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Free radical scavenging activity and HPLC-MS identification of major anthocyanin in ripe *Ziziphus mucronata* fruit collected in Burkina Faso

Arrounan NOBA[1], Constantin M. DABIRÉ[1,2*], Remy K. BATIONO[1,3], Bazoin Sylvain Raoul BAZIÉ[4], Paulin OUÔBA[5], Moumouni KOALA[1,6], Adama HEMA[1], Elie KABRÉ[4], Eloi PALÉ[1] and Mouhoussine NACRO[1]

[1]*Laboratoire de Chimie Organique et de Physique Appliquées (L.C.O.P.A.), Université Joseph KI-ZERBO, UFR/SEA, Département de Chimie 03 BP 7021 Ouagadougou 03 Burkina Faso.*
[2]*Laboratoire de Chimie et Energies Renouvelables, Université Nazi Boni, 01 BP 1091 Bobo 01, Burkina Faso.*
[3]*CNRST/IRSAT/ Département Substances Naturelles, 03 BP 7047 Ouagadougou 03 Burkina Faso*.
[4]*Agence nationale pour la sécurité sanitaire de l'environnement, de l'alimentation, du travail et des produits de santé (ANSSEAT, ex L.N.S.P) 09 BP 24 Ouagadougou 09, Burkina Faso.*
[5]*Laboratoire de Biologie et Écologie Végétales, Université de Ouagadougou, 03 BP 7021, Ouagadougou 03, Burkina Faso.*
[6]*Institut de Recherche en Sciences de la Santé, Centre National de la Recherche Scientifique et Technologique (IRSS/CNRST), 03 BP 7047 Ouagadougou 03, Burkina Faso.*
[*] *Corresponding author; E-mail: dabireconst@yahoo.fr, Tel.: +226 70 01 44 97.*

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ABSTRACT

In recent decades, interest in anthocyanin pigments has increased due to their possible use as natural food colorants, antioxidants, and anti-inflammatory agents. This study aimed to identify the major anthocyanins in ripe *Ziziphus mucronata* fruit harvested in the Niangoloko classified forest. HPLC-ESI-MS/MS was used to identify anthocyanins. The total phenolic content (TPC) and total flavonoids (TFC) were assessed using the Folin-Ciocalteu reagent and the spectrophotometric method of Khan (2012) respectively. Total anthocyanin content (TAC) was obtained using a differential pH method. Total antioxidant potential (TAC) was carried out using DPPH and ABTS tests. Cyanidin 3-O-glucoside and cyanidin 3-O-galactoside were major anthocyanins identified by HPLC-MS. Quantitative analysis showed that this fruit contains an average of 321 ± 11 mg gallic acid equivalent (GAE), 3815 ± 15 mg quercetin equivalent (QE), and 9.64 ± 0.70 mg cyanidin 3-O-glucoside equivalent per 100 g fresh fruit. Total antioxidant contents ranged between 206.57 ± 7.00 and 88.72 ± 0.87 mg TE/100 g fresh fruit. Free radical scavenging activity of *Ziziphus mucronata* fruit was found to be 342.90 µg/mL, while that of standard Trolox was 21.8 µg/mL. Therefore, *Ziziphus mucronata* fruit could be a potential source of natural bioactive substances of great interest to consumers.

Keywords: *Ziziphus mucronata* fruit, bioactive compound, anthocyanin, HPLC-MS, free radical scavenging.

INTRODUCTION

Anthocyanins are water-soluble pigments that can be used to color varied food products. Belonging to the flavonoid family, anthocyanins have many pharmacological properties, such as antioxidants, antidiabetic, antimicrobial, neuroprotective, antihypertensive, and anticancerous (Câmara et al.,

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2022; Chen et al., 2023). Recent studies reported by Chen et al. (2023) showed that anthocyanins are promising molecules for treating type 2 diabetes. As a result, there is growing interest in their beneficial effects. Known as a source of natural food colorants, anthocyanins are now recognized as physiologically functional dietary factors (Motohashi et al., 2009). However, the study of anthocyanins has mainly been limited to certain fruits such as grapes, berries, and sweet potatoes. Certain fruits, particularly wild fruits, which could be potential sources of anthocyanins, have still not been the subject of scientific studies on the identification or quantification of anthocyanins.

Ziziphus mucronata is a wild fruit growing in wes tern Burkina Faso belonging to the Rhamnaceae family. The fruit of this specie is rich in phenolic compounds, particularly flavonoids (Koïta et al., 2017). Ali et al. (2016) estimated that the fruit of *Ziziphus mucronata* can serve as a good source of protein, crude fat (lipids), and carbohydrates. The bioactive compound content of this fruit (proteins, carbohydrates, lipids, phenolic compounds, and vitamin C) could make it a potential source of nutrients for consumers. However, to our knowledge, the anthocyanin content, anthocyanin molecule structures, and free radical scavenging activity of this fruit have not yet been the subject of any scientific study. Thus, this study aimed to identify, by HPLC-ESI-MS, major anthocyanin in ripe *Ziziphus mucronata* fruit and to evaluate free radical scavenging activity.

MATERIALS AND METHODS

Standards and reagents

Folin-Ciocalteu reagent, 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox), quercetin, 2,2-diphenyl-picrylhydrazyl (DPPH), sodium acetate, gallic acid, sodium carbonate, Amberlite XAD-7, aluminum chloride was obtained from Sigma-Aldrich. Potassium chloride, sodium hydroxide, acetic acid, and chloroform were purchased from AnalaR NORMAPUR. Sodium nitrite was obtained from Labosi. Methanol, formic acid, acetone, and acetonitrile were obtained from Carlo Erba and hydrochloric acid was purchased from Fisher Chemical. The solvents used were analytical grade or HPLC grade.

