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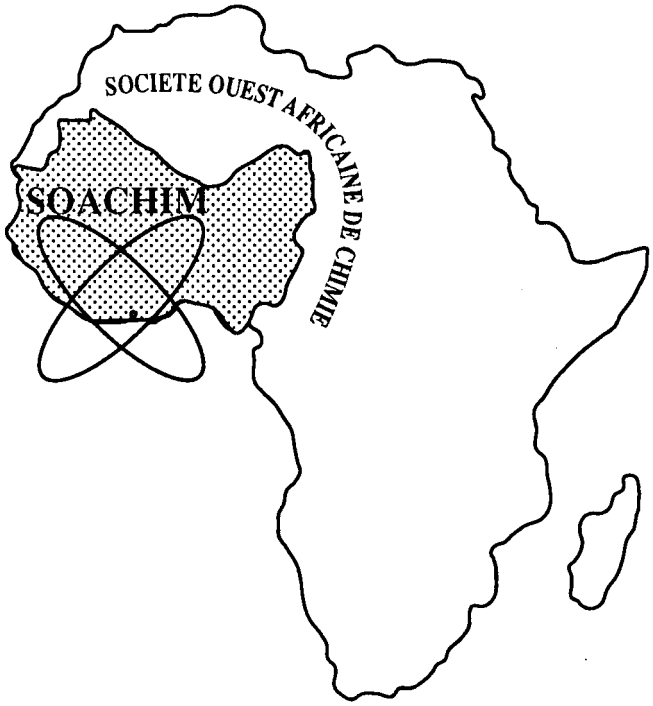

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Identification of major flavonoids using HPLC-MS/MS and determination of antioxidant potential of *Vigna radiata* seed extracts

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Résumé : Des graines de *Vigna radiata* importées d'Australie et acclimatées au Burkina Faso ont été étudiées pour leur teneur en composés phénoliques totaux, flavonoïdes, anthocyanes et caroténoïdes totaux. La méthode DPPH (2,2-diphényl-1-picryl-hydrazyl) nous a permis de déterminer leurs activités antioxydantes. Les résultats ont été comparés à ceux de *Vigna subterranea* et *Glycine max* cultivés au Burkina Faso. La diversité des flavonoïdes dans l'extrait brut de *Vigna radiata* a été également étudiée par chromatographie liquide haute performance-spectrométrie de masse en tandem (LC-MS/MS). Les teneurs en composés phénoliques, en flavonoïdes, en anthocyanes et en caroténoïdes étaient respectivement de 5,479±0,026 mg d'EAG/g, 4,482±0,456 mg d'EQ/g, 0,848±0,063 mg/g et 0,228±0,013 mg d'EBC/g. Les teneurs en antioxydants mesurées étaient similaires à celles de *Vigna subterranea* et de *Glycine max*. Enfin, sur la base de l'analyse LC-MS/MS, nous avons pu démontrer que les graines de *Vigna radiata* renferment majoritairement l'isovitexine, la vitexine et l'orientine. Ces composés C-glycosylés identifiés dans les graines de *Vigna radiata* sont moins répandus et font l'objet d'un intérêt de recherche croissant en raison de leur potentiel biologique élevé (anti-hypertension, anti-VIH, anti-plasmodial, anti-cancéreux et anti-fongique)

Mots clés : Fabaceae, Mung bean, Flavonoïdes, C-glycosylation, Antioxydant

Abstract: Seeds of *Vigna radiata* imported from Australia and acclimatized in Burkina Faso were studied for their total phenolics, flavonoids, anthocyanins and carotenoids contents. The DPPH (2,2-diphenyl-1-picryl-hydrazyl) method allowed us to determine their antioxidant activities. The results were compared with those of *Vigna subterranea* and *Glycine max* grown in Burkina Faso. The diversity of flavonoids in the crude extract of *Vigna radiata* was also studied by high performance liquid chromatography-tandem mass spectrometry (LC-MS/MS). The phenolics, flavonoids, anthocyanins and carotenoids contents were respectively 5.479 ± 0.026 mg GAE / g, 4.482 ± 0.456 mg QE / g, 0.848 ± 0.063 mg / g and 0.228 ± 0.013 mg of BCE / g. Total antioxidant contents measured were similar to those of *Vigna subterranea* and *Glycine max*.

Using LC-MS /MS analysis, it has been revealed that seeds of *Vigna radiata* contain isovitexin, vitexin and orientin. These C-glycosylated compounds identified in the seeds of *Vigna radiata* are less widespread and are the subject of increasing research interest due to their high biological potential (anti-hypertensive, anti-HIV and anti-plasmodial, anti-cancer and antifungal).

Key words: Fabaceae, Mung bean, Flavonoids, C-glycosylation, Antioxidant

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1. Introduction

Legumes play an important role in nutritional security around the world [1] as they contribute to the achievement of food security that encompasses both quantitative and qualitative aspects. They are excellent sources of protein, minerals, vitamins and bioactive compounds [2]. Their global production has increased significantly and the food processing industry is developing new uses of these legumes to create food products that have a beneficial effect on human health [3, 4].

Legumes are a good source of bioactive phenolic compounds for humans as they play an important role in many physiological and metabolic processes. In addition, diets in developing countries are mainly based on legumes and grain products [5]. Most phenolic compounds are concentrated in the coats of the seeds of these legumes [6,7].

