GSC Advanced Research and Reviews

eISSN: 2582-4597 CODEN (USA): GARRC2

Cross Ref DOI: 10.30574/gscarr

Journal homepage: https://gsconlinepress.com/journals/gscarr/

(RESEARCH ARTICLE)

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Phenolic compound and total antioxidant contents of seed extracts from 5 varieties of *Vigna subterranea* from Chad

Abel MBAIOGAOU [1,*], Michel NAITORMBAIDE [2], Bery Leouro MBAIOSSOUM [3], Severin MBAIHOUGADOBE [1] and Salomon Madjitoloum BETOLOUM [1]

[1] *Laboratory of Research on Natural Substances, Faculty of Pure and Applied Sciences (F.P.A.S.), University of N'Djamena, Chad.*
[2] *Chadian Institute of Agricultural Research for Development (CIARD), Bébédjia, Chad.*
[3] *Laboratory of Modelling, Mathematics, Computer Science, Applications and Simulation, Faculty of Pure and Applied Sciences (F.P.A.S.), University of N'Djamena, Chad.*

GSC Advanced Research and Reviews, 2023, 17(02), 001–009

Publication history: Received on 16 September 2023; revised on 01 November 2023; accepted on 03 November 2023

Article DOI: https://doi.org/10.30574/gscarr.2023.17.2.0405

Abstract

Vigna Subterranea is a plant whose seeds are widely consumed in Chad. Five seed varieties from the Chadian Institute of Agronomic Research for Development (CIARD) in, Chad. Total polyphenol and total anthocyanin contents were assessed by the Folin-Ciocalteu and pH-differential methods respectively. Total antioxidant levels were determined by DPPH and ABTS. The total polyphenol contents obtained were 11.647 mg GAE/g for B12 seed extract, 8.234 mg GAE/g for B16 seed extract, 0.98450 mg GAE/g for B9 seed extract, 1.625 mg GAE/g for B7 seed extract and 0.230 mg GAE/g for B3 seed extract. The total anthocyanin content of these extracts were 0.0100 mg/g, 0.0098 mg/g, 0.0063 mg/g, 0.0026 mg/g and 0.0013 mg/g for B16, B7, B12, B9 and B3 respectively for the five varieties. Results for antioxidant potency showed that the B12 seed extract contains most total antioxidants. Followed by seed extracts from varieties B16, B7 and B9 varieties. Seed extract from variety B3 contains less antioxidants.

Keywords: *Vigna Subterranea*; Total polyphenols; Total anthocyanins; Total antioxidants; Chad.

1. Introduction

Voandzou or *Vigna Subterranea* is a plant of the Fabaceae family. It is native to West Africa. Its seeds are widely consumed in many regions. This plant adapts easily to very difficult climatic conditions [1]. It tolerates acidic and slightly poor soils fairly well.

In Africa, *Vigna subterranea* plays a very important role in the diet, providing farmers with substantial income [2, 3, 4]. It also plays an important agronomic and medicinal role.

The seeds, roots and leaves of *Vigna subterranea* are used in Senegal and Nigeria to treat some diseases. In Senegal, the leaves are used to treat abscesses and infected wounds. The juice of the green leaves is applied to the eyes to treat epilepsy. The roots are used as an aphrodisiac, and the crushed seeds mixed with water are applied to the eyes to treat cataracts [5]. In Nigeria, the plant is used to treat venereal diseases [5]. In Chad, *Vigna subterranea* flour is used to treat high blood pressure.

Previous studies have shown that *Vigna subterranea* is a vitamin-rich plant, rich in mineral elements (Ca, Fe...) and highly balanced in protein [5, 6, 7, 8, 9]. Lipids, carbohydrates, β-carotene, thiamine, riboflavin, niacin and traces of

* Corresponding author: MBAIOGAOU Abel

ascorbic acid are found in ripe seeds. Amino acids such as tryptophan, lysine, methionine, phenylalanine, threonine, valine, leucine and isoleucine are also found in ripe seeds [10, 11]. *Vigna subterranea* seed oil contains the main fatty acids: palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid and behenic acid [11]. The seed coat contains phenolic compounds such as tannins and anthocyanic compounds [12].

