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FULL PAPER

Major flavonoids identification by HPLC-MS/MS and evaluation of antiradical potential of mung bean (*Vigna radiata*) seed extracts in Burkina Faso

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Mung bean is one of the most essential legumes in Burkina Faso due to its short maturation cycle (45 to 55 days) and its nutritional content. This work aims to contribute to a better knowledge of the bioactive compounds of the seeds of Beng Tigré, a mung bean cultivar originating from India and acclimatized in Burkina Faso. To do this, the contents of total phenolic compounds, flavonoids, and carotenoids were determined by colorimetric methods. The mineral elements were assayed by atomic absorption spectrometry after the seeds mineralization. The antiradical potential was evaluated by monitoring the disappearance of the stable radical DPPH• by UV-Visible absorption spectrophotometry at 517 nm and compared to standards. Major flavonoids were identified by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). Total phenolic compounds, flavonoids, and carotenoids were 4. 356±0.162 mg GAE/g, 3. 151±0.262 mg QE/g, and 0.302±0.012 mg BCE/g, respectively. Potassium, magnesium, and calcium were, respectively, the most abundant mineral elements in the seeds: K > Mg > Ca > Fe > Zn > Na > Mn > Cu. An antiradical activity of the Beng Tigré extract was observed with an IC_{50} of 209. 97±13. 55 µg of AO/g DPPH•. This activity is less effective than Gallic acid, ascorbic acid, and trolox. Finally, the HPLC-MS/MS analysis identified four compounds: naringenin 8-C-glucoside, luteolin 8-C-glucoside, apigenin 6-C-glucoside, and luteolin 7-O -rutinoside. Due to their high biological potential, these so-called bioactive compounds are the subject of growing research interest.

KEYWORDS

Antiradical potential; bioactive compounds; HPLC-MS/MS; mung bean.

Introduction

The Food and Agriculture Organization of the United Nations (FAO) has declared 2016 the International Year of Pulses [1]. In effect, legumes are particularly appreciated for their nutrient-rich products, especially the seed and, very often, the vegetable part (leaves and pods) [2]. They are an important source of proteins, various amino acids, vitamins, and essential

mineral elements. These plants also provide so-called bioactive compounds, including, among others, terpenoids, nitrogen compounds, phenolic compounds, etc.

In Burkina Faso, cowpea is the most widespread and consumed legume, alongside which we can mention voandzou, soybeans. However, with climate change, more than the pulses grown in our tropics may be needed to be increased to meet the ever-increasing demand of populations. Thus, the introduction into the Burkina Faso agriculture of mung bean, a legume, could contribute to people's resilience to the effects of climate change by increasing the supply of legumes, achieving food sovereignty, and helping to eradicate malnutrition.

Mung bean is a short-cycle (45-55 days) annual seed legume in the family Fabaceae and is widely grown in tropical and subtropical regions [3]. It is mainly grown in rotation with cereals. Mung bean also represents an opportunity to produce protein-rich foods for rural populations under changing climatic conditions [4-6]. Previous work on mung bean cultivars has shown that the seeds are excellent sources of protein, essential amino acids, minerals, vitamins, and phenolic compounds such as phenolic acids and flavonoids. These chemical substances, with very different physicochemical properties, exhibit various biological activities such as antitumor, antiviral, antifungal, antimicrobial, antioxidant, anti-parasitic, etc. [7]. These compounds are beneficial in preventing and curing many chronic ailments, namely cancer, diabetes, and cardiovascular diseases, due to their antiradical properties [8-10].

Recently, the interest in natural antioxidants has increased considerably in their therapeutic properties [11,12]. Natural antioxidants, the main representatives of which are carotenoids, ascorbic acid, tocopherols, and flavonoids, can protect the human body from free radicals and delay the progression of many chronic diseases as well as oxidative rancidity of lipids in food [13,14]. Thus, scientific research has been developed in various specialties to extract, identify, and quantify these compounds from several natural medicinal plants and food products.

The present work aims to identify the main flavonoids and evaluate the antiradical potential by assessing the contents of bioactive compounds in the extracts of mung bean seeds (Beng Tigré) popularized in Burkina Faso.

This study provides new scientific information on the mung bean (Beng Tigré) acclimatized and popularized in Burkina Faso. It fills the gap in the antiradical potential and phytochemical profiling of this little-studied variety in the sub-region.

1. Materials and methods

Plant Materials

The mung bean (*Vigna radiata*) seeds were the subject of our study (Figure 1). They were provided by the plant laboratoire de Génétique et Biotechnologies Végétales de l'Institut de l'Environnement et de Recherche Agricole (INERA) in Kamboinsé (Ouagadougou; Burkina Faso).

FIFURE 1 Mung bean seeds (Beng Tigré)

Chemical products

The chemicals used were: Folin-ciocalteu's reagent (RFC); quercetin; gallic acid; L-ascorbic acid; 2,2-diphenyl-1-picrylhydrazyl (DPPH); ferric 2, 4, 6-tripyridyl-s-triazine (TPTZ); and

6-Hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid (Trolox, a hydrophilic derivative of tocopherol). These products were purchased from Sigma-Aldrich.
Ultrapure water (18 MΩ) was prepared with a water purifier system (ELGA PureLab).
All other used chemical reagents were of analytical grade.

Methods

Extraction

The total phenolic compounds of the mung bean were extracted using different types of solvents: the acetone/water/acetic acid system (70:29.5:0.5; v/v/v), ethanol, methanol, and acetone [13,15].

The total carotenoid extracts were obtained from n-hexane, acetone, and an acetone/n-hexane mixture (50:50; v/v) [16]. Now, 1 gram of seed powder was extracted by maceration for 24 hours at 4 °C with 3 mL of each solvent. The extracts were filtered, and the residues were re-extracted twice with 2 mL of solvent for 24 hours. The filtrates are combined and stored at 4 °C to determine the contents of total phenolic compounds, flavonoids, and carotenoids [17].

In addition, the ground seed powder was exhausted by maceration with the acetone/water/acetic acid system (70:29.5:0.5; v/v/v). The filtrate obtained from the extract was concentrated almost to dryness under reduced pressure (T = 40 °C), and then dried in a desiccator under vacuum to have a crude extract which was used for the study of the antiradical activity and identification of flavonoids.

