). The solution upper layer (1 mL) was mixed with distilled water (1 mL) and $FeCl_3$ (150 µL, 0.1%) for 10 min and the absorbance measured at 700 nm in a spectrophotometer, with higher absorbance indicating greater reducing power.

Phenolic compounds identification and quantification

All procedures to identify and quantify phenolic compounds were performed using the method of Sakakibara et al. [29] with slight modifications.

The methanol solution (90% and acetic acid 0.5%) containing sample (50 mg), was allowed to stand in a sonicator for 1 min, and the supernatant was recovered by centrifugation at 1000×*g* for 10 min. After extraction three times, the extracts were dried with a centrifugal concentrator (VC-96N, Taitec Co., Saitama, Japan). The residues were dissolved in 0.25 mL of DMSO and filtered through a Millex-LG 0.2 µm membrane filter (Millipore Co., Bedford, MA) before HPLC analysis. The treatment was repeated independently three times.

HPLC

The HPLC system employed was an Agilent Technology 2001–2008 equipped with Chem station for LC 3D systhems Rev.B.03.02 [341] chromatography data station software, autosampler D 7200, and diode array detection (DAD) system to monitor at all wavelengths from 200 to 600 nm.

An Inertsil ODS (250 × 2.1 mm, 5 µm) column was used at 35 °C. Gradient elution was performed with two solutions: Solution A, composed of a formic acid solution (pH 3.3) and 10% methanol, and solution B, comprising 70% methanol, These were delivered at a flow rate of 0.25 mL/min as follows: initially, 100% of solution A; for the next 15 min, 70% A; for another 30 min, 65% A; for another 20 min, 60% A; for another 5 min, 50% A; and finally for 25 min, 0% A. The injection volume of the extract was 10 µL.

Polyphenol analyses. First, we made a library of HPLC retention times and spectra of aglycons, with a diode array detector for 33 standard chemicals and constructed the calibration curves of coumpounds identified (benzoic acid, o-hydroxybenzoic acid, m-hydroxybenzoic acid, p-hydroxybenzoic acid, protocatechuic acid, gallic acid, cinnamic acid, o-coumaric acid, m-coumaric acid, p-coumaric acid, caffeic acid, syringic acid, chlorogenic acid and ferulic acid). Sample extracts were then analysed using the same HPLC system. The detected polyphenol peaks were identified based on the retention times and spectra in the library. Quantitative analysis was performed by sample chromatography.

Statistical analysis

Data were analysed using ANOVA based on Tukey's HSD (Honestly Significant Difference) multiple comparison test with a threshold of 5%. Correlation analyses of antioxidant activity versus the total phenolic, flavonoid and anthocyanins content were carried out using the correlation and regression. RStudio software (version 1.2.1335 2009–2019) was used. The results were presented as mean ± standard deviation.

Results and discussion

Total phenolic compound, total flavonoid, total anthocyanin and ascorbic acid contents

The results (Table 1) showed that total phenolic compounds (TPC), total flavonoids (TF), total anthocyanins (TA) and ascorbic acid (AA) contents, extracted from *V. doniana* fruit pulp and peel, differed considerably ($P < 0.05$) from one locality to another. This corroborates other results that suggested that TPC content of *V. doniana* fruit in Côte d'Ivoire varied from region to region [18]. Thus, *V. doniana* fruit pulp and peel TPC contents were between 202.51 ± 4.19 and 259.75 ± 2.81 mg GAE/100 g DW and 225.84 ± 5.89 and 463.45 ± 6.85 mg GAE/100 g DW, respectively. The pulp and peel of fruit collected in Yamoussoukro exhibited the highest levels of TPC (259.75 ± 2.81 and 463.45 ± 6.85 mg GAE/100 g DW, respectively), while the lowest TPC levels occurred in those from Tiébissou and Ferke (202.51 ± 4.19

Table 1 Content of antioxidant compounds in black plum fruit pulp and peel

Samples	TPC (mg GAE/100 g DW)		TF (mg QE/100 g DW)		TF/TPC		TA (mg C3GE/100 g DW)		TA/TPC		AA (mg/100 g fresh weight)	
	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel
Ferke	227.47 ± 3.35b	225.84 ± 5.89a	79.43 ± 1.13b	103.86 ± 0.66a	0.35	0.46	5.52 ± 0.91b	8.28 ± 0.83b	0.02	0.04	2.54 ± 0.40a	nd
Tiébissou	202.51 ± 4.19a	383.03 ± 6.54b	75.71 ± 1.03a	145.55 ± 1.03c	0.37	0.38	4.26 ± 0.13b	5.83 ± 0.67a	0.02	0.03	2.08 ± 0.64a	nd
Yamoussoukro	259.75 ± 2.81c	463.45 ± 6.85c	77.95 ± 0.72ab	141.48 ± 0.66b	0.30	0.30	1.91 ± 0.08a	6.99 ± 0.97b	0.01	0.03	13.88 ± 2.77b	nd

