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# Radical-Scavenging activities of total extracts and identification of four anthocyanin isomers from two purple-fleshed varieties of *Ipomoea batatas* L. produced in Burkina Faso, by hplc-ms/ms

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## ABSTRACT

Anthocyanins are bioactive compounds which, due to their free radical scavenging properties, can protect the human body against oxidative stress that can cause many diseases, such as cancer, aging. The *Tuskegee purple Cap Vert* and *Tuskegee purple* sweet potato (*Ipomoea batatas* L.) varieties are rich in anthocyanins. Indeed, their total anthocyanin contents are estimated at 0.211 and 0.122 mg/g of fresh plant material, respectively. And the antiradical activities of the varieties *Tuskegee purple* and *Tuskegee purple Cap Vert* extracts were estimated at 0.06 and 0.05 mg/mL respectively. The identification of anthocyanins in the extracts of the two sweet potato varieties was performed by HPLC-MS-UV, and HPLC-MS/MS analyses. Four anthocyanin isomers were identified in *Tuskegee purple Cap Vert* variety. These are : cyanidin 3-sophoroside-5-glucoside ; peonidin 3-sophoroside-5-glucoside ; cyanidin 3-laminaribioside-5-glucoside ; peonidin 3- laminaribioside-5-glucoside.


**Keywords**: High Performance Liquid Chromatography, Mass Spectrometry, anthocyanins, *Ipomoea batatas*, radical scavenging properties.

## INTRODUCTION

Anthocyanins, a group of plant pigments widely used in fruits, vegetables and some flowers, are flavonoids that commonly produce blue, red or purple colours and are part of a class of water-soluble glycosides and



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anthocyanidin acylglycosides. Anthocyanins have two benzene rings connected by a three-carbon linear chain (C2 C4), represented as the C6-C3-C6 system. This particular chemical structure makes them very reactive to free radicals and to strong natural antioxidants (Jiu-Liang et al., 2014). Their structures vary according to the nature, number and position of the sugars attached to the aglycone; as well as the number of hydroxyle groups, aliphatic or phenolic acids that are attached to these different sugars (Lee et al., 2013). The six most common aglycones in plants are cyanidin, peonidin, delphinidin, pelargonidin, petunidin and malvidin (Kim et al., 2012). Among the six anthocyanidines, peonidin and cyanidin are usually found in purple-fleshed sweet potatoes (PFSP), but the most abundant anthocyanins reported from PFSP are peonidin derivatives (Jianteng et al., 2015 ; Zhang et al., 2015). In these varieties, glycosylated and acylated anthocyanins account for more than 93%(Jianteng et al., 2015 ; Truong et al., 2010). To date, nearly 40 anthocyanins have been identified in PFSP. These are mainly cyanidin 3-sophoroside-5-glucoside and peonidin 3-sophoroside-5-glucoside, mono- or diacylated with caffeic, febolic, *p*-coumaric, and *p*-hydroxybenzoic acids. Indeed, many works such as those of (Zhang et al., 2017 ; Wang et al., 2017 ; Luo et al., 2018 ; Claudia et al., 2017) revealed the presence of peonidin 3-cafeoyl-*p*-hydroxybenzoyl sophoroside-5-glucoside and peonidin 3-cafeoyl-feruloyl sophoroside-5-glucoside as the two major anthocyanins of the purple-fleshed sweet potatoes.

Anthocyanins play a very important role in plant physiology (Stintzing and Carle, 2004). Because of their diverse biological properties, they protect plants from the harmful effects of ultraviolet rays. They also play a very important role in human health. Studies showed that extracts of anthocyanins from the purple-fleshed sweet potatoes have beneficial effects for consumers due to their antioxidant activities (Zhu et al., 2010), antidiabetic (Zhao et al., 2013), antimutagenic (Yoshimoto et al., 2001), hepatoprotectants (Wang et al., 2017) and antihyperuricmiants (Jianteng et al., 2015). Acylated anthocyanins are known for their good stability and biological activities (Kamiloglu et al., 2015).

The use of synthetic colorants is increasingly decried by the food industry, particularly because their ingestion has been publicly connoted with adverse health effects. As a result, the tendency to maintain the naturalness of a food as much as possible, the principle of "coloring food with food" has gained enormous importance for manufacturers in recent years.

In order to contribute to the study of purple sweet potato anthocyanins, two PFSP varieties, *Tuskegee purple and Tuskegee purple Cap Vert* introduced and produced under the agroecological conditions of Burkina Faso, were used. This work aimed to determine the total anthocyanin levels and to identify anthocyanin structures of these varieties by coupling chromatographic and spectral methods (HPLC-ESI-MS/MS).

## MATERIALS AND METHODS

### Plant material

Plants materials studied consisted of tubers of two varieties of purple-fleshed sweet potato. These are varieties *Tuskegee purple* and *Tuskegee purple Cap Vert* grown in an experimental garden of Joseph Ki-Zerbo University in Burkina Faso. The cuttings of these varieties were provided by the Environmental and Agricultural Research Institute (INERA).

### Extraction

Acidified methanol 1% (hydrochloric acid) was used to extract anthocyanins from both varieties of PFSP. The raw extracts obtained were evaporated almost dry and then filtered and taken back with a minimum of acidified water. The water filter was deposited on the Amberlite XAD-7 in a column of about 24 cm. The extract was washed with a large amount of acidified water to get rid of impurities and free sugars contained in the raw extract. Total anthocyanin extracts were elucidated from the column with a minimum of acidified methanol. These total extracts were evaporated to dry and dissolved in a minimum of pure acidified methanol. Few drops of pure ethyl acetate from the previous extract was used to precipitate the anthocyanins, that whendried in a Speed dryer makes it possible

to obtain an amorphous powder of purple color. For HPLC-ESI-MS/MS tests, a 1 mg/mL concentration solution was prepared using the 95:5 (v/v) water-acid mixture as a solvent.

**Evaluation of total anthocyanin content and radical-scavenging activities**

The evaluation of radical-scavenging activities was carried out on the principle described by Brand-Williams et al. (1995). It was carried out using DPPH, the method of trapping free radicals. Thus, solutions of different concentrations of extract and standards were prepared in the ethanol-water solvent system (70:30; v/v). Then, 225 µL of a solution of the DPPH radical at the concentration of 0.25 mg/mL were added to 25 µL of each of the previously prepared solutions. The kinetic reaction was tracked for 1 hour and the absorbances are read every 1 min at 510 nm using a spectrophotometer (Serge et al., 2021) The percentage of remaining free radicals [$DPPH^{\circ}$] was determined using the following equation:

$$\mathrm{DPPH_{rem}} = \frac{A_e}{A_0} * 100 \quad (1)$$

$A_0$: Absorbance of the DPPH radical solution
$A_e$: Absorbance of DPPH after reaction with extract.