Assay of total phenolic compounds (TPC)

The phenolic compounds were determined by the Folin-Ciocalteu method [18]. This method, initially described by Slinkard and Singleton [19], allows for knowing the total phenolic content of a given sample. Briefly, 60 µL of the extract was added with 60 µL of the Folin-Ciocalteu reagent (diluted ten times). After 8 min, 120 µL of sodium carbonate (Na_2CO_3) was added at 7.5% (w/v). After 30 min of incubation (room temperature), the absorbance was read at 765 nm using a SAFAS-type spectrophotometer. The blank is prepared by replacing the Folin-Ciocalteu reagent with distilled water. A standard curve was established using gallic acid as a standard (Table 1) and the results are expressed in mg of equivalents of gallic acid (GAE) /g of dry seeds.

Assay of total flavonoids (TFT)

Total flavonoid contents were assessed using aluminum trichloride [19]. Briefly, 20 µL of quercetin with a concentration in the plage 0.001 and 0.5 mg/mL or extract was mixed with 10 µL of $NaNO_2$ (5%) and left to react for 5 min at room temperature, and then 10 µL of a solution of $AlCl_3$ (10%) was added. After 6 min of reaction at ambient temperature, 40 µL of NaOH (1 M) was added to the reaction medium. The mixture was diluted with 130 µL of ultrapure water and homogenized. The absorbance was read immediately at 510 nm using a SAFAS-type spectrophotometer.

The total flavonoid content of the extract expressed in mg of quercetin equivalent per gram of dry seeds (mg of QE/g) was obtained by relating the absorbances read on the standard curve established from quercetin (Table 1).

Assay of total carotenoids (TCT)

The total carotenoid contents of the extracts were evaluated according to the method described by McMurry *et al.* [16,20,21] but slightly modified. After suitable dilution, the absorbances of the extracts, kept at room temperature and protected from light, were read at 450 nm.

The total carotenoid contents were obtained by relating the absorbances of the extracts to a standard curve established using β-carotene as a standard (Table 1). The carotenoid contents

are expressed in Equivalents of β-carotene per gram of dry seeds (BCE/g).

Assay of mineral elements

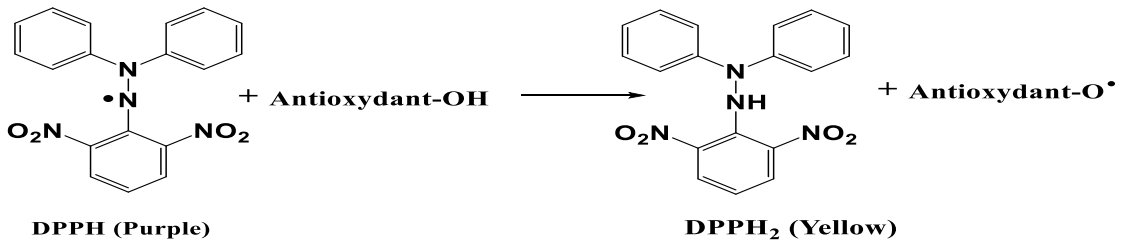
The contents of mineral elements were evaluated after the mineralization of the seeds of the Beng Tigré [22]. Indeed, the minerals were subjected to an air-acetylene flame atomic absorption spectrometer of the Analyst PinAAcle 900T type, of the Perkin Elmer brand. The wavelengths of the elements to be analyzed are initially defined on the device (324.75 nm for copper, 213.86 nm for zinc, 248.33 nm for iron, 285.2 nm for magnesium, 766.49 nm for potassium, 279.48 nm for manganese, and 589.0 nm for sodium). Next, the different readings of the calibration ranges made it possible to establish the calibration curves reflecting the absorbance as a function of the concentration. Finally, the samples to be analyzed are presented to the apparatus to determine their absorbances [23]. A blank is obligatorily passed between the passages of two different solutions. After the analysis of ten (10) samples, a control solution is passed to verify the effectiveness of the analysis results.

Anti-radical activity by the DPPH• radical test

The free radical scavenging activity of the seed extracts was measured using the method described by Burits and Bucar [24], with some modifications. The commercial radical of DPPH• was dissolved in methanol at a concentration of 0.2 mg/mL and kept at 4 °C protected from light. 50 µL of the crude extract solution dissolved in methanol at different concentrations ranging from 0.875 mg/mL to 6.8×10^{-3} mg/mL were mixed with 200 µL of a DPPH• solution. The absorbance was measured at the wavelength of 517 nm every 30 seconds for 30 min. The inhibition of the DPPH• free radical by each standard used ascorbic acid (vitamin C), gallic acid, and Trolox) was also analyzed at the same concentration to make the comparison. Antioxidants, with the property of donating hydrogen to the DPPH• radical lead to the discoloration of DPPH solution, which was taken on a color ranging from dark pink to pale yellow depending on the antioxidant content (Figure 2). The percentage of the DPPH• radical remaining after the radical reaction carried out by an antioxidant was calculated according to the formula [25]:

$$\%DPPH\bullet_{Rest} = \frac{Abs_{DPPH+antioxydant}}{Abs_{DPPH(T=0)}} X100$$

%DPPH•$_{Rest}$ is proportional to the concentrations of the antioxidant. The concentration that causes a decrease of the initial concentration of DPPH• by 50% was IC_{50}. This parameter is inversely proportional to the antioxidant capacity and is obtained graphically from the curve relating the percentage reduction %DPPH•$_{Rest}$ and the concentration of antioxidants. It can be further expressed in anti-radical effectiveness (EAR= $1/IC_{50}$).



FIFURE 2 Reaction of DPPH reduction with antioxidants

Identification of flavonoids by HPLC-MS/MS

Mung bean seed extract was subjected to HPLC coupled to tandem mass spectrometry equipped with a positive mode electrospray ionization source (ESI+) for the identification and characterization of flavonoids. Indeed, an HPLC system of the Agilent Technology infinitely better 1290 type was used for the chromatographic separation. It is equipped with a C18 reverse phase column of the zorbax sb type, 250 mm long, and 4.6 mm in diameter, and also the particle size is 5 µm. The elution was performed with a mobile phase A (water 5% formic acid v/v) and a mobile phase B (acetonitrile 5% formic acid v/v). The elution gradient versus time expressed as volume percent of mobile phase A and mobile phase B was programmed as follows: 0 to 5 min, 5% B; 5-15 min, 10% B; 15-25 min, 10% B; 25-35 min, 12% B; 35-50 min, 15% B; 50-60 min, 18% B; 60-80 min, 25% B and 80-90 min, 30% B. The flow rate was maintained at 0.6 mL/min, and the column temperature was at 25°C. The mass spectrometry scan was performed in positive mode with a 200-1200 m/z scan range. The nebulization was carried out at 200°C with a simultaneous flow of N_2 at 15 psi. The capillary voltages were set at 3.5 kV. Data were analyzed using HPLC/MS Data Acquisition for 6400 series triple quadrupole software version B.06.00 Bulld 6.0.6025.0.