Vigna radiata, also called mung bean is a legume of the Fabaceae family in the order of the Fabales. It has been cultivated in India since prehistoric times as a rotation crop with cereals such as wheat and rice. *Vigna radiata* seeds are consumed all over the world, especially in Burkina Faso (beng tigre) as it is traditionally known as an excellent source of vitamins, minerals, essential amino acids, carbohydrates and proteins [8]. This legume also contains phenolic compounds (phenolic acids, flavonoids) and carotenoids that are beneficial in curing and preventing many chronic ailments such as cancer, diabetes, cardiovascular disease because of their antioxidant properties [9]. Over the past decade, several studies have focused on the isolation and identification of bioactive compounds of *Vigna radiata*. These studies have shown that flavonoids are the most abundant secondary metabolites. Five subclasses of flavonoids, namely flavones, flavonols, isoflavonoids, flavanols and anthocyanins, were found in mung bean. They accumulate in plant tissues in the form of glycosylation or esterification conjugats, but sometimes they can also be found in the form of aglycones [10]. Flavones (vitexin, isovitexin, isovitexin-6'-o-α-L-glucoside and luteolin) and flavonols (quercetin, myricetin and kaempferol) were found to be the most abundant flavonoids detected in mung bean [11,12]. Flavonoids represent a large family of phenolic compounds found in fruits and vegetables in all parts of plants. They are known to have antioxidant properties [13,14]. This phytochemical family is also known to possess several biological properties, including anti-hypertensive [15], anti-HIV and anti-plasmodial [16], anti-cancer [17] and antifungal [18].

This study is part of our research program on health-beneficial phytochemicals. It aims to determine the total phenolics contents compounds, flavonoids, anthocyanins and total carotenoids of various extracts of *Vigna radiata*. Antioxidant levels were also measured by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical method and compared with those of black soybeans (*Glycine max*) and voandzou (*Vigna subterranea*) known for their health benefits. Finally, the majority flavonoids in *Vigna radiata* extracts were identified using high-performance liquid chromatography coupled with mass spectrometry in tandem mode (LC-MS/MS).

2. Materials and Methods

2.1. Plant and chemical materials

Plant materials: *Vigna radiata* seeds were supplied by the Laboratory of Vegetable Genetics and Biotechnology of the Institute of Environment and Agricultural Research (INERA) in Kamboinsin (Ouagadougou; Burkina Faso). Those of *Glycine max* and *Vigna subterranea* were reproduced in the experimental garden of the Laboratory of Organic Applied Chemistry and Physics (LCOPA) at the University of Ouagadougou.

Chemicals: the chemicals used were: the Folin-Ciocalteu reagent; quercetin; Gallic acid 2,2-diphenyl-1-picrylhydrazyl (DPPH); ferric 2.4.6-tripyridyl-s-triazine (TPTZ); 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox, a hydrophilic derivative of tocopherol); sulphuric acid. All other chemical reagents used were of analytical quality.

Vigna radiata seeds

Glycine max seeds

Vigna subterranea seeds

2.2. Methods

Extraction: Total phenolic compounds of mung bean, black soybeans and voandzou were extracted using acetone/water/acetic acid (70:29.5:0.5; v/v/v). Total anthocyanic extracts were obtained using methanol-hydrochloric acid mixture (99:1; v/v). As for carotenoids, acetone/hexane system (50:50; v/v) was used. In fact, 1 g of the crushed grains of each variety was extracted by maceration for 24 hours at 4°C with 3 mL of each solvent. Extracts were filtered and residues have been extracted again twice with 2 mL of solvent for 24 hours. The filters were collected and stored in the refrigerator at 4°C for the determination of total of phenolics, carotenoids contents and total antioxidant parameter [19].

***Determination of Total Phenolics Contents (TPC)*:** TPC of the extracts were demonstrated by the Folin-Ciocalteu method [20]. It consisted to a reaction of 60 µL studied sample with 60 µL of Folin-Ciocalteu reagent (diluted 10 times). After 8 min, 120 µL of sodium carbonate (Na_2CO_3) to 7.5% (p/v) are added. After 30 min, the absorbance was read at 765 nm. Blanks were prepared for each variety by replacing the Folin-Ciocalteu's reagent with distilled water. A standard curve was established using gallic acid as a standard (**Table I**) and the results were expressed in mg of Gallic Acid Equivalents (GAE)/g of dry material.

***Determination of Total Flavonoids Contents (TFC)*:** Total flavonoid contents were assessed using the aluminum trichloride method [21,22]. In short, 20 µL of standard (quercetin) concentration ranging from 0.001 to 0.5 mg/mL or extract was mixed with 10 µL of $NaNO_2$ (5 %) 96 wells and allowed to react for 5 min at room temperature. Then, 10 µL of an $AlCl_3$ solution (10 %) was added. After 6 min of reaction at room temperature, 40 µL of NaOH (1 M) were added to the medium. The mixture was diluted with 130 µL of bidistilled water and homogenized. The absorbances were read immediately at 510 nm using a SAFAS spectrophotometer. The total flavonoid contents of the extracts were obtained by reporting the absorbances read on the standard curve established from quercetin (**Table I**).