The results are expressed as mean ± deviation of three separate extractions and determinations. The data were analysed by ANOVA and in each column of each group (pulp and skin) different letters indicate statistically different values according to Tukey's HSD test at $p < 0.05$

TPC total phenolic compounds, *TF* total flavonoids, *TA* total anthocyanins, *AA* ascorbic acid

and 225.84 ± 5.89 mg GAE/100 g DW, respectively (Table 1). In addition, except for Ferke fruit pulp and peel, which showed no significant difference ($p > 0.05$), there were higher concentration of TPC in the *V. doniana* fruit peel than in pulp (Table 1). TPC in Tiébissou fruit peel was 1.89-fold higher than in pulp. TPC in Yamoussoukro fruit peel was 1.78-fold higher than in pulp. This result is consistent with results obtained by several authors [20, 30, 31] whose reported a higher phenolic compound content in fruit peel such as orange, grape and mango.

For TF levels, significant variation was observed ($P < 0.05$) between samples (Table 1). The TF of *V. doniana* fruit pulp and peel ranged from 75.71 ± 1.03–145.55 ± 1.03 mg QE/100 g DW. The highest amounts of TF were also found in peel (Table 1). Tiébissou peel (145.55 ± 1.03 mg QE/100 g DW) and Ferke pulp (79.43 ± 1.13 mg QE/100 g DW) showed high TF content. The results showed that the TF content of Ferke fruit peel was 1.30-fold higher than in pulp. For Tiébissou and Yamoussoukro fruits, TF contents were 1.92- and 1.81-fold, respectively, higher than those of pulp. Those results agree well with results reported by Levaj et al. [32] and Reza et al. [33].

Table 1 shows that the TF content of *V. doniana* fruit pulp and peel represent 34% and 38% of TPC, respectively, whereas anthocyanins represent only 1.66% and 3.33% of TPC. In this study, the fruit pulp and peel TF/TPC ratios are higher than those of carrot (0.28), tomato (0.17) and red pepper (0.08) and similar to okra (0.32) for the pulp [34]. In addition, these ratios are also higher than those of nine varieties of grenadine (0.114–0.288) for peel [33].

The total anthocyanin (TA) contents of black plum fruit were very low. These values were expressed in mg cyanidin-3-glucoside equivalent/100 g DW. They ranged from 1.91 ± 0.08 to 8.28 ± 0.83 mg C3GE/100 g DW. The anthocyanin contents in Ferke and Tiébissou fruit pulp differed notably from that of Yamoussoukro fruit. Ferke and Yamoussoukro peel anthocyanin contents showed significant differences from those of Tiébissou. However, the peel was still the richest (Table 1).

These TPC, TF and TA content variations may be due to many factors, such as the degree of fruit maturity at harvest, genetic differences, environmental conditions during plant growth, solar radiation and plant age [35–37].

Ascorbic acid levels ranged from 2.08 ± 0.64 to 13.88 ± 2.77 mg/100 g. The highest AA content was found in Yamoussoukro fruit (13.88 ± 2.77 mg/100 g), whereas Ferke and Tiébissou, which showed no significant difference between samples ($P > 0.05$), showed low AA contents. This difference in AA content within the same species fruit treated and stored under the same conditions could be explained by the light intensity and temperature during the plant growth period. Lee Seung and Kade [38] and Moor et al. [39] concluded that, among pre-harvest factors, light intensity and temperature are the most important factors influencing final AA content of the product. During the growing season, the more intense the light, the higher the AA content in the plant tissues. In this study, the AA content of Yamoussoukro fruit was similar to that published by Soro et al. [18] about *V. doniana* fruits harvested in three regions of Côte d'Ivoire and higher than fishery and nectarine (1.9–9.1 mg/100 g; [40]). However, AA levels in fruit from all localities are well below the average AA content of some common fruits, such as mango (31.7–56.7 mg/100 g [41];), papaya (74 mg/100 g) or guava (237.00 mg/100 g [42];).

Identification and quantification of phenolic compounds found in black plum (*V. doniana*) fruit pulp and peel

Figure 1 present an example of an HPLC/DAD chromatographic profile of phenolic compounds of *V. doniana* fruit pulp and peel collected in three localities of Côte d'Ivoire (i.e., Ferke), recorded at 250 nm. Based on comparing retention times (Table 2) and matching the UV spectra of the sample compounds and those of standards contained in our library (32 standards), only huit, all phenolic acids, were identified, i.e., gallic acid, protocatechuic acid, p-hydroxybenzoic acid, m-hydroxybenzoic acid, caffeic acid, chlorogenic acid, m-coumaric acid and cinnamic acid. Protocatechuic acid (10.65 ± 2.00 to 465.61 ± 22.96 mg/100 g DW), chlorogenic acid (42.97 ± 3.21 to 68.93 ± 9.56 mg/100 g DW) and cinnamic acid (743.93 ± 22.60–1798.65 ± 25.08 mg/100 g of DW) were identified in all samples (pulp and peel), which showed identical profiles. However, phenolic contents generally differed from locality to locality and from one fruit part to other part (Table 3).