Statistical analysis

All experiments were performed in triplicate, and data were represented as mean ± standard deviation. Analysis of variance (ANOVA) was performed using IBM SPSS Statistics version 25.0 statistical software. Differences were considered statistically significant if $P < 0.05$ and were calculated using the post hoc multiple comparison method, LSD.

Results and discussion

Contents of total phenolic compounds, total flavonoids, and total carotenoids

In this part, three solvents of increasing polarity, namely ethanol, acetone, and methanol, and a mixture of acetone/water/acetic acid (70:29.5:0.5; v/v/v) were used for the extraction of phenolic compounds from mung bean seeds. The results showed that the contents of total phenolic compounds varied according to the solvent used. The highest content (4.356±0.162 mg of GAE/g of dry seeds) was obtained from the acetone/water/acetic acid extract. Ethanol, methanol, and acetone showed contents of 3.863±0.164, 1.569±0.084, and 0.362±0.051 mg GAE/g of dry seeds, respectively. Comparison of these contents between the extraction solvents made it possible to establish the following order: acetone/water/acetic acid > ethanol > methanol > acetone. The content of total flavonoids measured in the acetone/water/acetic acid extract was estimated at 3.151±0.262 mg QE/g of dry seeds. Concerning the total carotenoids, two polar (acetone) and apolar (n-hexane) solvents, and also their acetone/n-hexane mixture (50:50, v/v) were used. The results showed that the highest content is measured in the acetone/n-hexane system extract with 0.302±0.012 mg of BCE/g of dry seeds, followed by the acetone extract (0.183±0.014 mg BCE/g) and n-hexane extract (0.054±0.002 mg BCE/g). The carotenoid extracts can be classified according to this order: acetone/n-hexane > acetone > n-hexane. The results show that the acetone/water/acetic acid system is the most appropriate solvent for extracting total phenolic compounds in mung bean seeds. This confirms the works of the literature, where this solvent system was unanimously used for the total phenolic compounds extraction [16]. Moreover, with this solvent system, the contents of total phenolic compounds (4.356±0.162 mg of GAE/g) and flavonoids (3.151±0.262 mg of QE/g) are comparable to those of Voandzou (≤ 4.536 mg GAE/g; 4.282±0.093 mg QE/g) and cowpea (5.584±0.037 mg GAE/g; 4.558±0.156 mg

QE/g) [26]. The total carotenoid assay results showed that the acetone/n-hexane system extracts carotenoids better. This could be explained by the fact that each solvent extracts a specific group of carotenoids. In fact, polar or slightly polar molecules, particularly xanthophylls, are soluble in acetone, and the group of apolar carotenes is more soluble in hexane. Thus, the acetone/n-hexane system extracts both xanthophylls and carotenes.

These results agree with several authors who revealed that mixed solvents efficiently extract bioactive compounds such as phenolic compounds and carotenoids [28-30]. Indeed, using mixed solvents results in a strong enrichment of the extracts in bioactive compounds. The advantage of composite solvents would be due to the increase in the solubility of bioactive compounds in the extracts obtained by these solvents compared to those obtained by pure solvents [29].

TABLE 1 Establishment of standard curves

Standard curves	Standards	Calibration equation	Correlation coefficient
CPT	Gallic acid	$y = 17{,}814x + 0{,}0485$	$R^2 = 0{,}9997$
TFT	Quercetin	$y = 0{,}767x + 0{,}0862$	$R^2 = 0{,}9963$
TCT	β-carotene	$y = 11{,}618x - 0{,}0075$	$R^2 = 0{,}9994$

Mineral elements content

The mineral element contents of the Beng tigré seeds are presented in Table 2. The results make it possible to distinguish the elements present in significant quantities, including Potassium (1140.2±14.851 mg/100 g), Magnesium (146.326±1.912 mg/ 100 g), Calcium (51.529±0.644 mg/100g), and those in minute quantities such as Iron (21.96±0.707 mg/100 g), Zinc (18.697±0.568 mg/100 g), Manganese (4.22±0.098 mg/100 g) and Copper (1.275±0.052 mg/100 g).

TABLE 2 Mineral element content of Beng Tigré seeds

Mineral elements	Contents (mg/100g)	PRI (mg/day)	SAM
Cu	1.275±0.052	1.1	0.6
Zn	18.697±0.568	7-9.5	4-5
Mn	4.22±0.098	1-10	-
Fe	21.96±0.707	1.5	-
Na	12.32±0.041	600-3500	-
K	1140.2±14.851	3100	1600
Ca	51.529±0.644	700	400
Mg	146.326±1.912	150-500	-

PRI: Population Reference Intake and SAM: Minimum Intake Threshold.

Sodium and potassium play the role of regulating the water content of human body and participate in maintaining the acid-base balance. The Na/K ratio in the seeds of the Beng tigré is equal to 0.01, less than 1. It is deduced that consuming this legume is beneficial in the fight against cardiovascular diseases [31,32]. Zinc and manganese are antioxidant trace elements that promote the elimination of excess free radicals in the body. Moreover, 100 g of Beng tabby learned appears sufficient to cover the daily requirements of iron, zinc, manganese, and copper. In any case, 200 g of Beng tigré covers the minimum intake threshold.

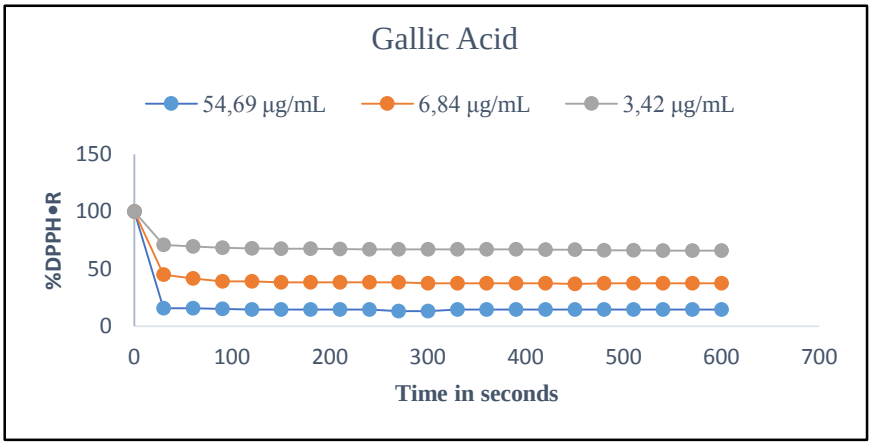
The mineral elements in our bodies play a crucial role in our daily lives. They compensate for daily losses (perspiration, urine, stools) and satisfy needs in certain physiological situations (bone growth, pregnancy, breastfeeding, and menstruation).