Extraction and sampling

Ripe *Ziziphus mucronata* fruit were harvested in the Niangoloko classified forest. Fruits were carefully washed and stored at -20°C for further analysis. Species identification was carried out at the NAZI Boni University in Bobo Dioulasso by Dr. Paulin OUOBA.

Extraction of total phenolic compounds

The extraction of phenolic compounds was carried out as Kim et al. (2002) previously reported with some modifications. 15 g of fresh fruit was extracted with 150 mL of acetone-water-acetic acid solvent system (70: 29.5: 0.5, v/v) for 24 h at 4°C. The operation was performed in triplicate. Non-phenolic compounds in the extract were removed with chloroform. The extract was stored at 4°C for analysis.

Anthocyanin extraction

Anthocyanins were extracted using the method described by Martinez-Cruz et al. (2011) with minor modifications. 10 g of fruit were extracted with 100 mL of acidified methanol (1% HCl, v/v) for 24 hours at 4°C. Extractions were performed in triplicate. Finally, the three extracts were pooled and filtered under vacuum. The filtrate was concentrated under reduced pressure (40 mbar) at 35°C using a rotary evaporator (Buchi Switzerland, Rotavapor R-300). The extract was recovered with a minimum of acidified distilled water (1% HCl, v/v) and stored at 4°C until further analysis.

Anthocyanin purification on Amberlite XAD-7

The aqueous extract obtained above was purified on Amberlite resin. The fixation of the extract on Amberlite XAD-7 was carried out in a column 24 cm (Amberlite) long and 3 cm in diameter. The amberlite resin was first prepared in ethanol. The resulting mixture was loaded onto the column and left to stand. The

column was rinsed with water (150 mL) The concentrated extract was carefully loaded onto the XAD-7 amberlite resin. The amberlite resin loaded with extract was washed with distilled water. Anthocyanins absorbed by the resin were then eluted with acidified ethanol (1% HCl, v/v). The collected eluate was concentrated to dryness under reduced pressure (40 mbar) using a rotary evaporator (Buchi Switzerland, Rotavapor R-300). This methodology was previously used for other edible fruits (Brito et al., 2014; Simirgiotis 2013a, 2013b). Identification of anthocyanin and free radical scavenging activity were carried out on the total anthocyanin extract.

Identification of anthocyanins by HPLC-MS/MS

The method described by Hutabarat et al. (2019) was used to identify anthocyanins with minor modifications. HPLC-ESI-MS analysis of extract was performed using the Agilent-1290 infinity HPLC system equipped with a 6430 triple quad LC/MS mass detector (MS) fitted with an electrospray ionization (ESI) interface (Agilent Technologies, USA) and an Agilent MassHunter version B.06.00 workstation for data processing. Separation was carried out in a Zorbax SB-C18 column with a particle size of 5 µm and 250 mm × 4.6 mm internal diameter. The column was thermostatically controlled at 25°C, and the flow rate was set at 0.6 mL/min. Solvent system was: water-formic acid (A, 95: 5, v/v) and acetonitrile-formic acid (B, 95: 5, v/v). Linear gradient solvent system in volumetric ratios was as follows: 0-5 min, 95% A; 5-15 min, 90% A; 15-25 min, 90% A; 25-35 min, 88% A; 35-50 min, 85% A; 50-60 min, 82% A; 60-80 min, 75% A; 80-90 min, 70% A; 90-100 min, 95% A and a final reconditioning the column with 95% solvent A isocratic for 10 min. Fifty microliters (50 µL) of sample previously filtered through a cellulose acetate membrane (Millipore 25 mm, 0.45 µm,) were injected. Ion mass spectra were recorded in positive ionization mode in the m/z range of 100-1200. Nitrogen was used as drying and nebulizing gas at a flow rate of 10 L/min and a pressure of 15 psi. The nebulizer temperature was set at 200°C, and a potential of 3.5 kV was used on the capillary. The collision energy was 10 eV.

Total phenolic content (TPC)

The total phenolic content was determined using the Folin-Ciocalteu reagent method (Singleton et al., 1999), with some modifications. To 60 µL of extract, 60 µL of Folin-Ciocalteu reagent was added. After 8 min of reaction at room temperature (25°C), 120 µL of 7.5% (w/v) aqueous sodium carbonate solution was added. The solution was incubated at 37°C for 30 minutes and the absorbance was read at 760 nm with a SAFAS MP96 spectrophotometer against a blank. The calibration curve was performed with gallic acid ($y = 24.941x + 0.1225$; $R^2 = 0.9995$) and the results were expressed as mg gallic acid equivalents/100 g of fresh fruit (mg GAE/100 g).

Total flavonoid content (TFC)

The total flavonoid content was performed as reported previously (Khan, 2012) with some modifications. To 50 µL of extract was added 15 µL of a 5% sodium nitrite ($NaNO_2$) solution. After 5 minutes, 15 µL of 10% Aluminum chloride ($AlCl_3$) solution was added and the mixture was allowed to stand for a further 6 minutes before adding 50 µL of 1 N sodium hydroxide (NaOH). The mixture was immediately diluted with 150 µL of deionized water. The absorbance of the pink solution obtained was measured immediately at 510 nm using a SAFAS MP96 spectrophotometer. Total flavonoid content was determined from a calibration curve ($y = 1.0409x + 0.3078$; $R^2 = 0.9974$) prepared with a series of quercetin standards. Result was expressed mg of quercetin equivalents /100 g of fresh fruit (mg QE/100 g).