Determination of Total Anthocyanins Contents (TAC): Total anthocyanin contents of extracts were estimated by the pH-differential method [23] using two buffer systems: potassium chloride solution, $pH_{1.0}$ (0.025 M) and sodium acetate solution, $pH_{4.5}$ (0.025 M). 0.5 mL of the extract are mixed with 3.5 mL of the corresponding tampons and the absorption was read in relation to the white at 510 nm and 700 nm, 15 min later using a monocuve SAFAS spectrophotometer.

Absorbance A is obtained from the following relationship:

$$\textbf{(1)} \quad : \mathbf{A = A_{510} - A_{700})pH1.0 - (A_{510} - A_{700})pH4.5}$$

And the contents were obtained by the following relationship:

$$\textbf{(2) : TAC (mg/ L)} = \frac{\mathbf{A*MW*DF*1000}}{\boldsymbol{\varepsilon * \ell}}$$

MW: Molecular weight of cyanidine 3-O-glucoside and ε its Molar extinction coefficient MW= 449.2 g/mol and ε = 26900 [24]. It was expressed in milligrams of cyanidin-3-glucoside per gram of dry grains.

Determination of Total Carotenoids Contents (TCC) : total carotenoids contents of extracts were assessed using the method described by McMurry and colleagues [25-27] but slightly modified. After proper dilution, the absorbances of extracts kept at room temperature and away from light, were read at 450 nm. Total carotenoid contents were obtained by reporting the absorbances of extract on an established standard curve using β-carotene as a standard (**Table I**). The contents of carotenoids were expressed in β-carotene equivalents per gram of dry plant material.

Determination of Total Antioxidant Status (TAS) : the method used was that of the direct measurement described by Stephanie et al [28]. The commercial DPPH radical was dissolved in methanol at a concentration of 4.10^{-2} mg/mL and kept at 4°C away from light. To each sample extract (50 µL) were added 200 µL of DPPH solution. Antioxidants with the property of ceding a single electron to radical DPPH, result in a discoloration of the DPPH solution that takes on a color ranging from dark pink to pale yellow depending on the antioxidant content (**Figure 1**). The absorbances were read after 10 min at 517 nm [28]. The results were expressed in mg of Trolox Equivalents per gram of plant material (**Table I**).

HPLC-MS/MS (LC-MS/MS) Flavonoids Identification : HPLC experiments coupled with tandem mass spectrometry equipped with a positive electrospray ionization (ESI) source were conducted for the identification and characterization of flavonoids. Indeed, a HPLC system of Agilent technology *Infinitely better 1290* was used for chromatographic separation. It has a zorbax sb-type in reverse C18 column of 250 mm length and 4.6 mm diameter with a particle size of 5 µm.

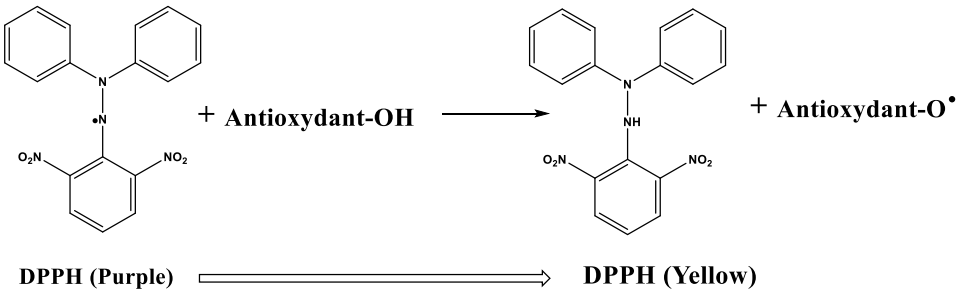


Figure 1: reaction of DPPH reduction with antioxidants

Table I: establishment of standard curves

Standard curves	Standards	Equations	Correlation coefficients
TPC	Gallic acid	y = 17.814x + 0.0485	R^2 = 0.9997
TFC	Quercetin	y = 0.767x + 0.0862	R^2 = 0.9963
TCC	β-carotene	y = 11.618x - 0.0075	R^2 = 0.9994
DPPH	Trolox	y = -16.096x + 0.5709	R^2 = 0.9995

The elution was achieved with a mobile phase A (water/5% formic acid v/v) and a mobile phase B (acetonitrile/5% formic acid v/v). The gradient based on the time expressed as a percentage of volume of the mobile phase A and the mobile phase B was programmed 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 triple quadrupole series version B.06.00 Bulld 6.0.6025.0.

Statistical analyses: all experiments were conducted in triple and data were represented as an average ± standard deviation. The analysis of variance (ANOVA) was performed using IBM SPSS Statistics 25.0. The relationship between different antioxidant assays has been described as Pearson's product-moment correlation coefficient (r). The differences were considered statistically significant if $P < 0.05$. Significant differences ($P < 0.05$) of averages were calculated using the post hoc multiple comparison method, LSD.

3. Results and Discussion

Three (3) different extracts were obtained from the grains of *Vigna radiata*, *Vigna subterranea* and *Glycine max*, with different solvents, in order to dispose of all the secondary metabolites studied.

Total phenolics, flavonoids and anthocyanins contents : the total phenolics contents of the three varieties are presented in **Table II**. Statistical analysis of these levels showed significant differences ($P < 0.05$) between the extracts. Indeed, *Glycine max* (black soy) contains more phenolics (5.584±0.037 mg of GAE/g) than *Vigna radiata* (mung bean) and *Vigna subterranea* that contain 5.479±0.026 mg of GAE/g and 5.397±0.224 mg of GAE/g, respectively.