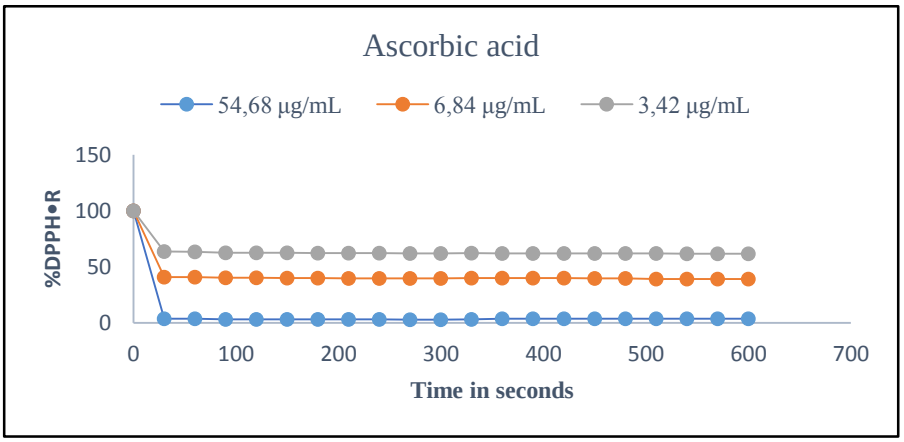
Study of antiradical activity

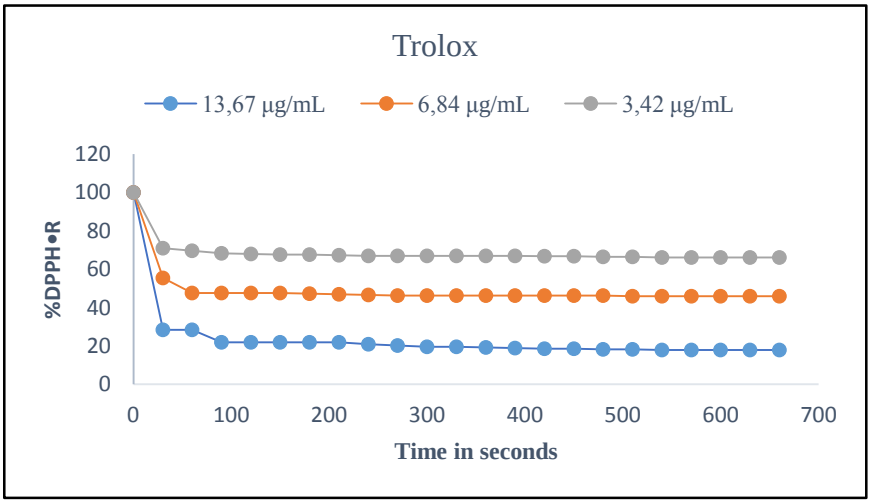
Antioxidant activity defines the ability of a compound to protect the body against the aggressiveness of free radicals. Its determination by the DPPH test is one of the methods for biologically and nutritionally evaluating food quality [33]. In addition, this test makes it possible to assess the kinetics of trapping free radicals. Indeed, in the presence of a hydrogen donor antioxidant, the free radical DPPH• is reduced to a stable molecule $DPPH_2$. This causes a decrease in the DPPH• concentration and a reduction in absorbance during the reaction time until the hydrogen-donating antioxidant capacity is exhausted. Thus, at different concentrations of the antioxidant tested, the kinetics of reduction of mDPPH• is monitored over time until an equilibrium is obtained, reflected by the presence of a plateau at the equilibrium time (Teq). The percentage of DPPH• remaining (%$DPPH•_{Rest}$) also decreases until it reaches a plateau. This kinetic varies according to the antioxidant used. The results are depicted in Figure 2. The reaction between DPPH and the reference hydrogen-donating antioxidants (gallic acid, ascorbic acid, and trolox) reaches equilibrium after a short time compared to the mung bean seed extract (Table 3). Indeed, the time required to reach equilibrium varies according to the concentrations. For gallic acid, equilibrium is reached in 30 seconds for a concentration of 54.69 µg/mL and 1 min 30 s for 6.84 µg/mL and 3.42 µg/mL. The equilibrium time of ascorbic acid is 30 seconds for the different concentrations tested (54.69 µg/mL, 6.84 µg/mL, and 3.42 µg/mL). Trolox has an equilibrium time of 1 minutes and 30 seconds in 13.67 µg/mL and 2 minutes in 6.84 µg/mL and 3.42 µg/mL. The equilibrium time observed for the mung bean seed extract is 1 minute and 30 seconds. The amount of antioxidants required to decrease the concentration of the free radical DPPH• by 50% (IC_{50}) was calculated from the equation of the curve of the percentage reduction of DPPH• as a function of the antioxidant concentration (Figure 3). The anti-radical efficacy EAR is deduced by the inverse of the IC_{50} ($EAR=1/IC_{50}$). The IC_{50} values of gallic acid, ascorbic acid, trolox, and mung bean seed extract are presented in Table 3. Statistical analysis of IC_{50} and EAR showed no significant difference between gallic acid, ascorbic acid, and Trolox. These antioxidants have similar efficacy but are more active than the mung bean seed extract, which restores the stability of DPPH with an IC_{50} of 209.97±13.55 µg/g. According to these results, it is proven that the reference antioxidants used (ascorbic acid, gallic acid, and trolox) are more effective compared to the extract of mung bean seeds. However, it should be noted that mung bean seed extract is a crude extract that contains bioactive compounds such as phenolics, flavonoids, carotenoids, mineral elements, etc.