Total anthocyanin content (TAC)

The assessment of total anthocyanin content (TAC) was carried out by the pH differential method according to AOAC as described by Giusti et al. (2015). This method uses two buffer systems (potassium chloride solution, pH = 1.0 (0.025 M) and sodium

acetate solution, pH = 4.5 (0.4 M)). Hence, 0.5 mL of extract was diluted in 3.5 mL of the two buffers: potassium chloride (pH = 1.0) and sodium acetate (pH 4.5). Deionized water was used as blank and cyanidin 3-glucoside as the standard. The absorbance of the mixture was read at 510 nm and 700 nm exactly 15 minutes later using a SAFAS MP96 spectrophotometer. Pigment concentration was expressed as mg cyanidin 3-glucoside equivalents/100 g fresh fruit (mg CE/100 g) and calculated using the formula Absorbance A was calculated as follows:

$$\text{TAC (mg/g)} = \frac{\text{A}\times\text{MW}\times\text{DF}\times\text{V}\times 1000}{\varepsilon\times\text{l}\times\text{m}}$$

where $A = (A_{\lambda vis-max} - A_{700})_{pH=1.0} - (A_{\lambda vis-max} - A_{700})_{pH=4.5}$; MW is the molecular weight of cyanidin 3-O-glucoside (449.2 g/mol); DF = dilution factor; ε is the molar extinction coefficient for cyanidin (26900 L/mol.cm) (Taghavi et al., 2022). M is the sample mass in g; 1000 is the factor to convert g to mg; V is the extract volume (L).

Total antioxidant content using the DPPH method

Total antioxidant content (TAC) of the extract was determined by DPPH assay as previously described by Zhang et al. (2015) with some modifications. DPPH solution was freshly prepared by dissolving 2 mg of the which reagent in 50 mL methanol. 50 µL of extract was added to 200 µL of DPPH solution. The mixture was shaken and allowed to react at room temperature in the dark for 10 minutes. The decrease in the absorbance of the solution was read at 517 nm using a SAFAS MP96 spectrophotometer. The calibration curve was performed with Trolox (concentrations ranging from 2 to 250 µg/mL, y = -17.101x + 0.3068; R^2 = 0.9989) and the results were expressed as mg Trolox equivalents/100 g fresh fruit (mg TE/100 g).

Determination of total antioxidant content using the ABTS method

The ABTS radical cation delocalization assay described by Re et al. (1999) was used to determine the total antioxidant content of the extract. ABTS was dissolved in water at a concentration of 7.46 mM. $ABTS^{\bullet+}$was produced by reacting the ABTS stock solution with 39.2 mM potassium persulfate and kept in the dark at room temperature for 16 hours before use. The blue-green solution of $ABTS^{\bullet+}$ was diluted to give an absorbance of around 0.7 at 734 nm. 50 µL of the extract was added to 200 µL of $ABTS^{\bullet+}$solution. The mixture was allowed to stand at room temperature in the dark for 10 minutes. The absorbance of the resulting solution was read at 734 nm using SAFAS MP96 spectrophotometer. The calibration curve was performed with Trolox (concentrations ranging from 2 to 250 µg/mL, y = -13.741x + 0.7782; R^2 = 0.9945)) and the results were expressed as mg Trolox equivalents/100 g fresh fruit.

Determination of free radical scavenging activity using the DPPH method

The free radical scavenging activity of the extract was determined using the DPPH method previously described by Popovici et al. (2009). A 25 µL of extract was mixed with 225 µL of the $DPPH^{\bullet}$ solution. The reaction mixture was stirred for 10 seconds. At regular time intervals (1 minute), absorbances at 517 nm were recorded (against methanol) using a SAFA MP96 spectrophotometer. $DPPH^{\bullet}$ radical reduction kinetics at different test sample concentrations were followed over time until a plateau was reached at the final time (Teq). The relative ratio of the residual concentration $[DPPH^{\bullet}]t=Teq$ remaining at the end of kinetics to its initial concentration assessed the free radical reduction:

$$\%\ DPPH^{\bullet} = \frac{[DPPH]_{(t=Teq)}}{[DPPH]_{(t=0)}} \times 100$$

From the curve plotting the relationship between the percentage reduction $\%(DPPH^{\bullet})_R$ and extract concentration, graphically interpolating of effective concentration (EC_{50}) and time T_{CE50} was done. The antioxidant activity is best when the EC_{50} value is small. Trolox solution was used as standard.

Statistical analysis

Data analysis was performed using Microsoft Excel 2016 and IBM SPSS Statistics

25.0. All experiments were performed at least three times. Results were expressed as the mean ± standard deviation. All data were analyzed using the ANOVA procedure with significance at the $p < 0.05$ level.

RESULTS

Identification of anthocyanins by HPLC-ESI-MS/MS

HPLC -ESI-MS/MS analysis showed two intense peaks indicating the presence of 02 anthocyanins. Figure 1 showed the chromatogram. Compounds identified are presented in Table 1. Figures 2, 3, 4 and 5 showed the mass spectra of the compounds, while Figures 6 and 7 showed their structures. Table 1 shows two anthocyanin molecules that could be identified as cyanidin derivatives. The structures of these anthocyanin molecules were identified mainly on the basis of their retention times and mass spectral data (Wu et al., 2005).

Total phenolic, total flavonoid, total anthocyanin and total antioxidant content

The phenolic content, flavonoids content, anthocyanins content and antioxidants content were 321±11 mg GAE/100 g, 3815±15 mg QE/100 g, 9.64±0.70 mg CE/100 g, 206.57±7.00 mg ET/100 g, 88.72±0.87 mg TE/100 g respectively (Table 2) ($p<0.05$).

DPPH radical scavenging activity

The concentration of radical reduced by anthocyanin extract for 40 minutes was measured. Thus, reactivity was estimated by the effective concentration EC_{50} of the extract, which corresponds to a 50% reduction in the activity (absorbance) of the DPPH radical in the reaction mixture. The smaller the EC_{50}, the greater the antioxidant capacity of compound. In addition, to better estimate the anti-free radical capacity of our extract, reduction kinetics was monitored by determining the $T_{EC_{50}}$ time required to reach the plateau (Figures 8 to 11).