Evolution curves in the rate of trapping DPPH free radicals over time were drawn. The trapping rate of DPPH free radicals corresponding to the reduction of 50% in the concentration of the radical DPPH was determined from the evolutionary curve of this rate in relation with the concentration of the extract according to the method described by Cristina et al. (2009). For the assessment of anthocyanin levels, the differential pH method using two buffer systems (potassium chloride solution, pH1.0 (0.025 M) and acetate solution, pH4.5 (0.4 M) was used. Indeed, 0.5 mL of the extract was mixed with 3.5 mL of the corresponding buffers and the absorption is read against a blank at 510 and 700 nm exactly 15 min later using a spectrophotometer.

Absorbance A is obtained from the following relationship:

$$\mathbf{A = (A_{510}\text{-}A_{700})_{pH1,0} - (A_{510}\text{-}A_{700})_{pH4,5}} \quad \mathbf{(2)}$$

And the grades are obtained by the following relationship:

$$\mathbf{TA\ (mg/\ L) = \frac{A * PW * DF * 1000}{\varepsilon * \ell}} \quad \mathbf{(3)}$$

MW: Molecular weight of Cyanidin 3-O-glucoside and **ε** its molar extinction coefficient and

MW = 449.2 g/mol and **ε** = 26900 ; DF : Dilution Factor

**Analysis by HPLC-DAD-MS-UV visible**

PFSP extracts were first analyzed using high-performance liquid chromatography coupled with mass spectroscopy equipped with a positive electrospray ionization source (ESI) and UV-visible spectroscopy. An agilent HPLC system with a "Column Oven (G7130A)" type was used. This was a 250 mm long C18 column. The elution profile consisted of isocratic (Byamukama et al., 2016). The elution was achieved with the mobile phase C (acetonitrile 5% formic acid /$H_2O$ 1:1 v: v) and the mobile phase D ($H_2O$ 5% formic acid v: v). The gradient of elution was 0 to 2 min, 20% C; 2 to 15 min, 40% C; 15 to 17 min, 50% C; 17 to 27 min, 50% C; 27 to 40 min, 95% C and 40 to 50 min, 95% C. The flow was maintained at 1 mL/min and the injection volume was 10 µL.

**HPLC-ESI-MS/MS Analysis**

HPLC coupled with tandem mass spectrometry by electrospray ionization in positive mode (HPLC-ESI-MS/MS) was used in this study for the identification of anthocyanins. Thus, a HPLC system of Agilent technology infinitely better 1290 was used for chromatographic separation. It had a C18 reverse-phase column with a length of 250 mm and a diameter of 4.6 mm, the size of the particles was 5 µm. The elution was achieved with the mobile phase A (water 5% formic acid v:v) and the mobile phase B (acetonitrile 5% formic acid, v:v). The gradient of elution according to the time expressed as a percentage of volume of the mobile phase A and the mobile phase B was computed as follows: 0 to 5 min, 5% B; 5 to 15 min, 10% B; 15 to 25 min, 10% B; 25 to 35 min, 12% B; 35 to 50 min, 15% B; 50 to 60 min, 18% B; 60 to 80 min, 25% B and 80 to 90 min, 30% B. The flow was maintained at 0.6 mL/min and the temperature of the column at 25°C.

Mass spectrometry scanning was performed in positive mode with a scanning interval of 200-1200 m/z. Nebulization was performed at 200°C with a simultaneous flow of $N_2$ to 15 psi. The hair strains were set to 3.5 kV. The data were analyzed using the LC/MS Data Acquisition software for 6400 series triple quadrupole version B.06.00 Bulld 6.0.6025.0.

## RESULTS

### Radical-Scavenging activities and Total anthocyanin content

The total anthocyanin extracts from *Tuskegee purple Cap Vert* and *Tuskegee purple* varieties were 0.211 and 0.122 mg of cyanidin 3-glucoside/g fresh material, respectively. The results of the kinetic reactions showed that for a concentration of 0.4 mg/mL of extract, the reaction was completed after 10 min for *Tuskegee purple Cap Vert* extract and 8 min for *Tuskegee purple* (Table1).

### HPLC-MS profiles of anthocyanin extract

The HPLC-MS analysis of our two excerpts showed the same chromatographic profile (Figure 3). Twelve peaks noted from 1 to 12 corresponding to 12 anthocyanin compounds were identified (Figure 4). Of these eleven peaks, only peaks 5, 8, 10 and 11 are major.

In ultraviolet-visible spectroscopy (UV), all the anthocyanin compounds in the extract exhibited the two absorption peaks characteristic of anthocyanins, particularly at about 280 nm and 530 nm. In addition, most of these compounds had a peak of absorption around 330 nm characteristic of the presence of an organic acid substitute. From the various UV-visible spectra recorded we noted that all the anthocyanin compounds of our extract were glycosylated in position 3 and 5. Indeed, the position and number of these acidic substitutes as well as the position of the different sugars can be deducted from the $A_{440}/A_{vis}$ and $A_{acyl}/A_{vis}$ ratios (Table 2). A ratio of $A_{440}/A_{vis}$ between 29% and 35% or 15% and 24% indicates respectively a monoside or a bioside. Three different cinnamic acids were found to acylate the anthocyanins of the studied extract. These are caffeic, *p*-hydroxybenzoic and ferulic acids.

### CLHP-SM/SM Profiles

High-performance liquid chromatography (CLHP) coupled with tandem mass spectrometry by electrospray ionization (ESI-MS/MS) on a triple-grid instrument were used to study the anthocyanins contained in our extract. The LC-ESI-MS in "select ion monitoring" SIM mode was used to obtain the chromatograms of all molecular ions already identified in the previous study (LC-MS-UV). After obtaining these chromatograms, the LC-ESI-MS/MS in "ion product" mode was used to obtain individual chromatograms for the purpose of molecular ion fragmentation.

Using the chromatographic conditions described above, and from the previous study, we obtained four isomer compounds corresponding to peaks 1, 2, 4 and 7 at the respective retention times RT = 12.26; 17,41 ; 48.97 and 57.09 min (Figure 4). The UV-visible electronic spectra corresponding to these compounds have absorption bands characteristic of anthocyanins (cyanidin and peonidin) which absorbances varied between 514 nm and 520 nm (Zhang et al., 2018).