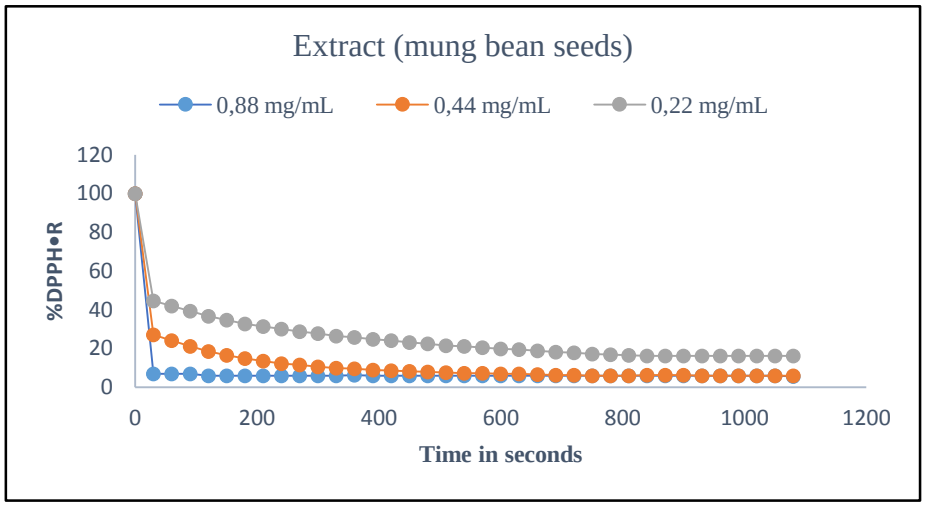
Identification of mung bean flavonoids by HPLC-MS/MS

The crude extract of the Beng Tigré subjected to HPLC-MS made it possible to observe several significant signals (Figure 4) corresponding to protonated molecules $[M+H]^+$. Four of these signals [**A**: t_R=37.64 min, **D**: t_R=47.32 min, **E**: t_R= 58.35 min, and **F**: t_R= 64.67 min] likely to correspond to flavonoids were subjected to fragmentation in MS/MS.







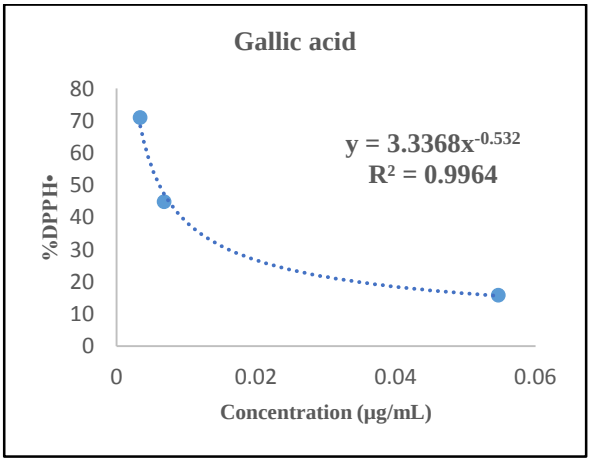


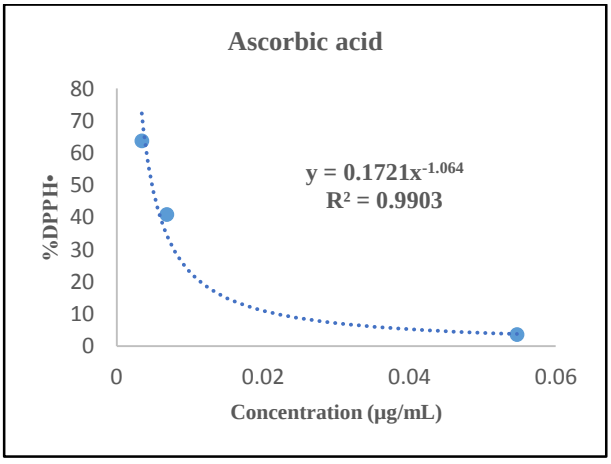
FIFURE 3 Kinetics of reduction of DPPH• by antioxidants

TABLE 3 Characteristic parameters of the DPPH• radical reduction kinetics

	Concentration (µg/mL)	Teq (min)	%DPPH$_R$ at equilibrium	Curve equation	Correlation coefficient	IC$_{50}$ (µg/g)	IC$_{50}$ (mean±standard deviation)	EAR (mean±standard deviation)
Gallic acid	54.69	0.5	15.84158416	$y = 3.3368x^{-0.532}$	$R^2 = 0.996$	6.17	6.17±0.43^a	$1.75.10^{-1}$±0.013^a
	6.84	1.5	39.24422442	$y = 3.4377x^{-0.518}$	$R^2 = 0.989$	5.69		
	3.42	1.5	68.31683168	$y = 3.2485x^{-0.522}$	$R^2 = 0.983$	5.32		
Ascorbic acid	54.69	0.5	3.630363036	$y = 0.1721x^{-1.064}$	$R^2 = 0.990$	4.84	4.80±0.04^a	$2.08.10^{-1}$±0.002^a
	6.84	0.5	40.92409241	$y = 0.1729x^{-1.062}$	$R^2 = 0.990$	4.79		
	3.42	0.5	63.69636964	$y = 0.1435x^{-1.095}$	$R^2 = 0.989$	4.77		
Trolox	13.67	1.5	21.78217822	$y = 5.7838x^{-0.445}$	$R^2 = 0.987$	7.8	6.79±0.93^a	$1.49.10^{-1}$±0.02^a
	6.84	2	48.18481848	$y = 3.4965x^{-0.531}$	$R^2 = 0.985$	6.6		
	3.42	2	67.98679868	$y = 2.0351x^{-0.625}$	$R^2 = 0.979$	5.98		
Extract (mung bean seeds)	880	1.5	6.270627063	$y = 6.6904x^{-1.342}$	$R^2 = 0.933$	223.4	209.97±13.55^b	31.10^{-5}±0.005^b
	440	5.5	9.900990099	$y = 6.2503x^{-1.333}$	$R^2 = 0.949$	210.2		

IC$_{50}$ and ERA results are expressed as mean ± standard deviation. Values are processed by IBM SPSS Statistics 25.0 software. The significant difference between the values is indicated by letters (P<0.05).





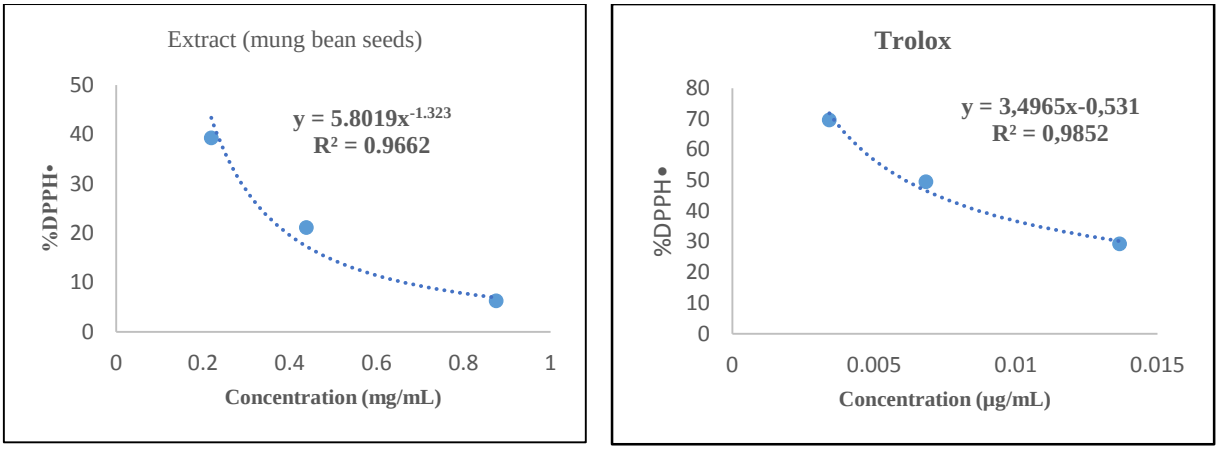


FIGURE 4 Percentage reduction of the DPPH• radical according to the concentration of the antioxidant