The anthocyanin extract studied had an EC_{50} of 342.90 µg/mL, compared with 21.8 µg/mL for Trolox, and a $T_{EC_{50}}$ time of 28 minutes (intermediate reaction) (Table 3). The $T_{EC_{50}}$ time was used to classify the reactivity of an extract. The reaction is rapid when $T_{EC_{50}}$ is less than 5 minutes, intermediate when $T_{EC_{50}}$ is between 5 and 30 minutes, and slow if $T_{EC_{50}}$ is greater than 30 minutes (Popovici et al., 2009).

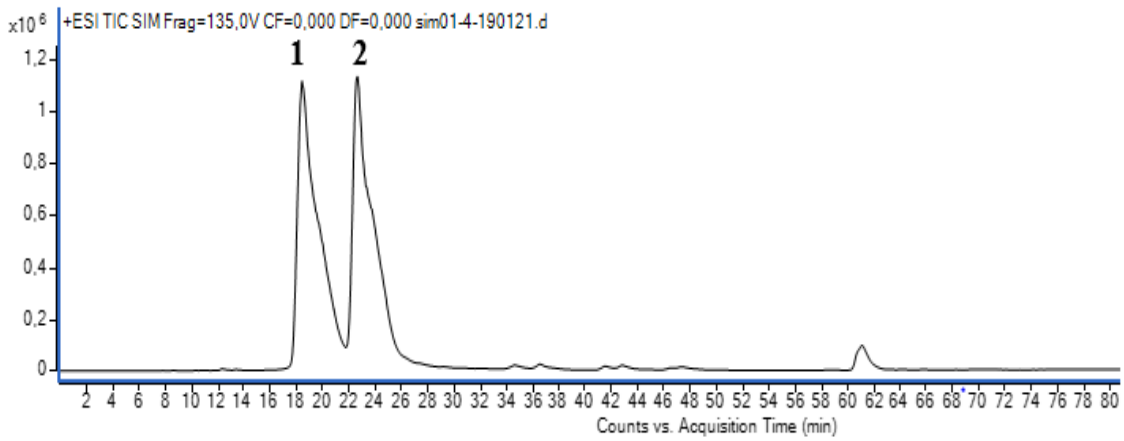

Figure 1: HPLC chromatogram of methanolic extract.

Table 1: Identification of anthocyanins in *Ziziphus mucronata* fruit.

Peak n°	Retention time (minutes)	Compound	$[M]^+$	$[M-162]^+$	Molecules identified
1	18.4205	1	449	287	Cyanidin 3-*O*-glucoside
2	22.6385	2	449	287	Cyanidin 3-*O*-galactoside

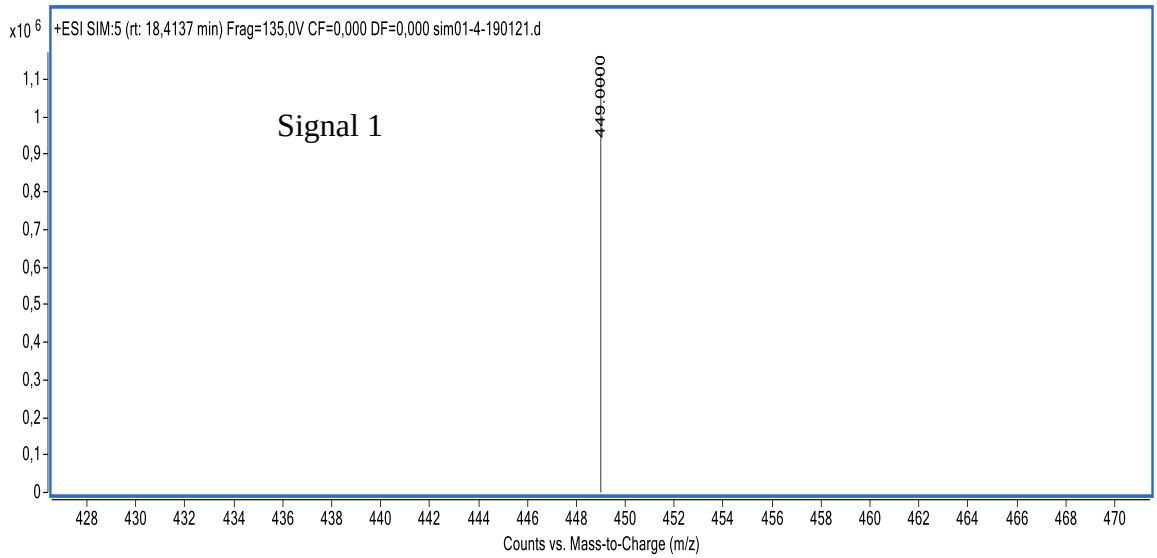

Figure 2 : Signal 1 mass spectra.