In Africa, where legumes of the genus *Vigna*, particularly *Vigna subterranea*, are widely consumed, it could be useful to have a database on their antioxidant content, with a view to treating diseases linked to oxidative stress, such as inflammatory and cardiovascular diseases, cancer, diabetes, Alzheimer's disease and cataracts [13, 14, 15]. Indeed, these data on total antioxidant content constitute additional nutritional information that will facilitate better integration and valorization of voandzou among functional foods like other fruits and vegetables [16]. In this aim phenolic compounds and total antioxidant were determined from seed extracts of 5 varieties of *Vigna subterranea*.

2. Material and methods

2.1. Plant material

Five (5) varieties of *Vigna subterranea* seeds from Chad harvested in October 2022 were used in the experiments. These seed varieties are supplied by the Chadian Institute of Agronomic Research for Development (CIARD) of Bebedja station, southern Chad (Figure 1)

2.2. Methods

2.2.1. Extraction of phenolic compounds from different seed varieties

The seeds of the different varieties of *Vigna subterranea* were ground. Three (3) grams of powder from each variety were extracted with 15 mL of a methanol-water-acetic acid solvent system (70:29.5:0.5) by maceration for 24 hours at 4°C. Extracts are filtered and residues are extracted twice more with 10 mL solvent for 24 hours. The filtrates are kept refrigerated at 4°C for determination of polyphenol and total antioxidant (Figure 2) [17].

2.2.2. Determination of total polyphenols

The phenolic compound content of seed extracts was determined using the Folin - Ciocalteu method [18]. It consists in reacting 1.05 mL of the sample with 5 mL of Folin reagent (diluted 10 times). After 8 min, 4 mL of 7.5% (w/v) sodium carbonate is added. After 30 min of incubation, the absorbance is read at 765 nm. Blanks are prepared for each sample by replacing Folin reagent with distilled water. Gallic acid is used as the standard (Table 1). Results are expressed as mg gallic acid equivalent (GAE/g) of dry material.

2.2.3. Measuring the antioxidant capacity of extracts

Two methods are used to assess total antioxidant content. These are the ABTS and DPPH methods. These two methods, based on different chemical mechanisms, were chosen to take account of the wide variability and range of action of individual antioxidants in the extracts studied. ABTS measures the capacity of antioxidants to trap radical-cation (ABTS+.) [19]. DPPH measures the trapping capacity of the stable commercial radical (DPPH.) [20]. Two standards are used for each method, namely Trolox and Quercetin.

2.2.4. DPPH method

The commercial DPPH radical is dissolved in methanol at 0.04 mg/mL and kept at 4°C protected from light. To each sample extract (1.5 mL) are added 2 mL of DPPH solution and the absorbance is read after 10 min at 517 nm. Results are expressed as mg TE/g and mg QE/g dry material (Table 1) [21].

2.2.5. ABTS method

The ABTS cation-radical is generated by mixing 1 mL of 39.2 mM potassium persulfate $K_2S_2O_8$ solution with 5 mL of 7.01 mM ABTS solution. The mixture is kept in the dark at 4°C for 16 hours, the time required to generate the cation-radical. The blue-green solution obtained is diluted to give an absorbance of 0.7±0.5 at 734 nm. To each sample (1.5 mL) are added 2 mL of ABTS solution and the absorbance is measured after 10 min at 734 nm. Results are expressed as mg TE/g and mg QE/g dry material (Table 1) [19].

2.3. Determination of total anthocyanins

The total anthocyanin content of extracts is estimated by the pH-differential method using two buffer systems: potassium chloride (KCl) solution, pH 1.0 (0.025 M) and sodium acetate (CH_3COONa) solution, pH 4.5 (0.4 M).