From these signals, the following compounds were detected: compound 1 at m/z 435 (**D** signal), compound 2 at m/z 449 (**A** signal), compound 3 at m/z 595 (**E** signal), and compound 4 at m/z 433 (**F** signal). These compounds corresponded to the raw formulas' calculated masses $C_{21}H_{23}O_{10}$, $C_{21}H_{21}O_{11}$, $C_{27}H_{31}O_{15}$, and $C_{21}H_{21}O_{10}$, respectively. These formulas can also check to those of flavonoids of the monoglycosylated (mono-*O*-glycosylated or mono-*C*-glycosylated) or diglycosylated flavone type. A previous study found that the most abundant flavonoids in mung bean possess the aglycone of apigenin or luteolin [34]. Furthermore, it should be remembered that the neutral loss of 132, 162, and 146 atomic mass units (u) make it possible to identify pentosides (xylose or arabinose), hexosides (glucose or galactose), and deoxyhexoside (rhamnose), respectively, in the case of *O*-glycosylated flavonoids [35]. Regarding *C*-glycosylated flavonoids, in negative ESI, the observation of a neutral loss of 120 u and 90 u corresponds to a hexose pattern, a loss of 104 u and 74 u corresponds to a deoxyhexose pattern, and a loss of 90 u and 60 u to that of a pentose motif. In positive ESI, the loss of 120 u shows the presence of a hexose [36]. Thus, analysis of the MS/MS spectrum of compound 1 m/z 435 (Figure 5) shows the presence of fragment ions characteristic of *C*-glycosylated flavonoids. Indeed, the presence of the ion at m/z 315 ($[^{0,2}X]^+$) commonly encountered among the fragment ions of *C*-glycosylated flavonoids and dependent on the presence of a hexose (glucose or galactose) is observed. This fragment ion, by loss of 42 u, makes it possible to deduce the nature of the aglycone of the flavonoid which is here naringenin ($[^{0,2}X-42]^+$ [25]. According to the literature, the *C*-glycosylation of flavonoids is carried out in position 6 or 8 of the aglycone [37]. Therefore, the presence or absence of certain fragment ions in the MS/MS spectrum makes it possible to distinguish the 8-*C* isomers from the 6-*C* glycosides [38]. In the case of 6-*C* glycosides, the ion at m/z [M+H-120 ; $^{0,2}X]^+$ fragments in turn to give, for example, the m/z ions $[^{0,2}X\text{-}CHO]^+$ and $[^{0,2}X\text{-}H_2O]^+$. These ions could not be observed in the fragmentation of compound 1. In addition, there is often a fragmentation of the *C* cycle in the 6-*C*-glycosides, thus giving fragment ions at m/z $[^{1,3}A]^+$ and $[^{0,2}B]^+$. These ions are also not observed. Therefore, compound 1 would likely be naringenin 8-*C*-glycoside. Since glucose is more common than galactose, compound 1 would be naringenin 8-*C*-glucoside. Compound 2 detected at m/z 449 corresponds to the molecule eluted after 37.64 min. The corresponding CID (Collision Induced Dissociation) spectrum is presented in Figure 6. On CID, the molecule undergoes mainly neutral losses of 120 u and 150 u leading to fragment ions at m/z 329 [M+H-

$C_4H_8O_4]^+$ and 299 $[M+H-C_4H_8O_4-CH_2O]^+$, respectively. As mentioned above, these losses are compatible with the characteristic fragment ions of a *C*-glycoside indicating the presence of a hexose (glucose) linked to a carbon of the genin. The 6-*C* and 8-*C* positions of the sugar attached to the genin in the *C*-glycosylated flavonoids can be determined by observing the intensity of the ion corresponding to the first loss of water [38]. In ESI+, if the ion $[M+H-H_2O]^+$ is the most abundant fragment peak of the MS/MS spectrum, the sugar is linked to carbon 8 of the genin. Otherwise, it is in position 6-C [25]. Also, the peak of 329 m/z is more abundant than that at 299 m/z in the case of an 8-C. The opposite is observed in the case of a 6-C [36]. These observations thus make it possible to deduce that compound 2 is probably luteolin 8-*C*-glucoside. Compound 3 (m/z 595) eluted after 58.35 min undergoes two consecutive losses of 146 u and 162 u, revealing the presence of a hexose residue (glucose) and a deoxyhexose residue (rhamnose) attached to the aglycone group. On the CID spectrum (Figure 7), the presence of the peak at 449 m/z ($[M+H-146]^+$) implies that one sugar unit is bound to another (osidic bond) and not directly to the aglycone [39]. This indicates an *O*-diglycosylated structure. A peak at 287 m/z ($[M+H-146-162]^+$) corresponding to the loss of a rutinoside is also observed. This peak at 287 m/z would correspond to the aglycone of luteolin or Kaempherol. A study showed that luteolin is more common in mung bean seeds [34]. Literature data also show that flavonoids could be glycosylated in any position, but *O*-glycosylation mainly occurs in position 7, as in flavones, isoflavones, flavanones, and flavonols [40,41]. Given these analyses, Compound 3 squeezed luteolin 7-*O*-rutinoside. Compound 4 eluted after 64.67 min corresponds to a compound detected at m/z 433. The MS/MS spectrum analysis does not show any characteristic fragment of the sugar loss (Figure 8). The sugar would therefore be linked to the aglycone by a carbon-carbon bond (*C*-glycosylation). Fragment ion analysis shows ions at m/z 283 $[^{0,2}X-CH_2O]^+$ and 313 $[^{0,2}X]^+$, which are characteristic of *C*-glycosylated flavonoids [42]. Peaks at m/z 270 $[^{0,1}X]^+$, 283 $[^{0,2}X-CH_2O]^+$ would be specific to 6-*C*-glycoside [37]. These fragment ions are observed on the MS/MS spectrum of compound 4. In addition, the fragment ions 415, 397, and 379 m/z corresponding to respective losses of one, two, and three molecules of H_2O are characteristic fragmentation pathways vitexin (Apigenin 8-*C*-glycosylated) or isovitexin (Apigenin 6-*C*-glycosylated) [43]. Considering all these results and the data from the literature, the probable structure of compound 4 is 6-*C*-glycosylated apigenin. Today, many techniques are employed for the structural analysis of bioactive compounds. These include UV-Visible spectroscopy, nuclear magnetic resonance spectroscopy, infrared spectroscopy, Raman spectroscopy, mass spectrometry, and X-ray crystallography. However, there is yet to be a universal analytical technique for analyzing chemical substances. Each method has its advantages and disadvantages. The application of one of these techniques will depend on several parameters, including the nature of the sample to be analyzed and the study's objective of the problem being addressed. These techniques are complementary and can be combined in different ways. This is the case of the HPLC-MS/MS combination used in this study. This technique is advantageous because it identifies non-volatile raw extract molecules without isolation steps. Moreover, although expensive, it is a reference technique for its flexibility, sensitivity, specificity, and speed.