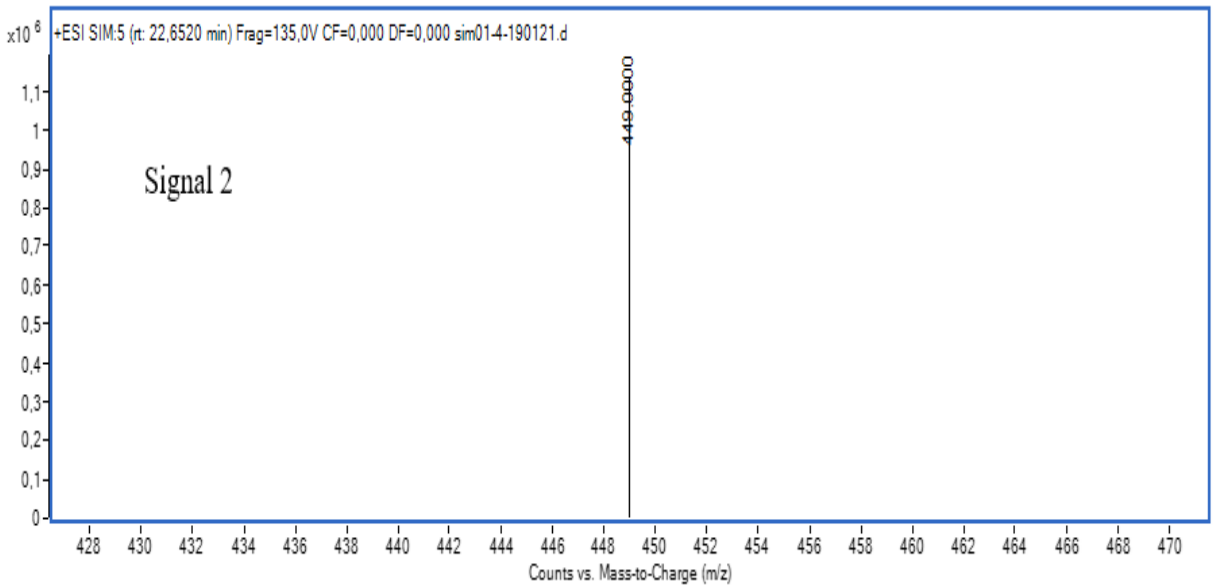

Figure 3 : Signal 2 mass spectra.

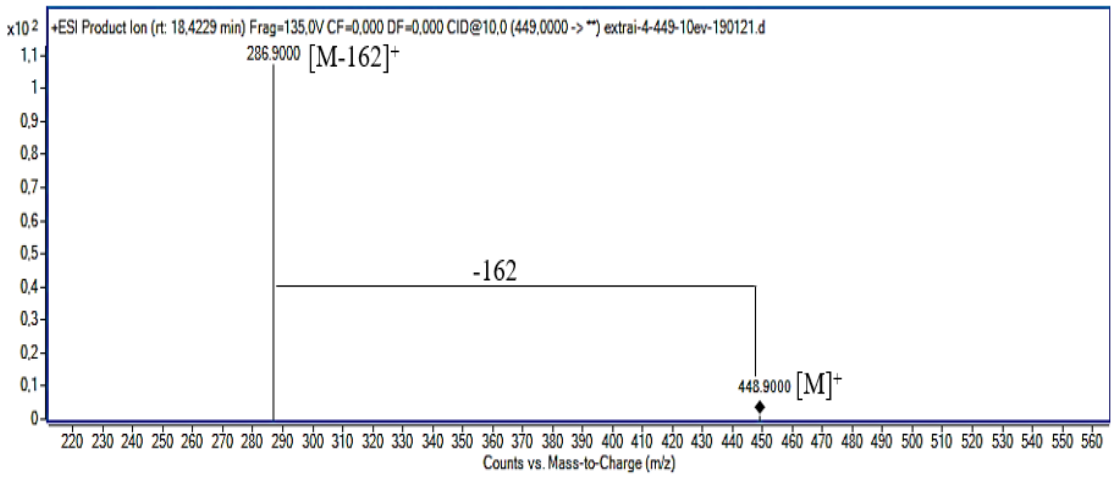

Figure 4: Mass spectra of compound 1.

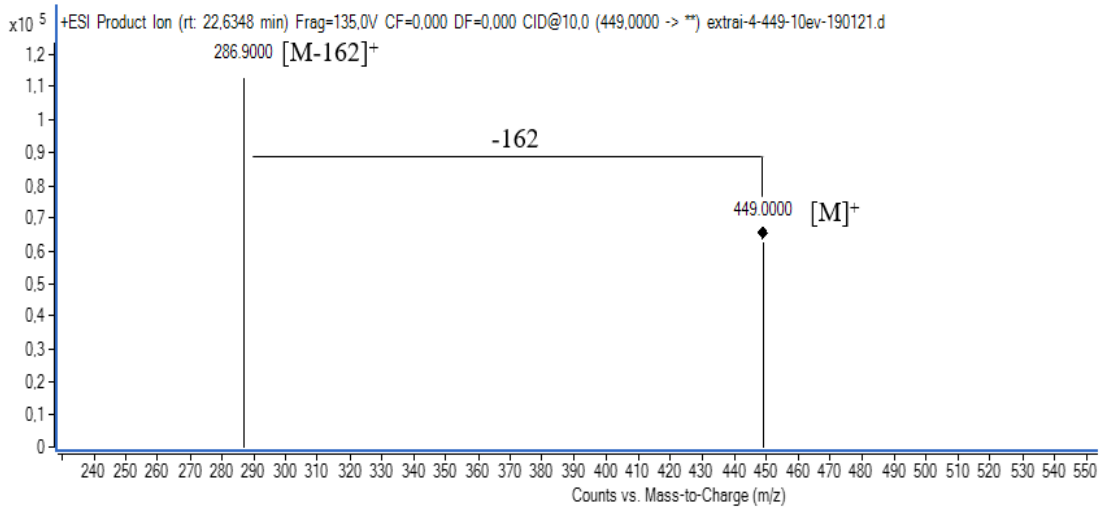

Figure 5: Mass spectra of compound 2.

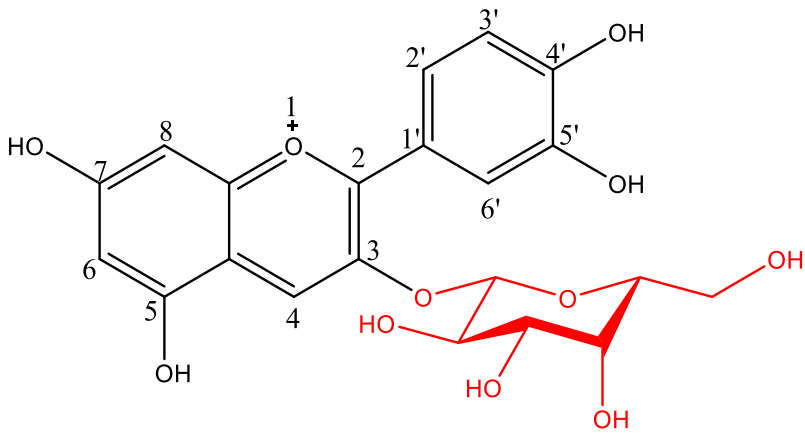

Figure 6 : Cyanidin 3-*O*-galactoside (compound 1).