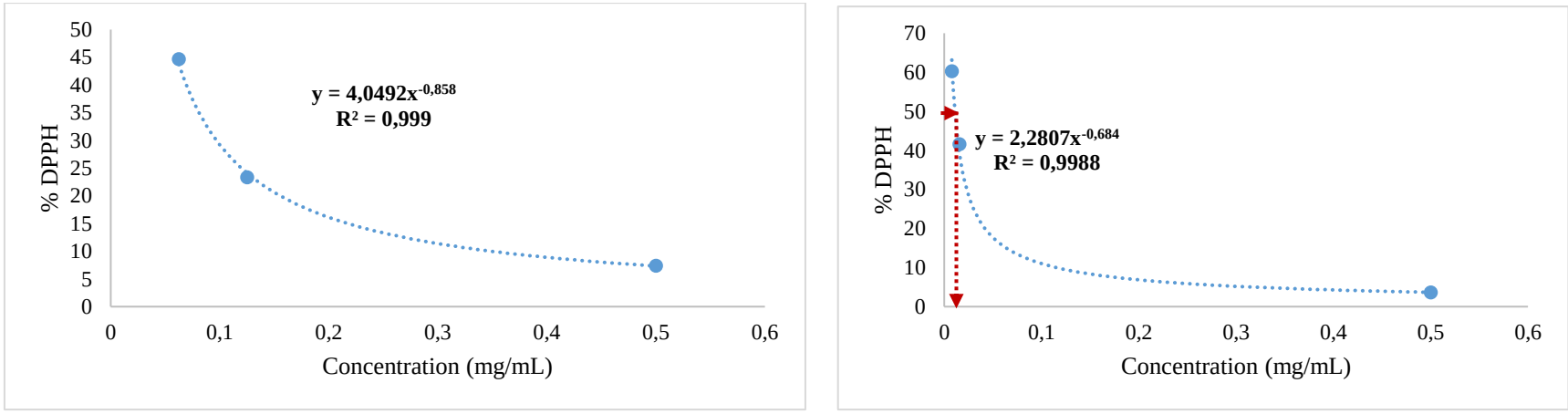
**Tableau 1**: total anthocyanins contents and I $C_{50}$.

| | ***Tuskegee purple Cap Vert*** | ***Tuskegee purple*** | **Gallic Acid** | **Ascorbic Acid** |
|---|---|---|---|---|
| $IC_{50}$ mg/mL | 0.05 | 0.06 | 0.004 | 0.01 |
| $IC_{50}$ mg/g | 200 | 240 | 16 | 40 |
| $1/IC_{50}$ | 10 | 4 | 100 | 80 |
| $TIC_{50}$ (min) | 10 | 8 | 1 | 1 |
| TAC (mg/g) | 0.211±0.004 | 0.122±0.001 | | |

**Tableau 2** : data obtained from HPLC-UV-MS chromatogram and UV-visible spectra of anthocyanic compounds.

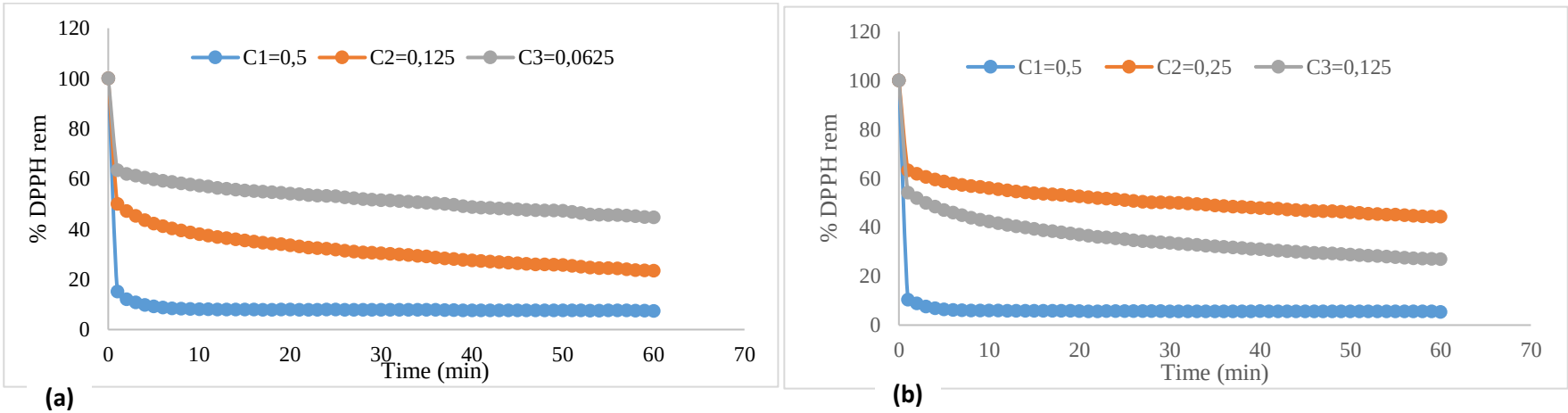
| Peak N° | Retention Time | % Area | $\lambda_{vis}$ | $\lambda_{acy}$ | $A_{440}/A_{maxvis}$ % | $A_{acy}/A_{vis}$ % | M+H | formulas | Identified Compound |
|---|---|---|---|---|---|---|---|---|---|
| **1** | 5.06 | 1.19 | 514 | | 18 | | 773 | $C_{33}H_{41}O_{21}$ | Cyanidin 3-sophoroside-5-glucoside |
| **2** | 6.5 | 2.09 | 514 | | 18 | | 611 | $C_{27}H_{31}O_{16}$ | Cyanidin 3,5-diglucoside |
| **3** | 7.92 | 3.59 | 514 | | 18 | | 787 | $C_{37}H_{38}O_{19}$ | Peonidin 3-sophoroside-5-glucoside |
| **4** | 12.32 | 3.67 | 520 | 330 | 17 | 30 | 907 | $C_{41}H_{47}O_{23}$ | Peonidin 3-O-(6”-O-*p*-hydroxybenzoyl sophoroside)-5-O-glucoside |
| **5** | 15.49 | 10.18 | 520 | 328 | 14 | 25 | 963 | $C_{44}H_{51}O_{24}$ | Peonidin 3-feruloyl sophoroside)-5-glucoside |
| **6** | 16.55 | 5.98 | 520 | 328 | 18 | 62 | 935 | $C_{33}H_{41}O_{21}$ | Cyanidin 3-sophoroside-5-glucoside |
| **7** | 17.17 | 3.9 | 518 | 328 | 18 | 51 | 1055 | $C_{49}H_{51}O_{26}$ | Cyanidin 3-caffeoyl-*p*-hydroxybenzoyl sophoroside)-5-glucoside |
| **8** | 17.73 | 1.90 | 514 | | 19 | | 773 | $C_{33}H_{41}O_{21}$ | Cyanidin 3-sophoroside-5-glucoside |
| **9** | 18.5 | 13.13 | 522 | 330 | 18 | 60 | 949 | $C_{43}H_{49}O_{24}$ | Cyanidin 3-sophoroside-5-glucoside |
| **10** | 19.15 | 3.78 | 526 | 330 | 15 | 49 | 1069 | $C_{50}H_{53}O_{26}$ | Peonidin 3-caffeoyl-*p*-hydroxybenzoyl sophoroside-5-glucoside |
| **11** | 19.65 | 14.72 | 520 | | 14 | | 787 | $C_{37}H_{38}O_{19}$ | Peonidin 3-sophoroside-5-glucoside. |
| **12** | 20.07 | 8.11 | 520 | 328 | 22 | 66 | 1125 | $C_{53}H_{57}O_{27}$ | Peonidin 3-caffeoyl-feruloyl sophoroside-5-glucoside |