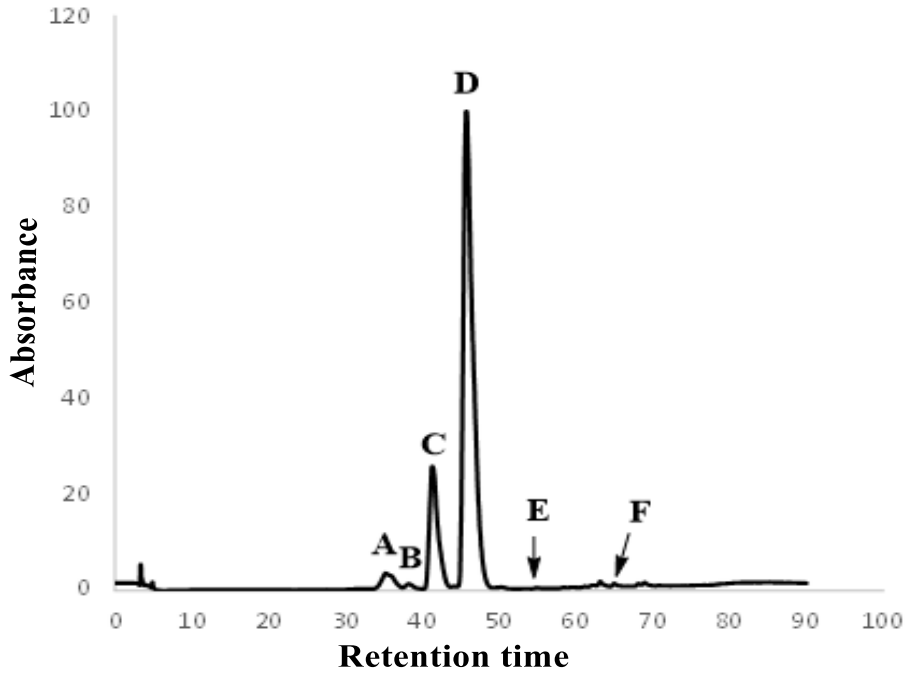
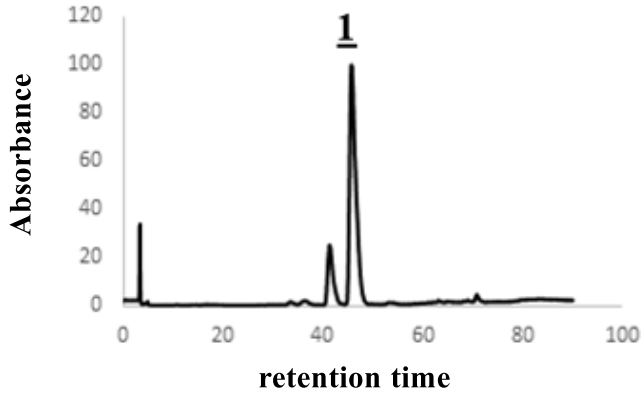


FIGURE 5 HPLC chromatogram of crude mung bean extract



Chromatogram of **D** signal

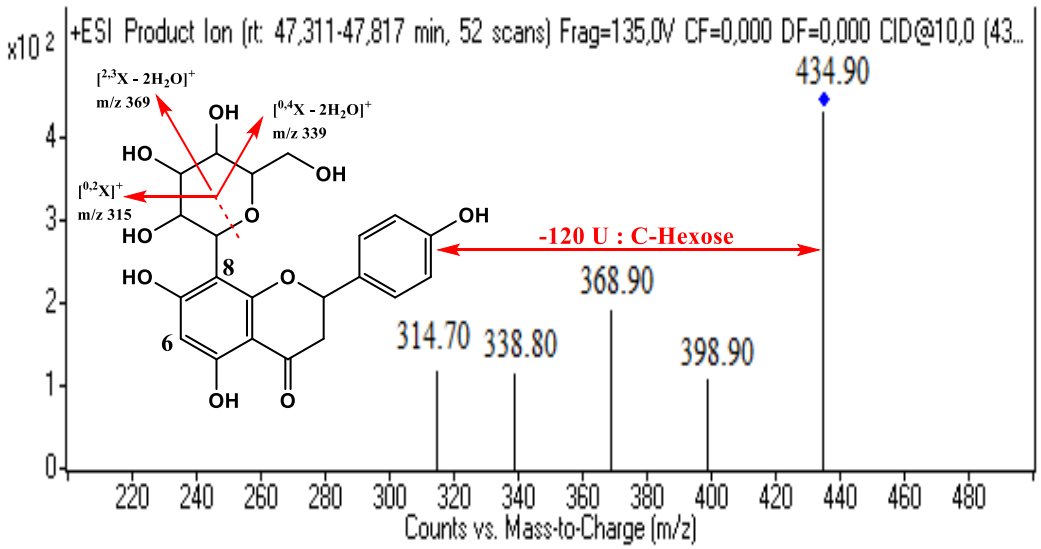
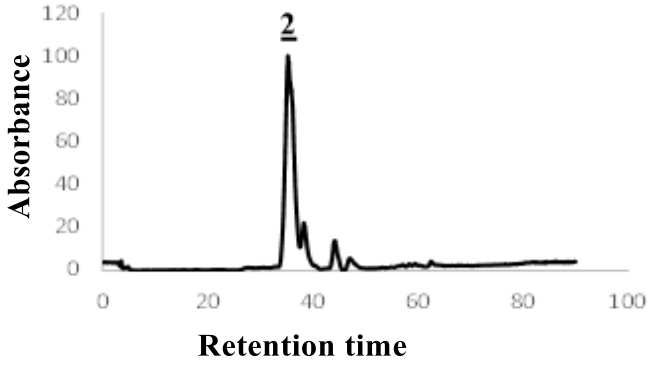