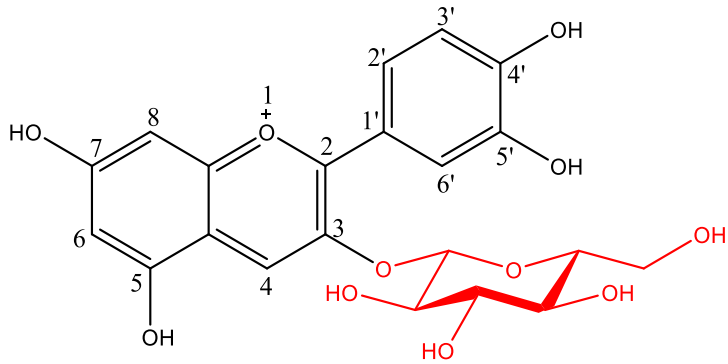

Figure 7: Cyanidin 3-*O*-glucoside (compound 2)

Table 2: Total phenolics, total flavonoids, total anthocyanins and total antioxidants contents.

	TPC	TFC	TAC	TAC	
				ABTS	DPPH
Content mg/100 g	321±11	3815±15	9.64±0.70	206.57[a]±7.00	88.72[b]±0.87

TPC: Total Phenolic content; TFC: Total Flavonoid content; TAC: Total Anthocyanin Content; TAC: Total Antioxidant; Significance levels: P <0.05

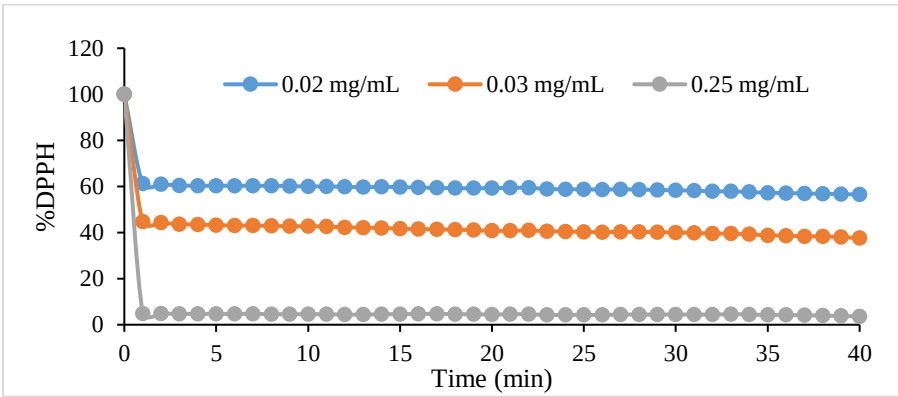

Figure 8: Curve showing the reduction of the DPPH• radical by Trolox as a function of time.

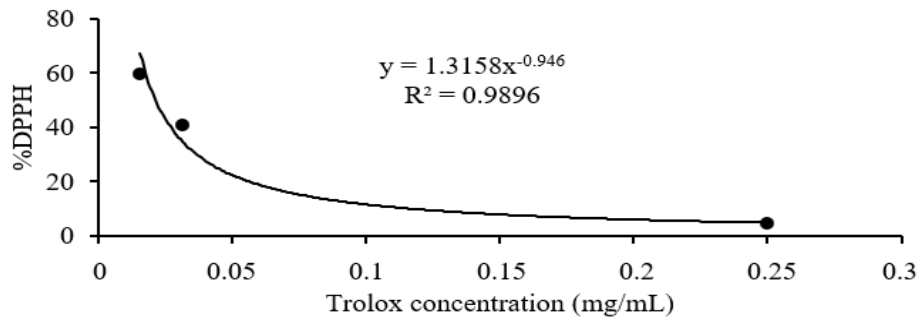

Figure 9: Determination of the effective concentration EC_{50} of the methanolic solution of Trolox.

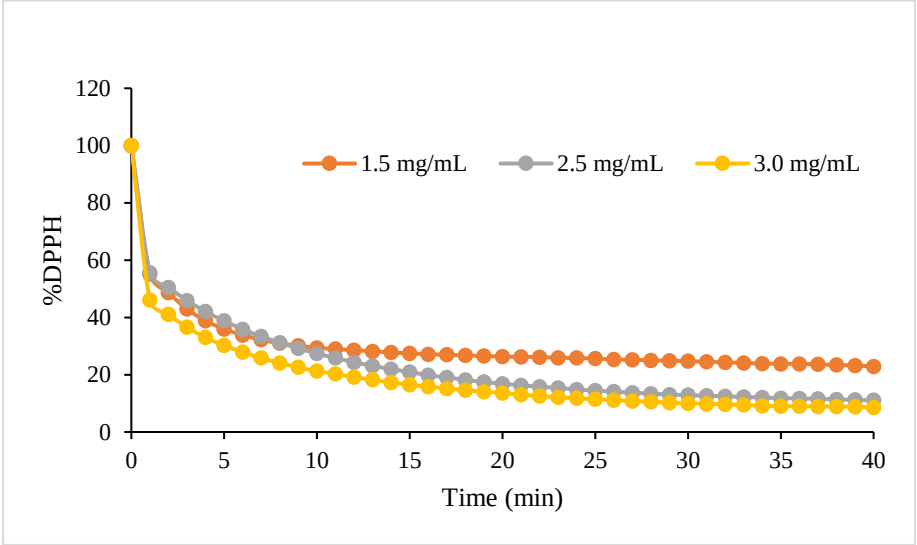

Figure 10: Curve showing the reduction of the DPPH• radical by extract as a function of time.