For flavonoids, contents are 4.482±0.456 mg of QE/g for *Vigna radiata*, 4.558±0.156 mg of EQ/g for *Glycine max* and 4.282±0.093 mg of QE/g for *Vigna subterranea* (**Table II**). No significant differences were observed. All varieties studied have approximately the same levels of total flavonoids.

Total anthocyanins from the extracts measured showed statistically significant differences ($P < 0.05$). *Glycine max* is the richest variety of anthocyanins with an average content of 1.205±0.027 mg/g followed by *Vigna radiata* (0.848±0.063 mg/g) and *Vigna subterranea* which has the lowest content (0.535±0.025 mg/g).

The results show that the total flavonoids content of *Vigna radiata* was comparable to that of *Vigna subterranea* and *Glycine max*. However, there was a variation of 36% of anthocyanins in *Glycine max* compared to *Vigna radiata*. As anthocyanins are one of the classes of flavonoids, the comparable flavonoids content is probably due to other types of flavonoids in *Vigna radiata* extracts. Previous studies have shown that *Vigna radiata* grains contained flavonoids such as vitexin and isovitexin [11]. It should also be noted that total phenolics content *Vigna radiata* was 10% lower than that of *Glycine max* and 8% richer than *Vigna subterranea.*

Total carotenoids content : total carotenoids contents expressed in mg of β-carotene equivalent per gram of dry grains (mg BCE/g) also show significant differences between the three studied varieties. *Vigna radiata* has the highest content (0.228±0.013 mg of BCE/g) which was 6 times higher than that of *Glycine max* (0.038±0.008 mg of BCE/g) and 15 times higher than *Vigna subterranea* (0.015±0.002 mg of BCE/g). Harina and ramirez have shown that *Vigna radiata* carotenoids are present in the form of β-carotene and xanthophylle [29]. These secondary metabolites are known as free radical trapping antioxidants.

Antioxidant contents using free DPPH Radical Scavenging method: the determination in foods, beverages and plant extracts [24] of total antioxidant status based on radical DPPH is one of the most popular spectrophotometric methods. In the DPPH method, the radical DPPH$^{\bullet}$ stable in methanolic solution was reduced to diphenyl-picrylhydrazine (yellow-colored solution) by antioxidants due to the formation of [DPPH-H] by transfer of hydrogen atoms to the DPPH free radical.

The results of antioxidant status are recorded in **Table II**. The results show that extracts from different varieties have similar antioxidant contents. Estimating the levels of total phenolics, flavonoids, anthocyanins and total carotenoids contents was important because it was often used to determine the antioxidant activity of a plant matter. Several studies have shown excellent linear correlations between "total phenolics content" and antioxidant activity [30, 31]. This antioxidant activity is also thought to be linked to the presence of flavonoids and anthocyanins. These observations may justify the good correlations between the dosage of DPPH radical scavenging and phenolics contents (**Table III**). The antioxidant properties of phenolics are related to their aromatic cycles carrying one or more hydroxyle groups and are therefore potentially able to extinguish free radicals by forming resonance-stabilized phenoxyle radicals. It is also well known that carotenoids have good antioxidant properties [32]. Indeed, these molecules are characterized by a strong conjugation of polyenes that allowed oxidation and photooxidation reactions [32]. They are then converted into stabilized radicals based on electron or hydrogen atom transfer reactions or electrophilic addition reactions.

The results obtained suggest that *Vigna radiata* seeds can be used as a functional ingredient with high antioxidant activity as they contain phenolics especially flavonoids that play a protective role against cardiovascular disease, cancer, type B diabetes, etc.

Identification of major flavonoids of Vigna radiata by LC-MS/MS: the HPLC of raw extract of *Vigna radiata* yielded three significant signals (**1**; RT = 45.76 min, **2**; RT = 41.78 min, **3**; RT=35.98 min) likely to correspond to flavonoids (**Figure 2**).

Table II : Total phenolics, flavonoids, anthocyanins, carotenoids and antioxidant contents

Varieties	TPC (mg /g)	TFC (mg/g)	TAT (mg/g)	TCC (mg/g)	TAS (mg/g)
Vigna radiata	5.479±0.026 ^{b}	4.482±0.456 ^{a}	0.848±0.063 ^{b}	0.228±0.013 ^{c}	2.059±0.043 ^{a}
Vigna subterranea	5.397±0.224 ^{a}	4.282±0.093 ^{a}	0.535±0.025 ^{a}	0.015±0.002 ^{a}	2.033±0.005 ^{a}
Glycine max	5.584±0.037 ^{c}	4.558±0.156 ^{a}	1.205±0.027 ^{c}	0.038±0.08 ^{b}	2.085±0.035 ^{a}

Table III : correlation between assays

	CPT	FT	TAT	TCT
DPPH	0.652*	0.896**	0.596*	0.41*
CPT	-	0.459*	0.927**	0.408

** The correlation is significant at level 0.01 (bilateral)

* The correlation is significant at level 0.05 (bilateral)