Table 3 reveals that Yamoussoukro fruit peel and Ferke pulp did not contain p-hydroxybenzoic acid and caffeic acid, respectively. This variability of phenolics in plant tissues depends on many factors, such as environmental conditions, including temperature, UV light, and nutrition [43, 44].

After identification, phenolic compounds were quantified (Table 3). Cinnamic acid was the main phenolic compound in all fruit pulp and Ferke fruit peel. Concerning pulp, Ferke samples contained high amounts of cinnamic acid (1291.51 ± 1.52 mg/100 g DW), followed by those of Tiébissou (1102.98 ± 5.50 mg/100 g DW) and Yamoussoukro (743.93 ± 22.60 mg/100 g DW). Gallic acid was main compound present in fruit peel from Tiébissou (2581.83 ± 11.09 mg/100 g DW) and Yamoussoukro (7770.14 ± 36.98 mg/100 g DW).

Zhang et al. [45] revealed that some cinnamic acid concentrations inhibit K562 leukaemia cell growth and proliferation in a time- and dose-dependent manner. In addition,

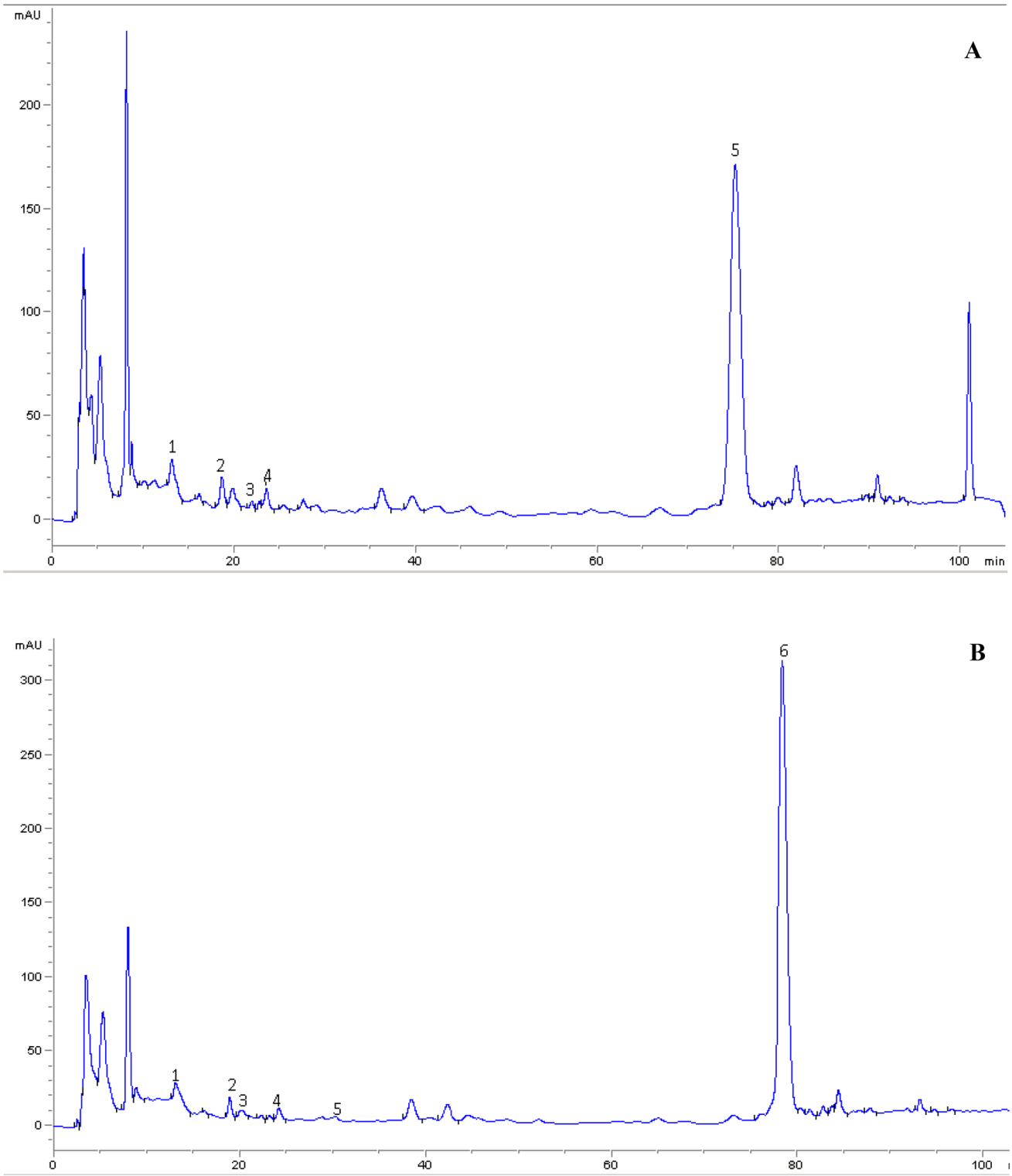


Fig. 1 HPLC chromatograms of phenolic compounds present in *V. doniana* fruit from Ferke: **a** Pulp: protocatechuic acid (1), p-hydroxybenzoic acid (2), m-hydroxybenzoic acid (3), chlorogenic acid (4), cinnamic acid (5) and **b** Peel: protocatechuic acid (1), p-hydroxybenzoic acid (2), caffeinic acid (3), chlorogenic acid (4), m-coumaric acid, (5) cinnamic acid (6). *%IB* inibition percentage, *BHT* butylhydroxytoluene, *AA* ascorbic acid

Table 2 Calibration curves of phenolic compounds identified and quantified in *V. doniana* fruit pulp and peel from three localities in Côte d'Ivoire

Analytes	Retention time (min)	Wavelength λ_{max} (nm)	Standard curve equations	Correlation coefficient (r^2)	Test concentration range (mg/mL)	Limit of detection (mg/mL)	Limit of quantification (mg/mL)
m-Hydroxybenzoic acid	21.79	250	y = 25430x − 17.614	0.9968	0.0033–0.0055	0.0003628	0.00076959
p-Hydroxybenzoic acid	18.55	250	y = 126839x − 7.7459	1.0000	0.0030–0.0166	0.00297985	0.00632089
Protocatechuic acid	13.26	250	y = 62185x − 20.655	1.0000	0.0018–0.1665	0.00232508	0.00493199