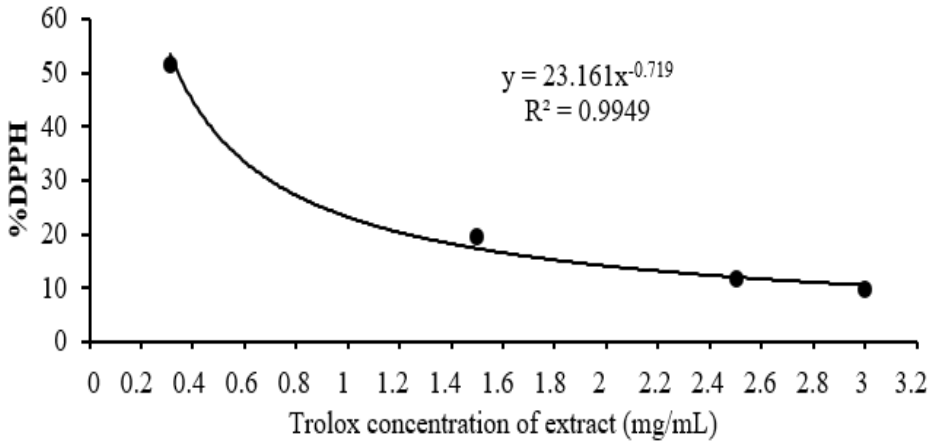

Figure 11: Determination of the effective concentration EC_{50} of the methanolic solution of extract.

Table 3: Antioxidant activities of total anthocyanin extract determined by DPPH method.

Total extracts	Curve equation	Correlation coefficient	Antioxidant capacity	
			EC_{50} (µg/mL)	T_{EC50} (minutes)
Trolox	$1.3158x^{-0.946}$	0.9896	21.38	11
Z. mucronata	$23.161x^{-0.719}$	0.9881	342.90	28

EC_{50}: effective concentration of the sample required to scavenge 50% of free radicals present in the test solution; $T_{EC_{50}}$: time required to reach the plateau

DISCUSSION

High-performance liquid chromatography (HPLC) is the most widely used tool for anthocyanin identification, in which individual anthocyanins can be separated according to their polarity (Lee et al., 2008). HPLC is usually coupled with mass spectrometry (MS) detectors (Swartz, 2010), which measure the mass-to-charge ratio (m/z) of individual ions, in order to obtain structural characterization by comparison with available published information on molecular and fragment ions, which generally form a single pattern. Electrospray ionization (ESI) is the most widely used interface in HPLC-MS configuration (Hutabarat et al., 2019).

Positive ESI analysis shows two types of ions: the molecular ion $[M]^+$ and a fragment ion $[M-X]^+$ resulting from the loss of the saccharide moiety (Abdel-Aal et al., 2006). Aglycones frequently found in nature include cyanidin, delphinidin, pelargonidin, petunidin, malvidin, and peonidin. The X value based on the difference between the molecular ion mass and that of the fragment gives an idea of the nature of the saccharide. Most known anthocyanins have a sugar in position 3. Glycosylation of the hydroxyl (OH) in position 3 has special properties and is essential for the stability of anthocyanin pigments (Hema et al., 2007)

HPLC-ESI-MS coupling is an effective technique for identifying unknown compounds with high selectivity and sensitivity (Yang et al., 2009).

As shown in Figure 1, two intense signals (1 and 2) at different retention times were recorded in *Z. mucronata* extract. Mass spectrometric detection of each signal yielded the mass spectra shown in Figures 2 and 3.

Tandem mass spectrometry analysis of each signal shows spectra with the same characteristics (Figures 4 and 6). Indeed, peaks 1 and 2 show molecular ions at m/z 449 ($C_{21}H_{21}O_{11}$) with identical fragmentation patterns, but the retention time in the HPLC system was 18 minutes for compound 1 and 22 minutes for compound 2. Their elution order characterized by their different polarities and MS characteristics indicate that they could be cyanidin 3-O-galactoside and cyanidin 3-O-glucoside, respectively. Indeed, the molecular ion at m/z 449 (Figures 4 and 6), through the loss of a 162 u unit, gives a fragment ion at m/z 287, which would correspond to the aglycone of cyanidin. Furthermore, in reverse-phase HPLC analysis, the most polar anthocyanidins were eluted first. On the other hand, literature data indicate that the elution sequence of anthocyanidin glycosides is such that galactose is eluted before glucose, which means that cyanidin 3-O-galactoside is more polar than its glucoside isomer. This would explain the difference in retention times between compounds 1 and 2 (Swartz, 2010 ; Wu et al., 2005 ; Huang et al., 2012 ; Cerezo et al., 2010 ; Filip et al., 2012). In short, we can say that compound 1 would correspond to cyanidin 3-O-galactoside (figure 5) and compound 2 would be cyanidin 3-O-glucoside (figure 7).