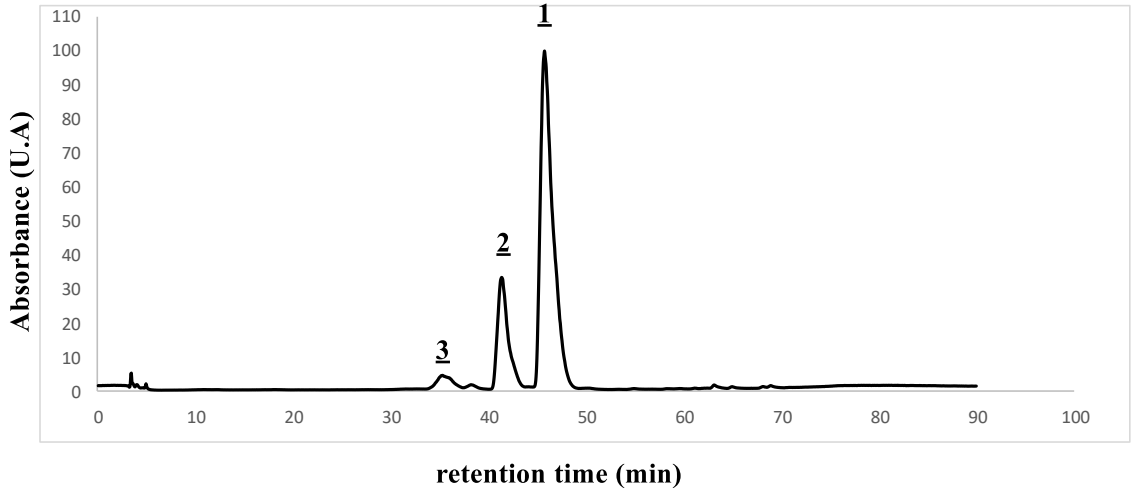


Figure 2 : HPLC chromatogram of crude extract of *Vigna radiata*

First, the three retention times observed correspond to protonated molecules $[M+H]^+$ detected respectively at m/z 433, 433, 449. Interestingly, we are then confronted with two different ion compositions that would correspond respectively to calculated masses of the raw formulas $C_{21}H_{21}O_{10}$ and $C_{21}H_{21}O_{11}$. These formulas may correspond to those of flavonoids of the type monoglycosyle flavones (mono-O-glycosyles or mono-C-glycosyles). A previous study found that the most abundant flavonoids in *Vigna radiata* were monoglycosyled and possess apigenin or luteoline aglycone [11].

An analysis by LC-MS/MS was conducted to identify the natures of aglycone and bound sugar. It should also be remembered that neutral losses of sugar-characteristic ions were observed in MS/MS. Indeed, in the case of mono-*O*-glycosylated flavonoids, there will be a loss of 162 u for an hexose, 146 u for deoxyhexose, 132 u for pentose and 176 u for a ground of carbohydrate acid [33-35]. In the case of mono-C-glycosylated flavonoids, in negative ESI, the observation of a neutral loss 120 u and 90 u corresponds to an hexose pattern, a loss of 104 u and 74 u corresponds to a reason for deoxyhexose and a loss of 90 u and 60 u to that of a pentose pattern. In positive ESI, the loss of 120 u corresponds to a hexose [33-35].

During the ESI collision activation, the molecular ion m/z 433 suffers a loss of 120 u $[M+H-C_4H_8O_4]^+$ revealing the presence of an hexose residue attached (in the C-glycoside position) to the aglycone grouping (**Figure 3 and 4**). As showed in **Table IV**, compounds **1** and **2** are isomers. LC-MS/MS data of $[M+H]^+$ ions of these isomers are similar and show characteristic losses of 120 u. However, the MS/MS spectrum of compound **2** indicates fewer fragments than compound **1**. The ions that appeared at m/z 313 and 283 correspond to the cyclical cleavage of glycosyl by a loss of 30 u (CH_2O) [36]. Fragment ions m/z 415, 397 and 379 corresponding to losses of one, two and three molecules of H_2O respectively are characteristic fragmentation pathways of vitexin (apigenin 8-C-glycosyle) or isovitexin (apigenin 6-C-glycosyle) [36]. The m/z 271 $[M+H-162]^+$ ion product of the spectrum of compound **1** corresponds to the loss of a glucose molecule. These results show that these isomers correspond to vitexin and isovitexin.

According to the literature, the position of C-glycosyle sugar can be determined by observing the intensity of the ion corresponding to the first water loss. If in positive ESI mode, $[M+H-H_2O]^+$ ion was the most abundant fragment peak in the MS/MS spectrum, then sugar is linked to the carbon 8 of the aglycon, otherwise it is in the C-6 position [37]. This hypothesis is not in agreement with our case because the spectra of the two isomers all have the most abundant m/z 415 ion. Moreover, the relative abundance of ions cannot be based on the relative abundance of ions, since depending on the type of analyzer with which fragmentation experiments were conducted, the relative abundances of all ions will not always be equivalent. Thus, one of the most likely hypotheses is that related to the polarity of molecules. Because vitexin is more polar than isovitexin, it will be detected before isovitexin in polar HPLC mobile phase. In addition, studies have shown that isovitexin is present in higher quantities than vitexin in *Vigna radiata* seeds [9,38]. This corresponds well to the chromatogram obtained (**Figure 2**). Based on all these results as well as the reported data from the literature [36], we identify compound **1** as apigenin 6-C-glycosyle (isovitexin) and compound **2** as apigenin 8-C-glycosyle (vitexin).