$\lambda_{vis}$ : visible UV wavelength ; $\lambda_{acy}$ : acylated wavelength ; $A_{440}$ : absorbance at wavelength 440 ; $A_{maxvis}$ : maximum visible uv absorbance ; $A_{acy}$ : acylated absorbance ; % : percentage




**(a)** **(b)**

**Figure 1**: Determination of $IC_{50}$ from extract of *Tuskegee purple Cap Vert* variety (a) and Ascorbic Acid (b).




**(a)** **(b)**

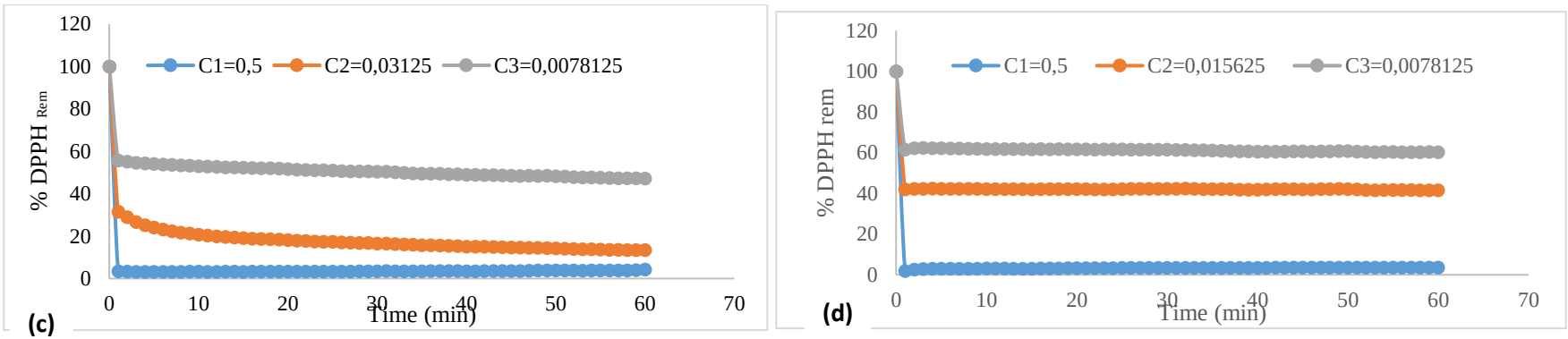



**Figure 2**: Inhibition of DPPH radical in relation with time of *Tuskegee purple Cap Vert* (a); *Tuskegee purple* (b); Gallic acid (c) et Ascorbic Acid (d).