Gallic acid	7.83	280	y = 48555x + 4270.3	0.9984	0.333–2.664	0.11659045	0.24731308
m-Coumaric acid	36.18	280	y = 162423x − 76.264	0.9967	0.0012–0.0033	0.00043907	0.00093135
Caffeic acid	22.24	320	y = 116621x − 54.934	0.9994	0.0024–0.0333	0.00013178	0.00027954
Chlorogenic acid	22.61	320	y = 62017x − 226.65	0.9954	0.0066–0.0333	0.00310377	0.00658375
Cinnamic acid	69.26	280	y = 173660x + 2059.1	0.9993	0.333–1.665	0.06677249	0.14163861

Table 3 Analysis of phenolic compounds *V. doniana* fruit pulp and peel from three locaities from Côte d'Ivoire

Phenolic compound	Pulp			Peel		
	Ferke	Tiébissou	Yamoussoukro	Ferke	Tiébissou	Yamoussoukro
Phenolic acids						
Benzoic acid	nd	Nd	nd	nd	nd	nd
o-Hydroxybenzoic acid	nd	Nd	nd	nd	nd	nd
m-Hydroxybenzoic acid	20.21 ± 5.50	Nd	nd	nd	nd	nd
p-Hydroxybenzoic acid	Trace	26.13 ± 6.00b	22.03 ± 2.45b	17.36 ± 1.00a	25.14 ± 5.23b	nd
Protocatechuic acid	49.69 ± 3.78b	13.61 ± 1.56a	10.65 ± 2.00a	67.88 ± 2.95b	465.61 ± 22.96c	13.52 ± 2.00a
Gallic acid	nd	Nd	nd	nd	2581.83 ± 11.09a	7770.14 ± 36.98b
Cinnamic acid	1291.51 ± 1.52c	1102.98 ± 5.50b	743.93 ± 22.60a	1798.65 ± 25.08c	936.18 ± 8.15a	1069.73 ± 12.45b
o-Coumaric acid	nd	Nd	nd	nd	nd	nd
m-Coumaric acid	nd	34.20 ± 1.71	nd	5.77 ± 1.00	nd	nd
p-Coumaric acid	nd	Nd	nd	nd	nd	nd
Caffeic acid	nd	24.34 ± 2.30a	48.96 ± 10.09c	16.02 ± 3.76a	42.28 ± 4.12b	32.28 ± 3.36b
Syringic acid	nd	Nd	nd	nd	nd	nd
Chlorogenic acid	42.97 ± 3.21a	60.18 ± 11.67b	68.93 ± 9.56c	48.07 ± 6.33a	64.95 ± 2.08b	52.71 ± 2.51a
Ferulic acid	nd	Nd	nd	nd	nd	nd
Σ Phenolic acids	**1459.92 ± 8.51**	**1235.31 ± 28.74**	**872.47 ± 46.7**	**1936.39 ± 40.12**	**4090.85 ± 53.63**	**8937.65 ± 57.3**

The results are expressed as mean ± standard deviation of three separate extractions and determinations. The data were analysed by ANOVA and in each column of each group (pulp and skin), different letters indicate statistically different values according to Tukey's HSD test at $p < 0.05$

these authors observed a disappearance of the K562 cell stacking growth state after treatment with cinnamic acids, suggesting that contact inhibition of cell growth could be restored.