Table IV: HPLC and ESI$^+$-MS/MS data from raw extract from *Vigna radiata* seeds

Compounds	Retention Time (min)	Molecular ions $[M+H]^+$	ESI$^+$-MS/MS (m/z)
1 ($C_{21}H_{21}O_{10}$)	45.76	433	**433** $[M+H]^+$; **415** $[M+H-H_2O]^+$; **397** $[M+H-2H_2O]^+$; **379** $[M+H-3H_2O]^+$; **313** $[M+H-C_4H_8O_4]^+$; **283** $[M+H-C_4H_8O_4-CH_2O]^+$; **271** $[M+H-glc]^+$
2 ($C_{21}H_{21}O_{10}$)	41.78	433	**433** $[M+H]^+$; **415** $[M+H-H_2O]^+$; **313** $[M+H-C_4H_8O_4]^+$
3 ($C_{21}H_{21}O_{11}$)	35.98	449	**449** $[M+H]^+$; **431** $[M+H-H_2O]^+$; **413** $[M+H-2H_2O]^+$; **395** $[M+H-3H_2O]^+$; **329** $[M+H-C_4H_8O_4]^+$; **299** $[M+H-C_4H_8O_4-CH_2O]^+$

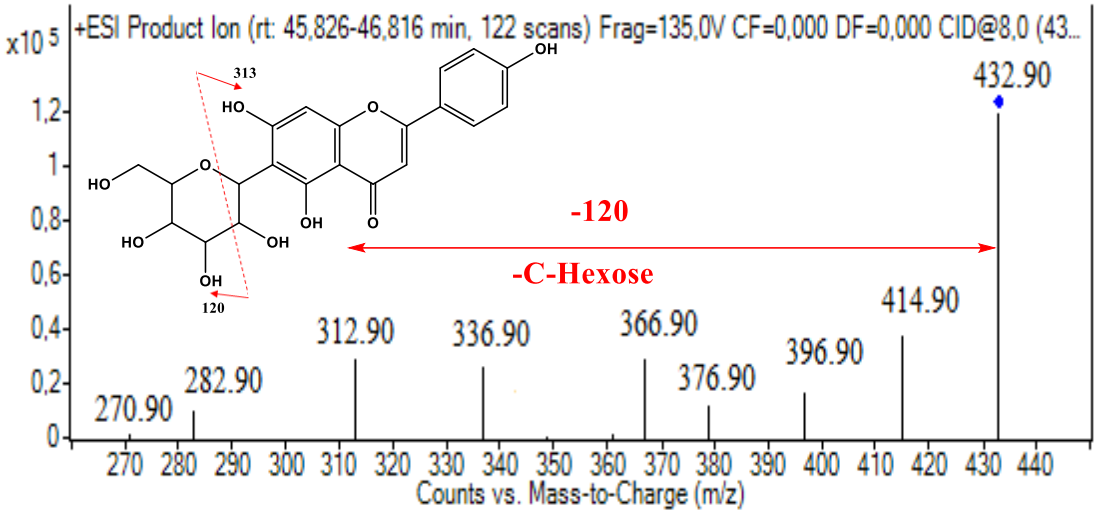


Figure 3: collision-induced dissociation (CID) mass spectrum of m / z 443 (RT = 45.76min)

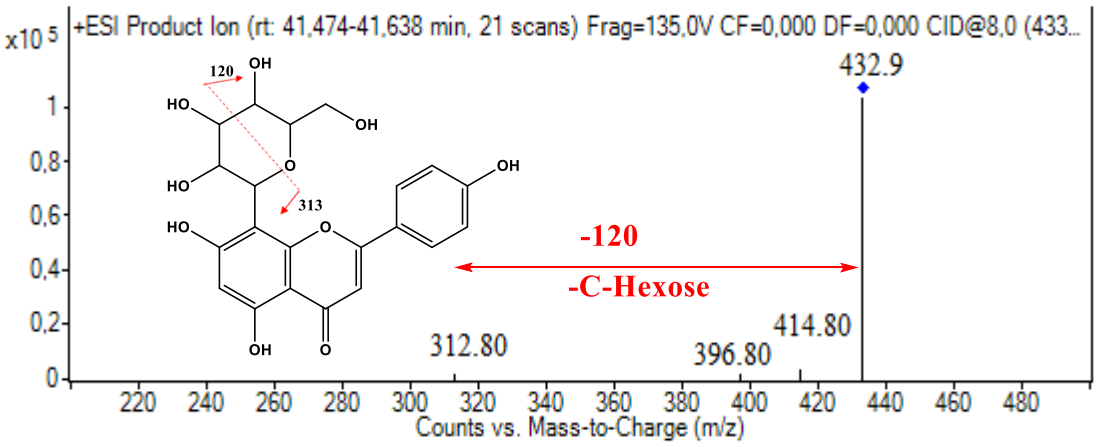


Figure 4: collision-induced dissociation (CID) mass spectrum of m / z 443 (RT = 41.78 min)