To 1.2 mL of the extract, 10.8 mL of the corresponding buffers were added and the absorbance was read against the blank at 510 nm and at 700 nm 15 minutes later. Absorbance A was calculated as follows: $A = (A_{510} - A_{700})_{pH\ 1,0} - (A_{510} - A_{700})_{pH\ 4,5}$. The monomeric concentration of anthocyanin dyes in the extract is calculated as cyanidin-3-glucoside $\left(mg/L = \frac{A \times MW \times DF \times 1000}{\varepsilon \times l} \right)$ [22] where A: absorbance; MW: molecular weight; (449.2); DF: dilution factor; ε: molar absorptivity (26900). Total anthocyanin contents are expressed in micrograms of cyanidin-3-glucoside per gram of dry material.

2.4. Statistical analysis

All data obtained were treated statistically by Excel software at the 5% probability threshold. All experiments were performed in triplicate. Results are expressed as mean ± standard deviation. Values of $p < 0.05$ were considered statistically significant [23].

3. Results and discussion

The standard curves and the correlation between the different methods are shown in Table 1 and Table 2 respectively. The total polyphenol (TPC), antioxidant (TAO) and total anthocyanin (TAC) contents of the five *Vigna subterranea* seed varieties are shown in Table 3.

The polyphenol, total anthocyanin and total antioxidant contents of five *Vigna subterranea* varieties supplied by CIARD of Bebedja (Chad) were determined. These five varieties are characterized by the creamy-yellow, purple-black or variegated color of their pericarp. The differences between the antioxidant, polyphenol and total anthocyanin contents (Table 3) of these voandzou varieties are often wide. This variation in content is probably linked to the color of the seeds.

3.1. Total polyphenol content

The total polyphenol contents of the various *Vigna subterranea* seed extracts were assessed using the Folin - Ciocalteu method. The results in Table 3 show that the B12 seed extract is the richest in total polyphenols (11.6476 mg GAE/g). It is followed by B16, B7, B9 and B3 seed extracts with respective contents of 8.2348, 1.6258, 0.9845 and 0.2304 mg GAE/g. The total polyphenol contents of the B12 and B16 extracts obtained from Chad are higher than those of the seed extracts of the Burkina Faso varieties studied by Abel et al. On the other hand, B7, B9 and B3 seed extracts had lower polyphenol contents than the Burkina Faso varieties KVS225 (4.406 mg GAE/g), KVS360 (3.595 mg GAE/g) and KVS312 (4.536 mg GAE/g) studied by the same author [24].

The color of the pericarp of the *Vigna subterranea* varieties certainly partly explains the differences observed between these voandzou studied. It is known that polyphenols are responsible, through intra- or intermolecular copigmentation phenomena [25; 26], for the diversity of colors observed (red, blue, mauve, purple, etc.) in plant leaves, roots, stems, bark, fruit and flowers [27; 28]. Moreover, they are almost entirely responsible for the antioxidant activities of botanical extracts [29; 30]; this was confirmed in this study by the good correlation coefficients (R~0.9825) observed between TPC and TAO (Table 2).

3.2. Total antioxidant content (TAO)

The total antioxidant content of *Vigna subterranea* extracts was determined by two methods: ABTS and DPPH. For each method, two standards were used: Trolox and Quercetin. Results are expressed as milligram Quercetin equivalent per gram (mg QE/g) and milligram Tolox equivalent per gram (mg TE/g) of dry material.

By the ABTS method, using Trolox as standard, the results obtained (Table 3, Figure 4) showed that the seed extract of B12 (5.890 mg TE/g) is the richest in total antioxidants than the other seed varieties. It was followed respectively by the seed extracts of the following varieties B16 (4.069 mg TE /g), B7 (2.009 mg TE /g), B9 (0.889 mg TE /g) and B3 (0.373 mg TE /g). Varieties B12, B15 have higher antioxidant contents than seed extracts of varieties KVS97 (2.242 mg TE /g), KVS153 (2.107 mg TE /g) KVS312 (2.239 mg TE /g), KVS67 (2.038 mg TE /g) from Burkina Faso measured by

the same method. However, seed varieties from Burkina Faso have higher levels of total antioxidants than seed varieties B7, B9 and B3 from Chad [31].