Furthermore, in vitro data showed that cinnamic acids had the highest antioxidant activity compared to their benzoic equivalents, resulting in a strong ability to neutralise peroxyl radicals [46].

Regarding gallic acid, in vitro and in vivo studies showed high cytotoxicity of this polyphenol against cancer cells, inducing cell death by apoptosis. Strlič et al. [47] reported that gallic acid has anti-carcinogenic effects through one or more molecular mechanisms of pleiotropic action on cell cycles, apoptotic processes, angiogenesis, invasion and metastasis.

Concerning the total amount of acidic phenols, it can be concluded that peel are the parts containing the highest amounts of phenolic compounds (1936.39 ± 40.12–89 37.65 ± 57.3 mg/100 g DW). Important amounts of phenolic acids were measured in fruit pulp and peel in the following decreasing order: Yamoussoukro peel > Tiébissou peel > Ferke peel > Ferke pulp > Tiébissou pulp > Yamoussoukro pulp. The samples containing highest phenolic acid content were therefore those of Yamoussoukro fruit peel (8937.65 ± 57.3 mg/100 g DW), while Yamoussoukro pulp samples contain low amounts of phenolic acids

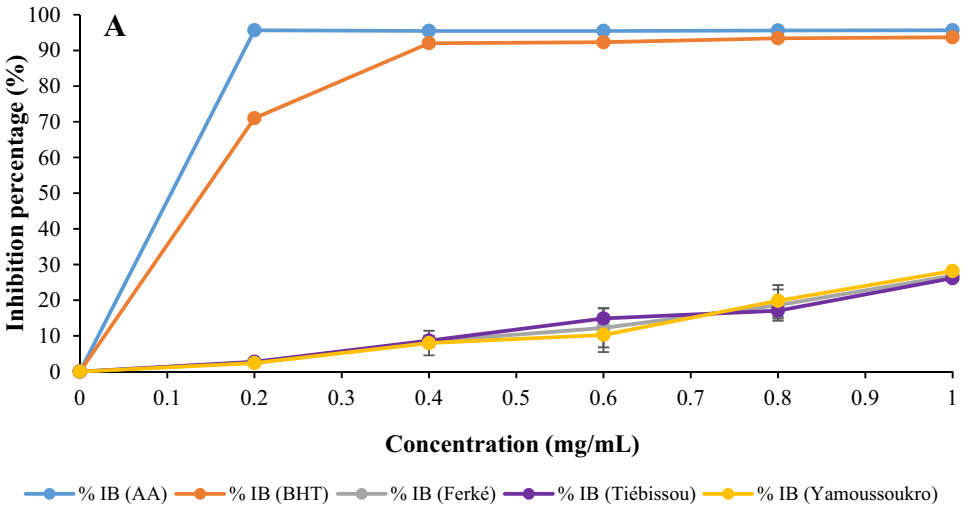
(872.47 ± 46.7 mg/100 g DW). Yamoussoukro fruit peel was reported to contain more than tenfold higher concentrations of phenolic acids than the pulp. Moreover, it contained more than twice the phenolic acids of Tiébissou fruit peel and at least four-fold higher than Ferke peel.

According to Anttonen and Karjalainen [43], variation in phenolic acid content would depend on plant nutritional status and genotype. In addition, high phenolic compound content in *V. doniana* fruit peel could be also attributed to temperature increase and soil moisture deficit encountered during fruit development and maturation [48–51]. In Côte d'Ivoire, the climate is tropical, with a dry season and a rainy season each year. The high phenolic compound content of peel would protect fruit against high oxidative stress under the prevailing climatic conditions. However, among peel samples, the low Ferke phenolic acid content would be induced by higher temperatures (average annual temperature: 33 °C) during the dry season compared to the other localities (Tiébissou: 30 °C and Yamoussokro: 25, 9 °C). Indeed, work by Pereira et al. [52] showed that excessive temperatures induce the degradation of some phenolic compounds in grapes Jeyaramraja et al. [53] reported that increased soil moisture deficiency resulted in decreased phenylalanine ammonia lyase activity and, consequently, a reduction in the synthesis of phenolic compounds including gallic acid and caffeine in tea. Thus, the absence of gallic acid in Ferke fruit pulp and peel is in line with Jeyaramraja et al. [53]'s results. In conclusion, *V. doniana* fruit is a good source of gallic and cinnamic acids, and its consumption could contribute to preventing degenerative diseases such as cancer, diabetes, Parkinson's disease, Alzheimer's diseases, rheumatoid arthritis and osteoporosis.