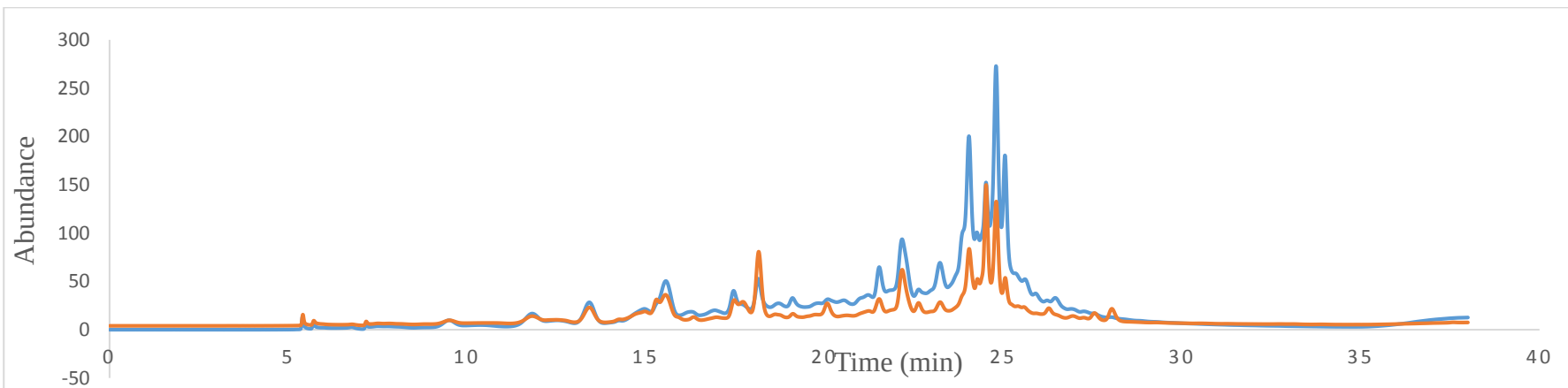



**Figure 3**: Cchromatographic profiles of *Tuskegee purple* (blue) et *Tuskegee purple Cap Vert* (red) varieties.

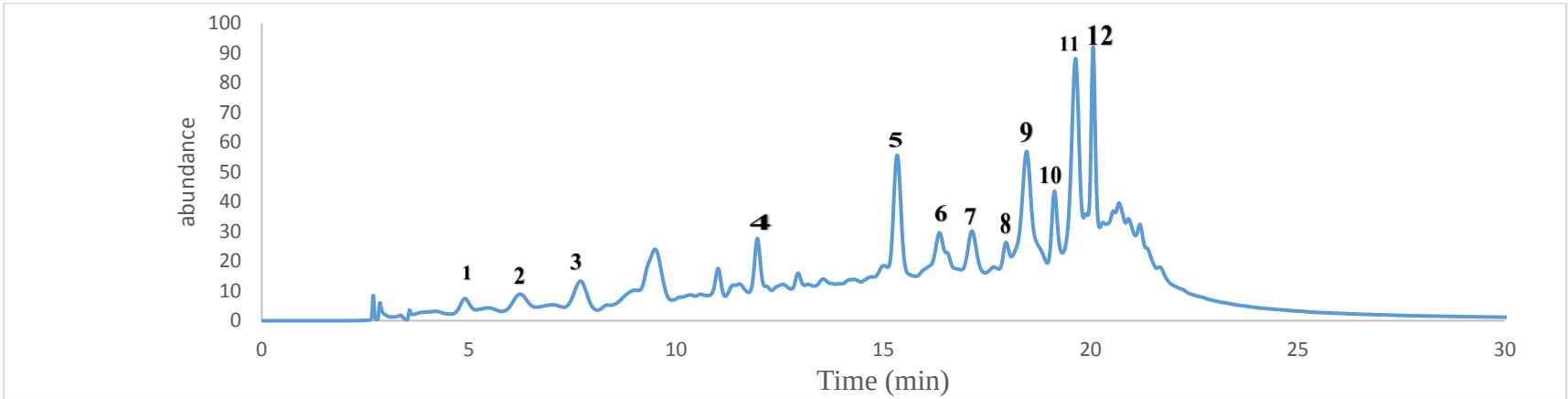



**Figure 4a**: HPLC/MS chromatogram of the crude extract *Tuskegee purple Cap Vert* variety.

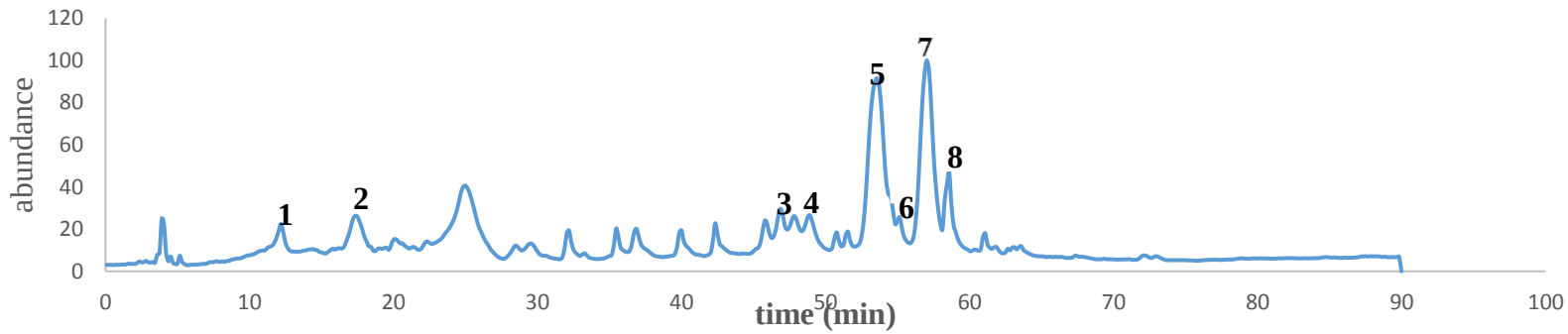



**Figure 5b**: HPLC/MS-MS chromatogram of the crude extract *Tuskegee purple Cap Vert* variety.

## DISCUSSION

### Radical-Scavenging activities and Total anthocyanin content

The total anthocyanin content of extracts from *Tuskegee purple Cap Vert* and *Tuskegee purple* varieties obtained in our study is comparable to that of the Okinawa varieties which ranged from 10 to 21.1 mg/100g and to other red flesh potato genotypes (2 to 40 mg/100 g fresh weight) (Rodriguez et al., 1998). However, our results showed low anthocyanin content compared to that reported on blackcurrant (322 - 476 mg/100 g fresh weight) and blueberry (0.57 mg/g fresh material) (Laurie and Truong, 2010). Compared to other plants such as grapes (27 to 120 mg/100g) and eggplants (86 mg/100 g fresh weight) the total anthocyanin content of these varieties remains high (Alasalvar et al., 2001).

Radical-Scavenging activities of extracts were assessed by the DPPH method and the percentage of DPPH radicals trapped was expressed based on the concentration of extract (Table 1). Figure 2 shows that the lower the concentration of the extract, the lower the trapping activity of DPPH radicals of sweet potato anthocyanins. The antioxidant activity of trapped radicals increases with increased concentrations. Indeed, DPPH is a stable free radical centered on nitrogen, which color switches from purple to yellow during a reduction by hydrogen or electron donation. Substances that are capable of performing this reaction can be considered antioxidants or compound possessing radical-scavenging activities. The radical-scavenging activity of an antioxidant is therefore defined as the amount of antioxidant needed to reduce the initial concentration of DPPH ($IC_{50}$) by 50% (Dieng et al., 2017 ; Kokou et al., 2014). In addition, antiradical power is defined as the inverse of antiradical activity. The higher the antiradical power, the more efficient the antioxidant. The results show that the antiradical power of the extract of *Tuskegee purple Cap Vert* variety is almost double of that of *Tuskegee purple* variety, but remains low compared to standards such as gallic acid and ascorbic acid (Table 1). The good free-radical scavenging activity of the extract from *Tuskegee purple Cap Vert* variety may be due to its high total anthocyanins content. According to the work reported by Hua et al. (2008), there is a high correlation between the levels of flavonoids (anthocyanins) and free-radical activity. These results are consistent with those of Yuzhi et al. (2012). Indeed, Yuzhi et al. (2012) reported that the antiradical power of purple-fleshed sweet potato extract was low compared to that of ascorbic acid.

### Identification of compounds 1 and 4

Based on the visible UV spectra obtained in the previous study, compound **1** and **4** at the respective retention times RT = 12.26 and 48.97 min, give two characteristic bands of the anthocyanin compounds. One in the UV spectra around 278 and 280 nm respectively due to the presence of the aromatic cycle B and the other in the visible around 514 nm due to the presence of the eight conjugated double bonds of the aromatic cycles. $A_{440}/A_{max}$ ratios equal to 18% and 19% (Table 2) obtained from their electronic spectra show that these compounds are glucosylated in position 5 (Wu et al., 2006 ; Hua et al., 2008). In addition, the absence of a shoulder around 330 nm shows that these compounds are not acylated.