The analysis of the total phenolics contents results indicates that the fruit of this species would contain an average 321 ± 11 mg GAE/100 g of fresh fruit. Kwape et al. (2013) reported that the fruit of this species contains a high content of phenolic compounds, with an average value per gram of extract estimated at 250 mg of GAE. This difference could be explained by several factors such as the harvesting environment, fruit ripeness, and analysis method. In addition, the phenolics content of the fruit is significantly higher than that of the other parts of the plant (Kubola et al., 2011; Olajuyigbe et al., 2020). Interestingly, the TPC in this fruit is comparable to that of previously studied fruits, expressed as mg GAE/100 g, e.g. 126-247 in guava, 125 in plums (Koley et al., 2016). Furthermore, TPC is significantly better than those obtained in cultivars of the species *Ziziphus mauritiana Lamk* (172 to 328.61 mg GAE/100 g fresh fruit) (Koley et al., 2016). In addition, the fruit of this species is much richer in total flavonoids than the fruit of *Dacryodes edulis*, both in the seed and in the mesocarp (511 mg GAE/mg MS and 248 mg GAE/mg MS) (Etou Ossibi et al., 2023). Phenolic compounds in plants have multifunctional properties and can act as singlet oxygen quenchers and free radical scavengers. The

presence of substantial amounts of phenolic compounds in this fruit indicate that it is an important source of antioxidants with potential health benefits for consumers.

The total flavonoid content of this fruit was 3815±15 mg of quercetin equivalents per 100 gram of fresh fruit. Previous studies have reported the presence of flavonoids in the fruit of this species (Koïta et al., 2017). Studies also showed that the *Ziziphus* genus is rich in flavonoids, with contents varying from one species to another (Koley et al., 2016). This would explain the significant flavonoid content of this fruit. Compared with certain wild fruits consumed in Burkina Faso, such as *Ximenia americana*, *Vitellaria paradoxa*, *Tamarindus indica*, *Saba senegalensis*, *Lannea microcarpa*, and *Adansonia digitata*, the flavonoid content of this fruit is not outdone (Lamien et al., 2008 ; Noba et al., 2020). The total phenolic contents and total flavonoid contents in the fruit of *Ziziphus mucronata* were much higher than those in the fruit of *Saba thompsonii* according to the results obtained by Mensah et al. (2023) (4.783 mg GAE/100 g). However, this fruit studied have higher amounts of phenolic contents than edible wild fruits of *Uapaca kirkiana* (255.38 mg GAE/ 100 g fresh sample) and *Syzygium guineense* (137.37 mg GAE/ 100 g fresh sample) (Mapunda et al., 2019).

Anthocyanins, belonging to the flavonoid family (Kabore et al., 2022), are an alternative to artificial food color, and research suggests potential health benefits due to their antioxidant properties (Li et al., 2017). Quantitative analysis of *Z. mucronata* extract indicates that this fruit contains an average of 9.64 ± 0.70 mg cyanidin 3-glucoside equivalent per 100 g fresh fruit. The anthocyanin content of this fruit is higher than that of fruits such as pomegranate (3.42 mg/100 g), black tomato (4.17 mg/100 g), purple grape (5.38 mg/100 g), eggplant (1.4 mg/100 g), and tubers such as purple sweet potato (5.47 mg/100 g) (Horbowicz et al., 2008). Studies carried out by Mpiana et al., (2008, 2010) indicated that anthocyanins have an antihemolytic activity on the erythrocytes of SS blood, sickle cell disease (SCD) that affects more than 50 million people. Thus, *Z. mucronata* fruit could be an important source of beneficial anthocyanins for consumers.

Antioxidant assays are becoming increasingly important in the nutritional field as they provide useful information about the health and functional quality of raw materials without the need to analyze individual antioxidants (Scalfi et al., 2000). This parameter takes into account the presence of effective oxygen radical scavengers such as vitamin C and phenolic compounds, and their synergistic and/or antagonistic effects. In this study, two in vitro tests were used to evaluate the antioxidant potentials of *Z. mucronata* fruits, namely the ABTS test and the free radical scavenging test using the $DPPH^{\bullet}$ radical. Using these two methods, the antioxidant content of the extract was estimated at 206.57 ± 7.00 and 88.72 ± 0.87 mg ET/100 g fresh fruit ($p<0.05$) respectively for the ABTS and $DPPH^{\bullet}$ methods. These contents are far higher than those found in *Saba senegalensis* fruit (Noba et al., 2020), a wild fruit widely consumed in Burkina Faso.

The extract had a higher EC_{50} than the Trolox used as reference. However, the extract exhibited intermediate reactivity, just like the standard used. The complexity of the anthocyanin molecules making up this extract is thought to be responsible for its low anti-free radical activity and reactivity. Indeed, the steric hindrance of DPPH radical site mentioned in the literature may be a factor in the underestimation of free radical scavenging activities. However, work to identify the majority of anthocyanins showed that cyanidin monoglucosides were present in the extract. Also, the various constituents of the extract may act synergistically, each influencing the overall antioxidant capacity to a greater or lesser extent. Furthermore, total anthocyanin extracts are complex mixtures whose antioxidant activities can't be explained by structural considerations of anthocyanin constituents alone. There may be non-anthocyanin compounds that affect the free radical scavenging activity in one way or another. The number of hydroxyl and sugar groups contributes to increasing or decreasing

the stability, thus affecting antioxidant potential. It also depends on the free radical chosen to assess the antioxidant capacity. In addition to the anti-free radical activity of anthocyanins, they have enormous biological properties such as anti-inflammatory, anti-cancer and anti-diabetic activity, and have a major impact on the prevention and control of cardiovascular disease (Swamy, 2020).

Conclusion

Two anthocyanin molecules were identified in *Z. mucronata* fruit: cyanidin 3-O-glucoside and cyanidin 3-O-galacotside. Micronutrient evaluation revealed that this fruit is rich in bioactive compounds (phenolics, flavonoids and anthocyanins). This bioactive compound content is by far comparable to certain wild fruits consumed in Burkina Faso. In addition, the free radical scavenging activity and the DPPH$^{\bullet}$ radical reduction kinetics of the anthocyanin-rich extract of *Z. mucronata* fruit were evaluated. The extract showed lower free radical scavenging activity (342.90 µg/mL) than the standard used (Trolox 21.38 µg/mL). Nevertheless *Z. mucronata* wild fruits may constitute a potential source of natural bioactive substances of great interest for the prevention and treatment of many pathologies.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

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