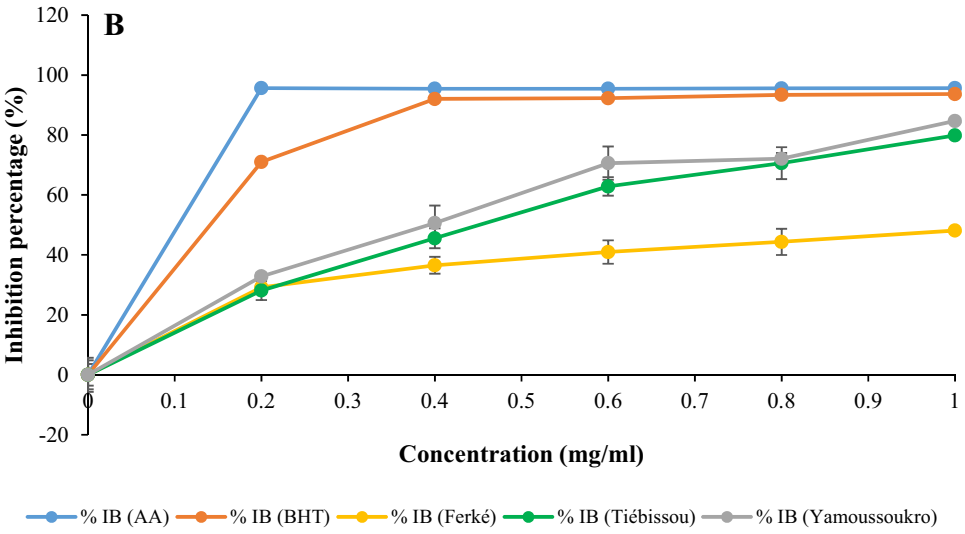
Antioxidant activities of black plum (*V. doniana*) fruit pulp and peel DPPH radical scavenging activity

The •DPPH radical-scavenging activity of peel and pulp extracts was tested and compared to ascorbic acid (AA) and butylated hydroxytoluene (BHT). The results showed that *V. doniana* fruit pulp and peel extracts could act as antioxidants to eliminate free radicals (Fig. 2a, b). Significant activities of removing •DPPH radical of peel and pulp extracts were evident at all tested concentrations. In addition, a dose-related reaction was observed between DPPH radical reactions and extracts reactions. It is also apparent that the radical-scavenging activity of Yamoussoukro and Tiébissou fruit peel extracts was comparable to those of AA and BHT at 0.8 and 1 mg/mL (Fig. 2b) of total polyphenols. Yamoussoukro and Tiébissou fruit peel inhibited •DPPH radical to more than 70% at 0.8 mg/mL (Fig. 2b), while AA and BHT trapped 95.5 and 93.5% of •DPPH from 0.8 mg/mL of polyphenols (Fig. 2b), respectively. Concentrations ranged between 0.8 and 1 mg/mL, a difference of 11–25% (Fig. 2b) was reported. However, AA and BHT presented high antioxidant power.

As shown in Fig. 2b, peel extracts exhibited antioxidant activity at least 3- to 11-fold higher than pulp. Furthermore, peel IC_{50} values ranged from 0.48 ± 0.01 to 0.91 ± 0.09 mg/mL of extract, while those pulp ranged between 1.90 ± 0.27 and 2.13 ± 0.18 mg/mL (Table 4). However, there was a significant difference ($P < 0.05$) between the IC_{50} of the fruit parts. IC_{50} is inversely related to a compound's antioxidant capacity because it expresses the amount of antioxidant required to decrease free-radical concentration by 50% [54]. Among peel, those with the strongest antioxidant capacity were derived from Tiébissou (0.53 ± 0.06 mg/mL) and Yamoussoukro (0.48 ± 0.01 mg/mL). However, no significant difference was observed between the IC_{50} values of pulp ($P > 0.05$). Total polyphenol content and •DPPH radical-scavenging showed a negative correlation ($r = -0.7997$, Table 5). This means that the IC_{50} of an extract containing high amounts of polyphenols should be lower, which was not the case in this study. The total polyphenol content of extracts differed significantly ($P < 0.05$). Therefore, this result could be attributed to the types or structures of phenolic compounds contained in the extracts. Levett [55], Gao and Emin [56] and Lien et al. [57] demonstrated that the phenolic compounds antioxidant activities vary with molecular structure and the number and position of –OH groups. Natella et al. [46] showed that, for example, cinnamic acids have a high antioxidant capacity compared to their benzoic acid counterparts. They were found to be quite potent lipid peroxidation inhibitors [13]. On the other hand, the total flavonoid content and the ·DPPH radical-scavenging activity of extracts exhibited a strong negative correlation. Similar to the present study, Sariburun et al. [58] reported that flavonoid content in raspberry and mulberry fruit was more correlated with ·DPPH radical-scavenging activity than total polyphenol content. The IC_{50}s of fruit peel extracts from Yamoussoukro and Tiébissou were only about 1/4 of those of pulp extracts. This variation in antioxidant capacity between peel and pulp extracts is due to differences in phenolic compounds, the amount contained in extracts and their efficiency as antioxidants [59]. These results confirm those presented in Table 3. The study by Palafox-carlos et al. [60] demonstrated that gallic acid was the phenolic compound with the highest contribution (70%) into the antioxidant activity of ripe mango. Only Tiébissou and Yamoussoukro peels contained gallic acid, which was also the majority compound. Gallic acid is able to reverse oxidative damages induced by lead (Pb) and prevents ketamine-induced oxidative damages [61–63]. This could justify their high antioxidant power compared to pulp.