The LC-MS/MS chromatogram shows that both compounds **1** and **4** have respective retention times at RT=12.26 min and RT = 48.97 min (Figure 5). This result shows that these two molecules are of different polarities. Since the elution solvent is polar, the compound with the lowest retention time is the most polar. The MS/MS spectra of these compounds give the same molecular ion to m/z 773 but different fragmented ions (Figure 11 and 13). The presence of fragmented ions at m/z 611, 449 and 287 show the loss of three hexoses. These compounds are therefore triglycosylated. According to El-Sayed et al. (2006) the stereochemistry of sugar hydroxyls can affect polarity. As a result, the sophoroside

will be more polar than laminaribioside. According to his work, a less polar sugar increases the retention time. This explains why cyanidin 3-sophoroside will have a relatively low retention time compared to that of cyanidin 3-laminabioside. In addition, it has been reported that when the sugar fractions are different in position 3 and 5, the fraction of sugar of greater molecular weight tends to fix in position 3 (Qingguo et al., 2005). Sugars attached to aglycons in position 3 of compounds **1** and **4** could be sophoroside and laminaribioside, respectively. The molecular ion of compound **1** gives two fragmented ions. The ion fragment at m/z 448.9000 corresponding to the loss [M+H-sophoroside] as demonstrated by Qingguo et al. (2005) and the ion fragment at m/z 286.9000 corresponding to the aglycon of cyanidin. The molecular ion of compound 4, corresponding to the tR= 48.97 min retention time, also yields two fragmented ions. A fragment ion at m/z 610.9000 corresponding to the mass calculated from the formula $C_{27}H_{30}O_{16}$. The intensity of this peak shows that this fragment ion is stable. It could therefore correspond to the loss of glucose from the laminaribioside and the ion fragment m/z 286.9000 corresponding the mass of the aglycon of the cyanidin. Compound **1** could be tentatively identified as cyanidine 3-sophoroside-5-glucoside (Figure 19) and compound **4** could be cyanidin 3-laminaribioside-5-glucoside (Figure 20).

**Identification of compounds 2 and 7**

The visible UV spectra of these compounds in MeOH-HCl 0.01 N reveal 2 characteristic bands of anthocyanin compounds. One in the UV around 280 nm and the other in the Visible around 520 nm. The values of the $A_{440}/A_{max}$ equal screw ratios 18 and 14% (Table 2) obtained from these electronic spectra show that these compounds are glycosylated in position 5. As before, the absence of a peak at 330 nm shows that these compounds do not possess an acyl fraction.

The LC-MS/MS chromatogram shows that these two compounds **2** and **7** have retention times RT=17.41 min and RT= 57.09 min (Figure 8). This result shows that these two molecules are of different polarities. The MS/MS spectra show that these two compounds have the same molecular ion at m/z 787 (Figures 15 and 17). So they are isomer compounds. The molecular ions corresponding to these two compounds give the same fragmented ions but at different intensities. The fragmented ions corresponding to the precursor ion 625 correspond to the loss of a hexose. This fragment ion corresponds to the mass calculated from the crude formula $C_{28}H_{34}O_{16}$. And two other fragmented ions at m/z 462 and 301 respectively. The presence of these two fragmented ions shows that compounds **2** and **7** are derivatives of paeonidin. Comparing the retention times of compounds **1** and **2** on the one hand and those of compounds **4** and **7** on the other hand, reveals that, the one of compound **1** is lower than that of compound **2** and that of compound **4** is lower than that of compound **7**. This may be explained by the presence of the methyl group on compounds **2** and **7**. These results are in accordance with the work of El-Sayed et al. (2006) according to his works, methylation increases retention time. These results confirm the fact that compounds **2** and **7** are derivatives of peonidin. The fragment ion to m/z 462 corresponds to $[M-2xglycose]^+$ and while the fragment ion to m/z 301 corresponds to the aglycone of the peonidin. Compound **2** could be tentatively identified as peonidin 3-sophoroside-5-glucoside (Figure 21) while compound **7** could be peonidin 3-laminaribioside-5-glucoside (Figure 22).