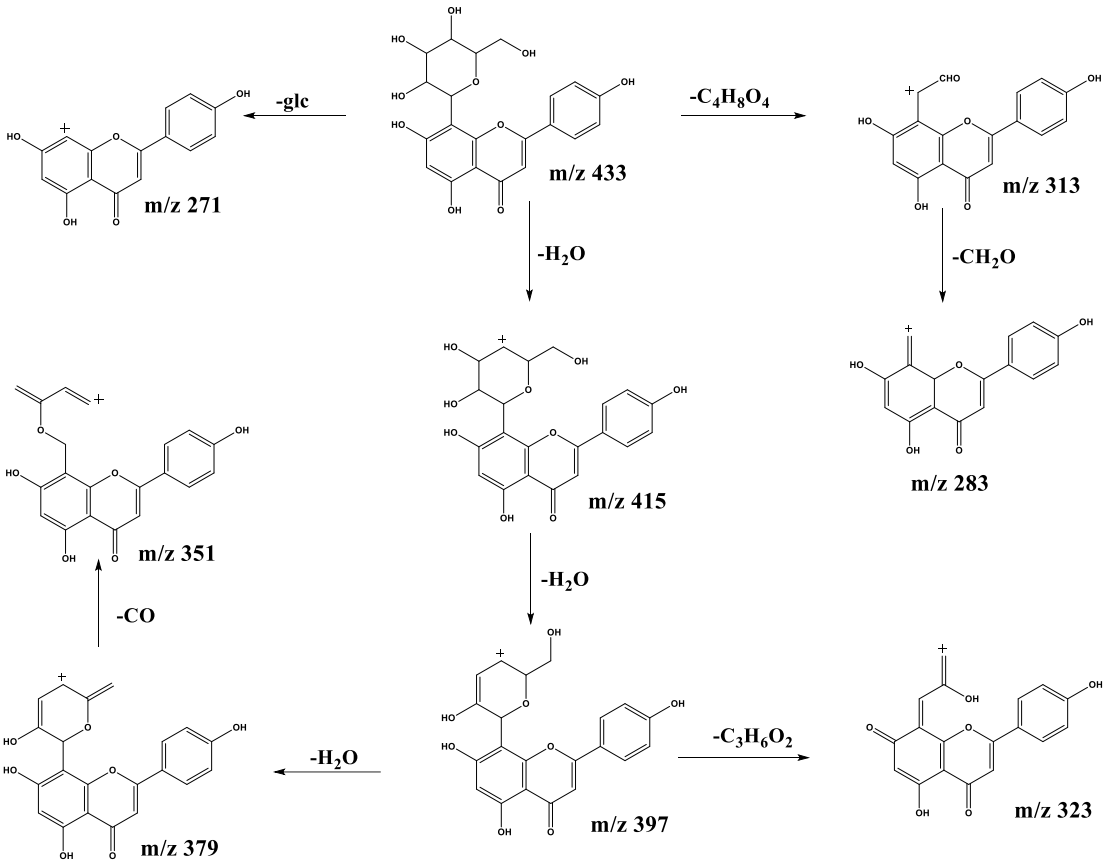


Figure 5 : the cleavage voices of vitexin in positive mode [39]

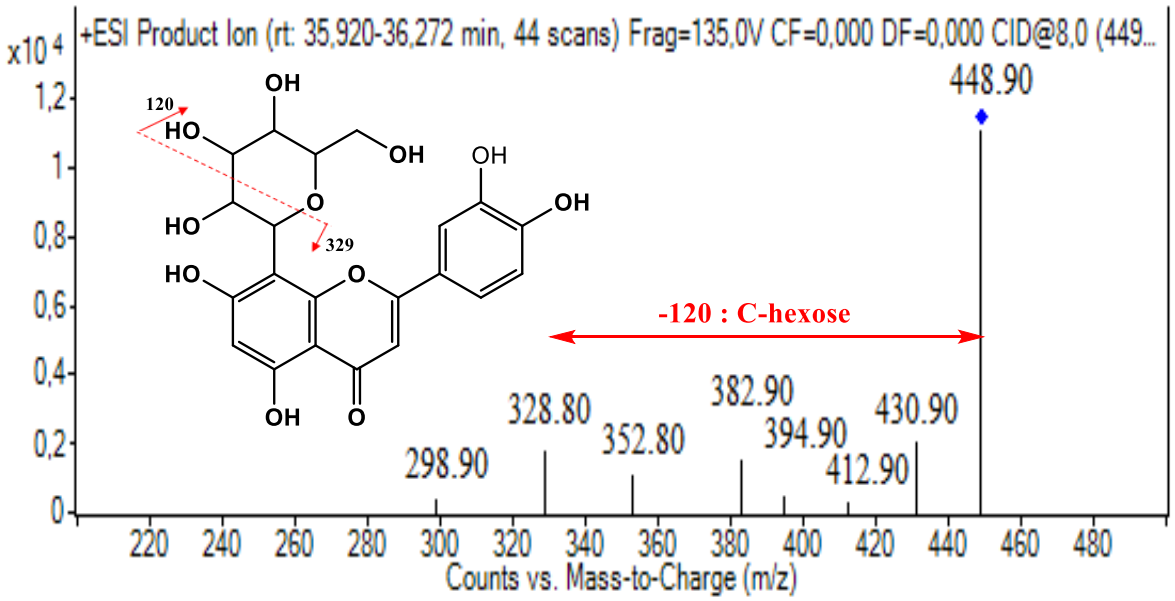


Figure 6: collision-induced dissociation (CID) mass spectrum of m / z 449 (RT = 35.98 min)