%IB: Inibition percentage; BHT: Butylhydroxytoluene; AA: Ascorbic Acid



%IB: Inibition percentage; BHT: Butylhydroxytoluene; AA: Ascorbic Acid

Fig. 2 **a** Antioxidant activity of pulp extracts of black plum fruit (*V. doniana*) from Ferke, Tiébissou and Yamoussoukro by DPPH method. %IB Inibition percentage, BHT Butylhydroxytoluene, AA Ascorbic Acid. **b** Antioxidant activity of peel extracts of black plum fruit (*V. doniana*) from Ferke, Tiébissou and Yamoussoukro by DPPH method. *FPU* ferke pulp, *TPU* tiébissou pulp, *YPU* yamoussoukro pulp, *FPE* ferke peel, *TPE* tiébissou peel, *YPE* yamoussoukro peel, *BHT* butylhydroxytoluene, *AA* ascorbic acid

Table 4 Antioxidant activities of pulp and peel extracts of black plum (*V. doniana*) fruits expressed as IC_{50} (mg/mL) and reduction capacity (mg Ascorbic Acid Equivalent (AAE)/g dry extract)

Samples	IC_{50} (mg/mL)		Reduction capacity (mg AAE/g dry extract)	
	Pulp	Peel	Pulp	Peel
Ferke	1.90 ± 0.27^{a}	0.91 ± 0.09^{a}	5.80 ± 0.00^{a}	10.89 ± 0.00^{a}
Tiébissou	2.13 ± 0.18^{a}	0.53 ± 0.06^{b}	8.03 ± 0.01^{a}	23.12 ± 0.02^{b}
Yamoussoukro	1.94 ± 0.06^{a}	0.48 ± 0.01^{b}	9.11 ± 0.01^{a}	27.67 ± 0.03^{b}

Results are expressed as mean ± standard deviation of three separate samples. In the columns, values with the same letter mean that they are significantly the same

Ferric reducing antioxidant power (FRAP)

The FRAP test measures the ability of an antioxidant compound to be reduced the TPTZ-Fe(III) complex to its ferrous form, TPTZ-Fe(II). Similar to the results of the DPPH method, Fig. 2a and b show that all extracts act dose-dependently. *Vitex doniana* fruit portion extracts appear to be active in reducing Fe(III), indicating their antioxidant

Table 5 Correlation coefficient between antioxidant activities and phytochemical compounds in extracts of *V. doniana* fruit pulp and peel

Phytochemical compounds												
Antioxidant tests	Pulp						Peel					
	Total phenols		Total flavonoids		Total anthocyanins		Total phenols		Total flavonoids		Total anthocyanins	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
DPPH	−0.80	0.056	−0.96	0.002	−0.70	0.121	−0.88	0.002	−0.94	0.001	−0.71	0.032
FRAP	0.97	0.001	0.95	0.003	0.40	0.436	0.94	0.001	0.91	0.004	0.60	0.076

activity (Fig. 3). The ferric reducing power of *V. doniana* extracts varied, but all values were lower than those of standards (BHT and AA). The FRAP values, expressed in mg AAE/g dry extract, are used to compare the reducing power of extracts (Table 4). According to the Table 4, FRAP values of pulp extracts are not significantly different. This average 7.64 ± 0.00 mg AAE/g dry extract. On the other hand, among peel extracts, Yamoussoukro extracts (27.67 ± 0.03 mg AAE/g dry extract) and Tiébissou (23.12 ± 0.02 mg AAE/g dry extract) express the highest reduction capacity and express the lowest reduction capacity by Ferke extract (10.89 ± 0.00 mg AAE/g dry extract). Black plum fruit peel extracts had highest significant FRAP value than pulp. These results indicate that the most reactive antioxidants are present in the peel fraction, which are in agreement with works realized on apples [64–66], guava, mango and apricot [67] and pear [68].