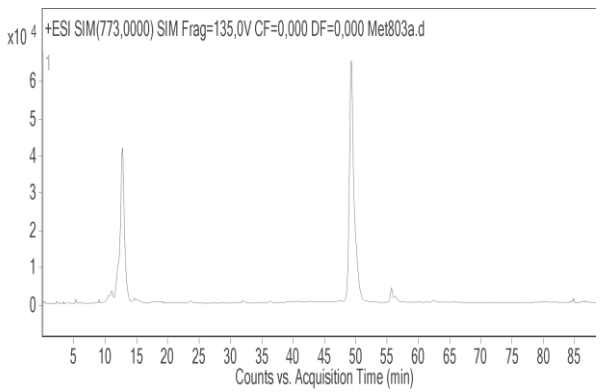



**Figure 6**: HPLC Profile of compounds **1** et **4.**

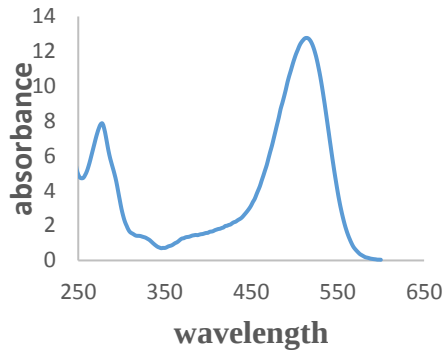



**Figure 7***:* UV-visible spectrum of compound **1.**

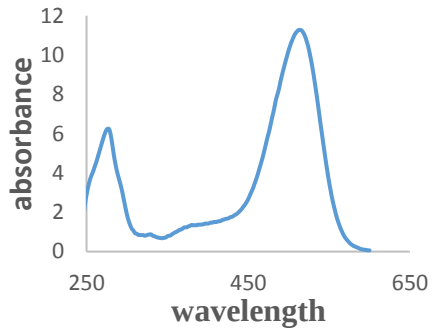



**Figure 8**: UV-visible spectrum of compound **4.**

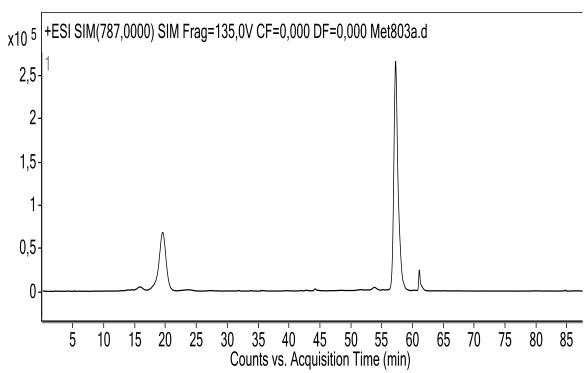



**Figure 8**: HPLC Profile of compounds **2** et **7.**

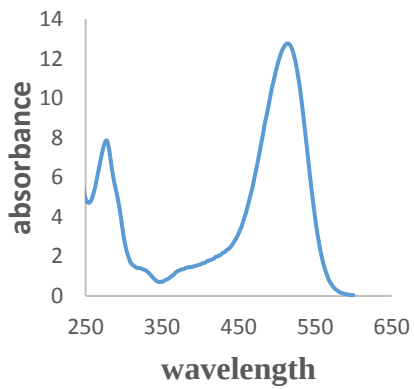



**Figure 9**: UV-visible spectrum of compound **2.**

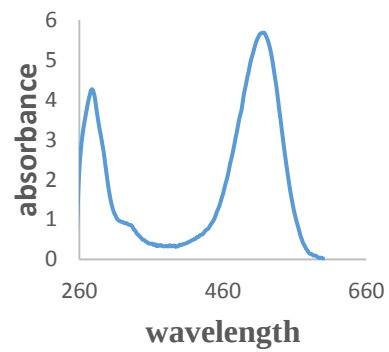



**Figure 10**: UV-visible spectrum of compound **7.**

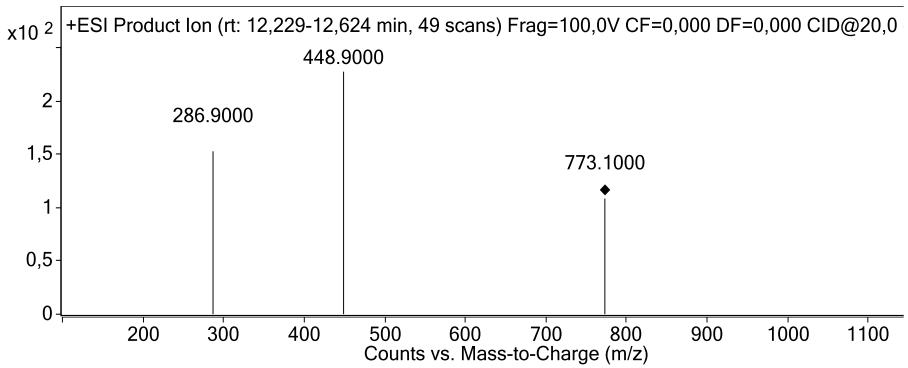



**Figure 11**: MS/MS spectrum of compound **1.**

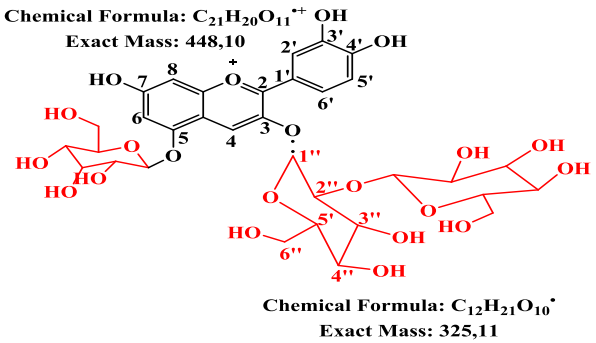



**Figure 12**: Fragmentation of compound **1.**

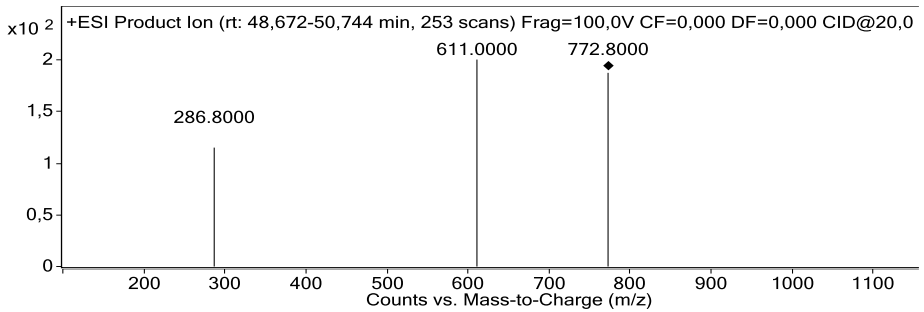



**Figure 13**: MS/MS spectrum of compound **4.**

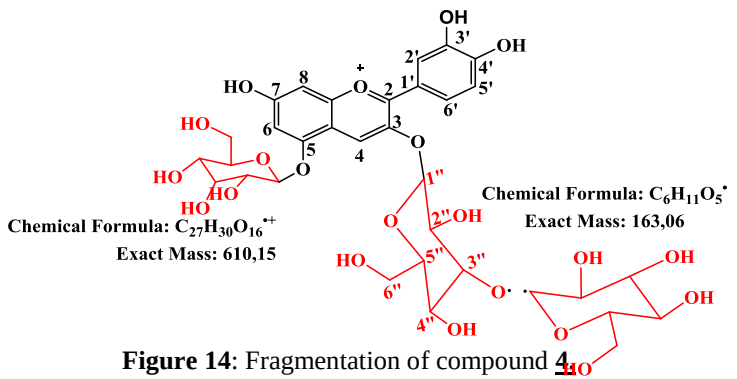



**Figure 14**: Fragmentation of compound **4.**

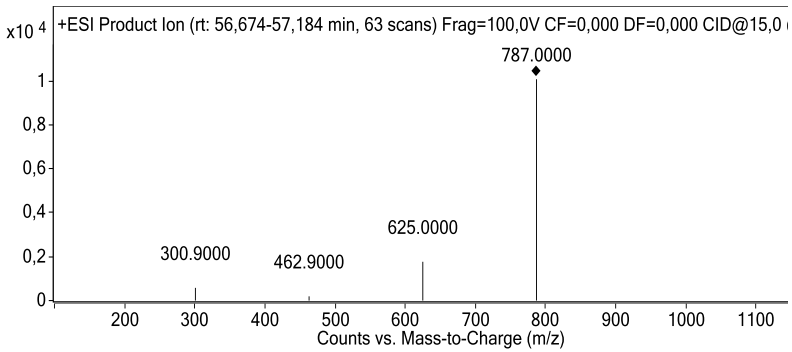



**Figure 15** : MS/MS spectrum of compound **2.**

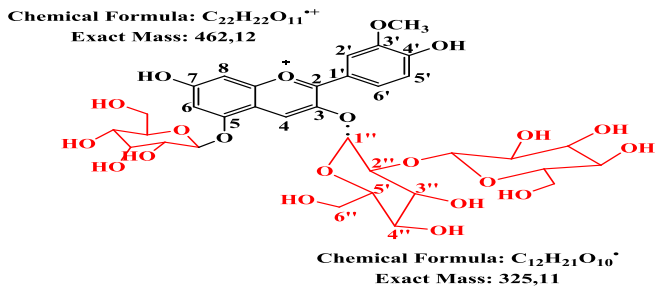



**Figure 16**: Fragmentation of compound **2.**

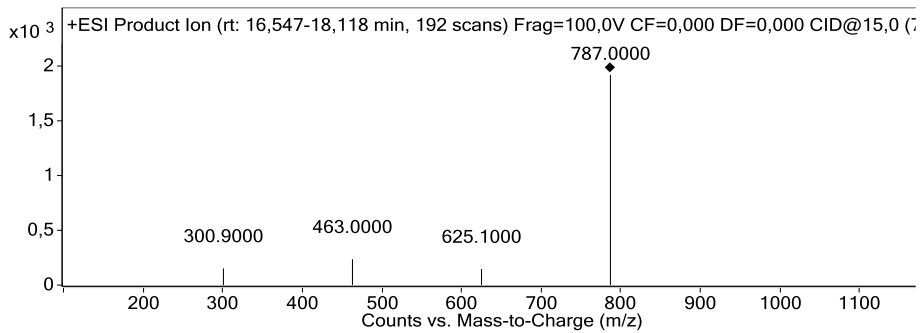



**Figure 17** : MS/MS spectrum of compound **7.**

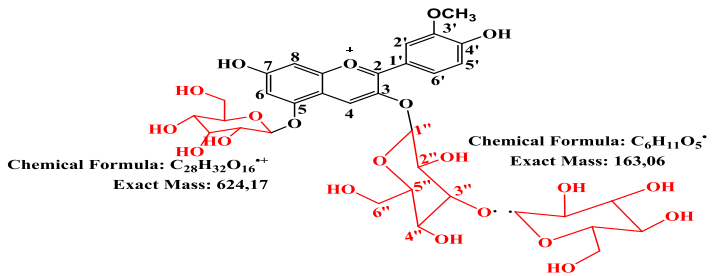



**Figure 18** : Fragmentation of compound **7.**

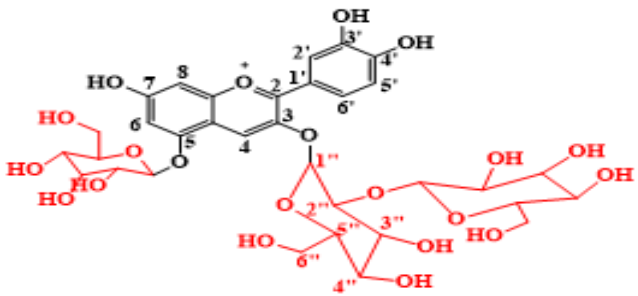


**Figure 19** : Proposed structure of compound **1.**

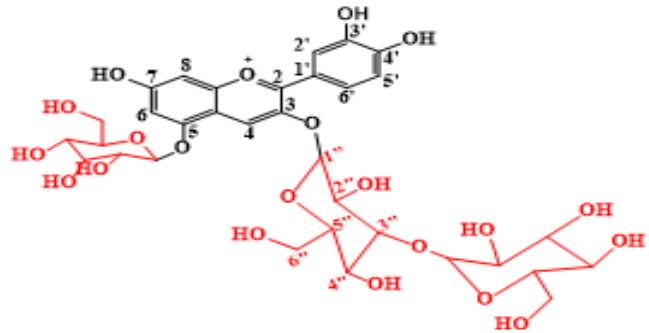


***Figure 20***: Proposed structure of compound **4.**

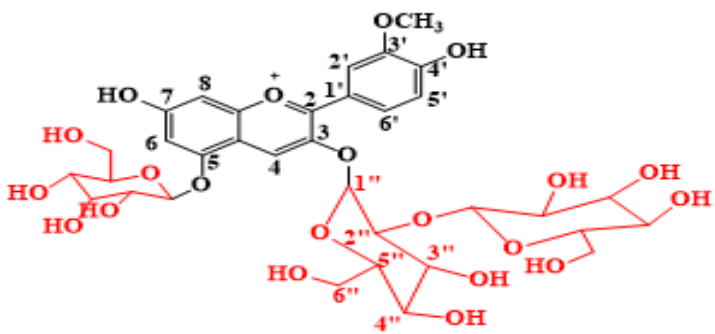