FIGURE 6 ESI+-MS/MS spectrum of compound **1** (m/z 435)



Chromatogram of **A** signal

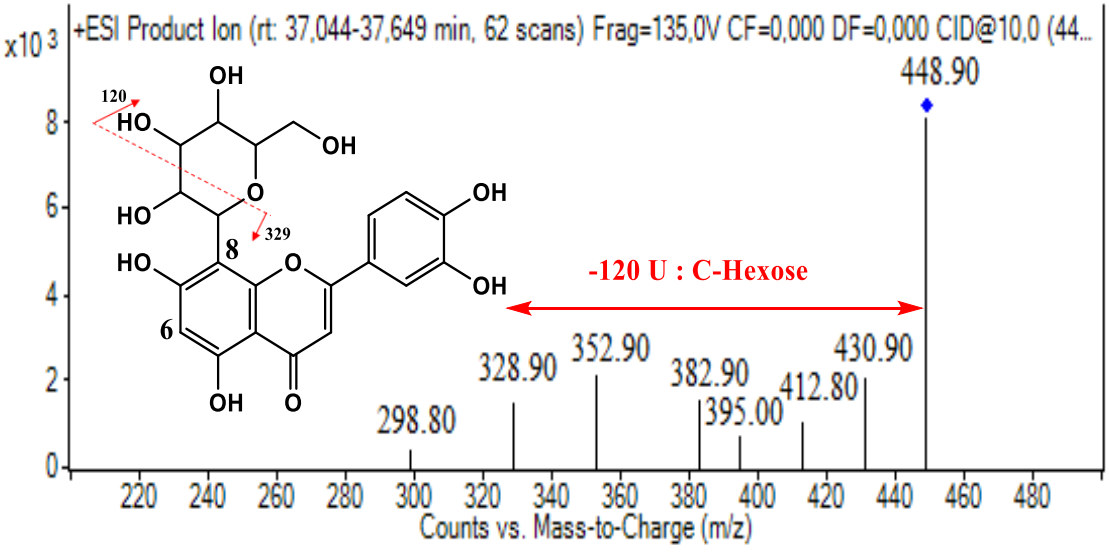
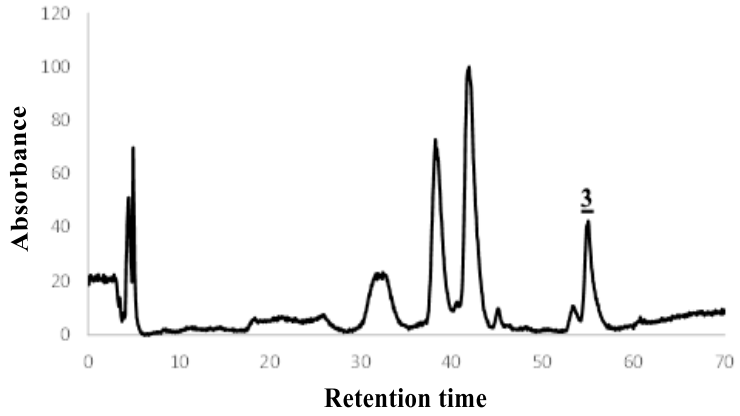


FIGURE 7 ESI+-MS/MS spectrum of compound **2** (m/z 449)



Chromatogram of **E** signal

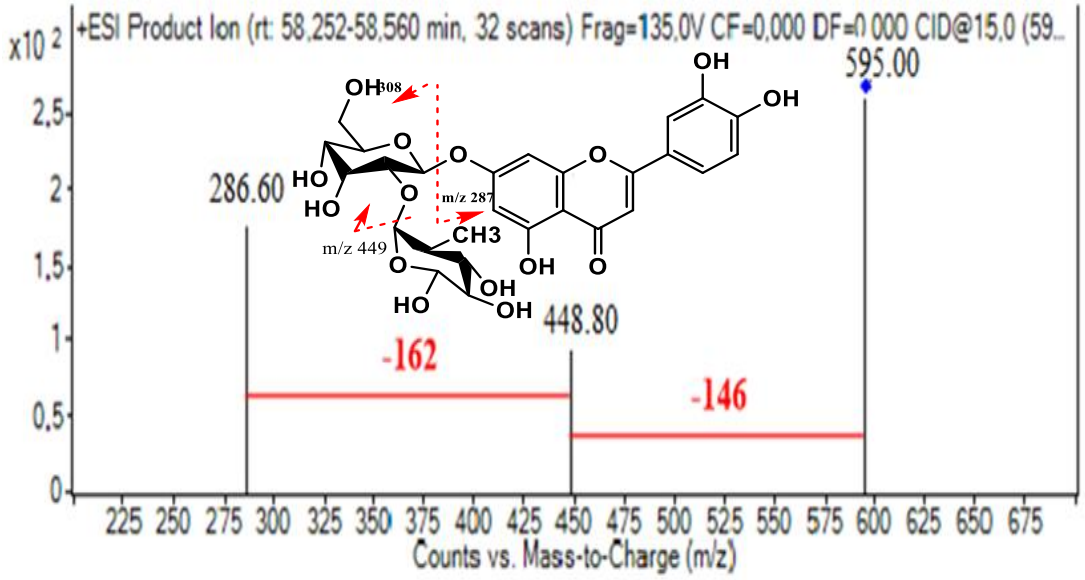


FIGURE 8 ESI+-MS/MS spectrum of compound **3** (m/z 595)

Conclusion

In this work, we analyzed the bioactive compounds and their antiradical activity, and also identified some flavonoids of the seeds of Beng Tigré to enhance this legume and promote its consumption. This study showed that Beng Tigré is a potential source of phenolic compounds, flavonoids, carotenoids, and mineral salts. According to the results, the crude extract using acetone/water/acetic acid solvent system of the seeds showed significant antiradical activity (IC_{50} = 209.97±13.55 µg/g). This indicates that the culture of Beng Tigré is vital in nutrition, and its regular consumption naturally contributes to preventing or curing specific chronic ailments such as cardiovascular diseases, cancers, diabetes, etc. The major molecules of flavonoids identified, namely naringenin 8-C-glucoside, luteolin 8-C-glucoside, apigenin 6-C-glucoside, and luteolin 7-O-rutinoside also show that Beng Tigré could be a source natural supply of colorants or food additives, pharmaceutical and cosmetic active ingredients, to the pharmaceutical, cosmetic and food industries.

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Conflict of Interest

There are no competing interests to declare that are relevant to the content of this article.

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