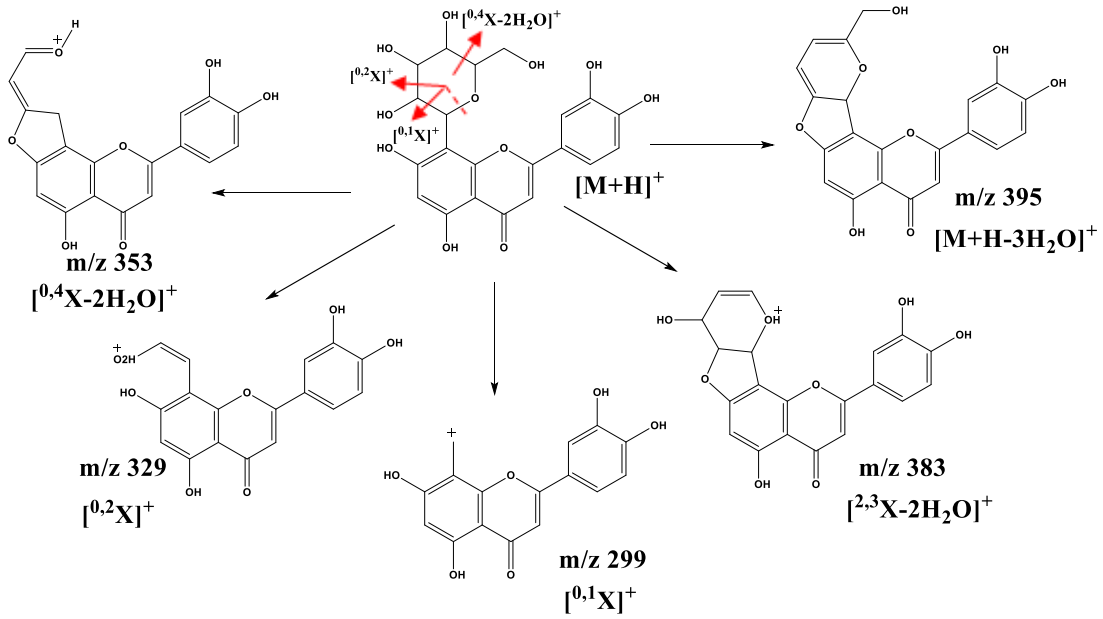
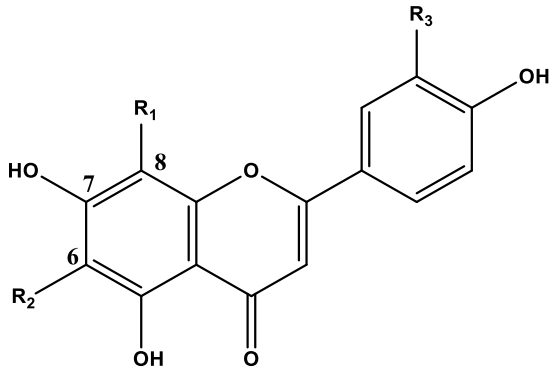


Figure 7: Orientin fragmentation scheme in positive ionization mode [39]



Compound **1** : R_1 = Glc ; R_2 = H et R_3 = H
Compound **2** : R_1 = H ; R_2 = Glc et R_3 = H
Compound **3** : R_1 = Glc ; R_2 = H et R_3 = OH

Figure 8: structure of compounds **1**, **2** et **3** identified in seeds of *Vigna radiata*

The CID spectra of compound **3** is presented in **Figure 6**. On CID, the m/z 449 suffers 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 already remembered, these losses are consistent with the characteristic ions of a C-glycoside indicating the presence of hexose (in this case, glucose) associated with a carbon of the aglycon. These ions also correspond to the cyclical cleavage of glycosyl by a loss of 30 u as in the case of vitexin. The m/z 329 ion corresponding to the ion $[Aglycone +42]^+$ allows to calculate the mass of the aglycon which gives 287 u, or a luteolin [40]. The fragment ions at m/z 431, 413, 395 correspond respectively to losses of one, two and three molecules of H_2O. Compound **3** could be luteolin 8-C-glycosyle (orientin) or luteolin 6-C-glycosyle (isoorientin). The difference is noted by observing the presence of peak m/z 431 corresponding to the first loss of H_2O and the intensity of peaks m/z 329 and 299. Indeed, a recent study showed that in positive ionization mode, the m/z 431 ion corresponding to the first loss of H_2O is present for the isomer in C-8. For the C-6 isomer, the m/z 329 ion is more intense than the m/z 299 ion and vice versa for C-8 [40]. Thus, compound **3** is identified as luteolin 8-C-glycoside (Orientin).

4. Conclusion

Vigna radiata seeds are not only seen as a protein-energy source but also as a source of phytochemicals

with beneficial effects on consumers health. Its antioxidant content is comparable to that of *Vigna subterranea* and *Glycine max*. LC-MS/MS analysis of raw *Vigna radiata* extracts has led to the identification of three flavonoids including vitexin, isovitexin and orientin. These C-glycosylated compounds are less common and are the subject of increasing research interest due to their high anti-hypertensive, anti-HIV and anti-plasmodial, anti-cancer and antifungal properties.

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