**Figure 21** : Proposed structure of compound **2.**

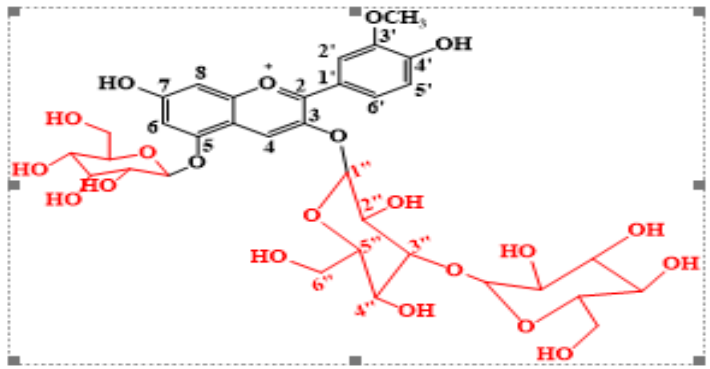


**Figure 22**: Proposed structure of compound **7.**

## Conclusion

The characterization and quantitative analysis of anthocyanins in *Tuskegee purple Cap Vert* et *Tuskegee purple* varieties of sweet potato grown in a garden at Joseph Ki-Zerbo University were carried out. Total anthocyanin contents are estimated at 0.211 and 0.122 mg of cyanidine 3-glucoside/g extract for *Tuskegee purple Cap Vert* and *Tuskegee purple* varieties, respectively. While their $IC_{50}$ are estimated at 0.05 and 0.06 mg/ mL respectively. Only the *Tuskegee purple Cap Vert* variety was analyzed in HPLC-ESI-MS/MS. Twelve anthocyanins (five are non-acylated anthocyanins and 7 are acylated) were identified ; four of which were identified in HPLC-ESI-MS/MS. To our knowledge, two of them could, for the first time be reported in sweet potatoes. This is cyanidin 3-laminaribioside-5-glucoside and peonidin 3-laminaribioside-5-glucoside. *Tuskegee purple Cap Vert* and *Tuskegee purple* varieties of sweet potato are rich in anthocyanins and can be used as an antioxidant.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

## AUTHORS' CONTRIBUTIONS

DSK is the principal author who did the bench work concerning this study. AH coordinated the work and is the corresponding author. EK and RB performed the HPLC-MS/MS analysis. KS provided the cuttings of the varieties / plant breeder. EP and MN are the laboratory managers. ITS is a collaborator in the project. PD performed the HPLC-UV-MS analysis.

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