FRAP values showed strong positive correlation with TPC content (r = 0.97, P < 0.05) and flavonoids (r = 0.95, P < 0.05) (Table 5). As may be seen in Table 5, TPC content of black plum extracts contributes significantly to ferric reducing and DPPH radical scavenging (r = −0.80). It means that the phenolic compounds contained in the fruit extracts of black plum have greater capacity to reduce Fe(III) than to scavenge free radicals. They act more as reducers than as radical scavengers. Similar results were obtained by Lamien-Meda et al. [69], Ma et al. [70] and Sokamte et al. [71]. Therefore, black plum fruit pulp and peel extracts properties to chelate the metal chelating may be important in oxidative stress conditions where a transition metal ion is involved. One of such conditions is atherosclerosis [72]. Polyphenolics and flavonoids seem to be the antioxidant compounds which contribute to black plum (pulp and peel) antioxidant activity as might be concluded from the highest values of Pearson coefficients between TP and DPPH (r = −0.80; r = −0.88), TP and FRAP (r = 0.97; r = 0.94), TF and DPPH (r = −0.96; r = −0.94) and, TF and FRAP (r = 0.95; r = 0.91). Flavonoids such as quercetin inhibits the oxidation of LDL cholesterol. Flavonoids play also a key role in neutralizing free radical species which occurs by the mechanism of electron transfer [73–75].

The results from this study highlight the importance of the TPC, TF and TA content and antioxidant capacities of *V. doniana* fruit pulp and peel from Côte d'Ivoire.

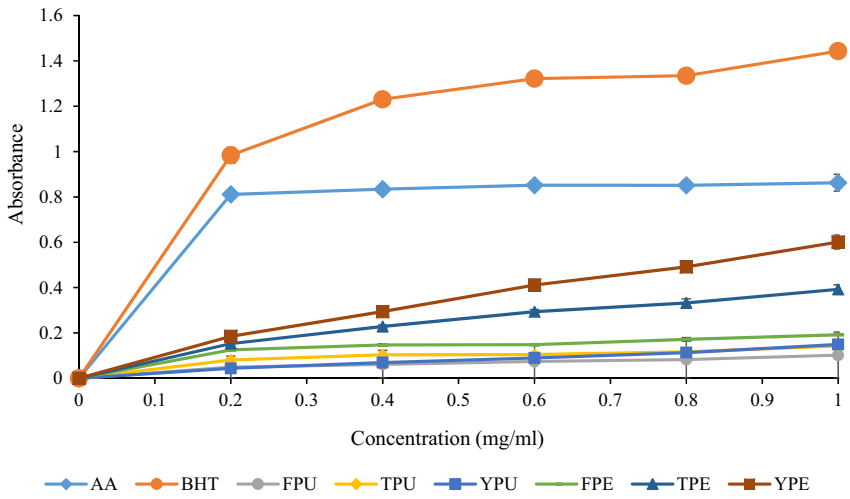


Fig. 3 Antioxidant activity of fruit pulp and peel extracts of black plum (*V. doniana*) from Ferke, Tiébissou and Yamoussoukro evaluated by the FRAP method

FPU : Ferke pulp ; TPU : Tiébissou pulp ; YPU : Yamoussoukro pulp ; FPE : Ferke peel ; TPE : Tiébissou peel ; YPE : Yamoussoukro peel; BHT : Butylhydroxytoluene; AA: Ascorbic Acid

This study provides data for health professionals and food policy makers in Côte d'Ivoire to encourage the population to consume more wild edible fruits, in particular *V. doniana* fruit, as well as to promote the preservation of this fruit species.

Conclusions

In summary, a detailed study of phenolic compound content, ascorbic acid content and antioxidant activities and their correlations in *V. doniana* fruit pulp and peel extracts from Côte d'Ivoire (Ferke, Tiébissou and Yamoussoukro) was carried out. The fruit pulp and peel from Yamoussoukro showed the highest phenolic compound content among the fruits from the three zones. It has also been shown that extracts of *V. doniana* fruit peel contain higher concentrations of phenolic compounds than does the pulp. The higher capacity for scavenging of ·DPPH radical and ferric reduction were observed in Yamoussoukro and Tiébissou fruit peel. Therefore, the consumption of *V. doniana* fruit with peel is recommended. Moreover, significant correlations were observed between antioxidant capacities and the total polyphenol and flavonoid contents. Preliminary analysis of peel and pulp extracts by HPLC revealed the presence of some phenolic acids with good biological activities, which could explain their antioxidant activity. Thus, owing to their ability to trap free radicals and their reducing power, *V. doniana* fruit peel could contribute to disease prevention related to abnormally high levels of ROS in the body. Consumption of black plum peel is therefore encouraged and recommended.

However, further research should be conducted to identify those phenolic compounds present in *V. doniana* fruit with high HPLC peak areas not identified during analysis. In addition, an evaluation of the inhibitory effect of lipid peroxidation of phenolic compounds in *V. doniana* fruit peel should be profitable.

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Compliance with ethical standards

Conflict of interest The authors declared that they have no conflict of interest.

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