By the same method, with Quercetin as standard, the B12 seed extract (11.068 QE/g) is always the richest (Table 3, Figure 5). It is followed in order by seed extracts of varieties B16 (7.578 mg QE/g), B7 (3.631 mg QE/g), B9 (1.485 mg QE/g) and B3 (0.496 mg QE/g).

With either Trolox or Quercetin as standard, the B12 seed extract is the richest in antioxidants. This is followed by B16 extract, B7 extract, B9 extract and B3 extract.

The antioxidant content of these extracts is ranked in decreasing order as follows: B3< B9<B7<B16<B12

Table 1 Establishment of the curves – standards

Calibration curves	Standard	Equations	Correlation coefficients
RFC	Gallic acid	y = 11.046x + 0.0997	R^2 = 0,998
ABTS	Trolox	y = -22.677x + 0.7789	R^2 = 0.9994
	Quercetin	y = -11.834x + 0.7659	R^2 = 0.9704
DPPH	Trolox	y = -16.341x + 0.5964	R^2 = 0.9915
	Quercetin	y = -25.451x + 0.6703	R^2 = 0.9955

Table 2 Correlation(R) between different methods

Methods	Standard	RFC
ABTS	Trolox	R = 0.9694
	Quercetin	R = 0.9694
DPPH	Trolox	R = 0.9865
	Quercetin	R = 0.9918

Table 3 Total polyphenols contents (TPC), total antioxidants (TAO) (by ABTS and DPPH methods), and total anthocyanins contents (TAC) of extracts

Method		Varieties				
	Standard	B16	B12	B9	B3	B7
RFC	Gallic acid	8.234±0.014^b	11.647±0.03^a	0.984±0.003^c	0.230±0.01^e	1.625±0.012^d
ABTS	Trolox	4.069±0.006^b	5.890±0.062^a	0.889±0.012^c	0.373±0.03^e	2.009±0.062^d
	Quercetin	7.578±0.011^b	11.068±0.11 ^{a}	1.485±0.023^c	0.496±0.03^e	3.631±0.083^d
DPPH	Trolox	5.39±0.0103^b	6.889±0.008^a	0.310±0.017^d	0.040±0.02^e	0.677±0.008^c
	Quercetin	4.041±0.066^b	5.004±0.005^a	0.493±0.002^d	0.049±0.01^e	0.780±0.011^c
TAC		0.0100±0.002^a	0.0063±0.002^c	0.0026±0.002^d	0.0013±0.01^e	0.0098±0.00^b

Superscripted values with the same letters in the columns were not significantly different ($p < 0.05$) according to Duncan's multiple comparison test.

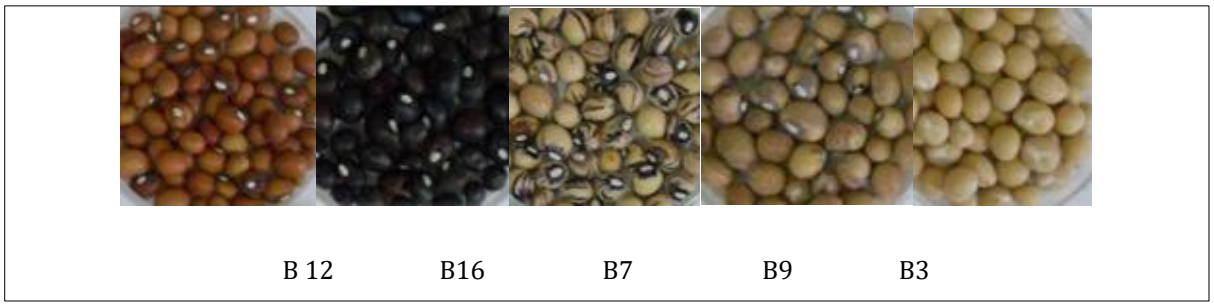

Figure 1 Different varieties of *Vigna subterranea* seeds

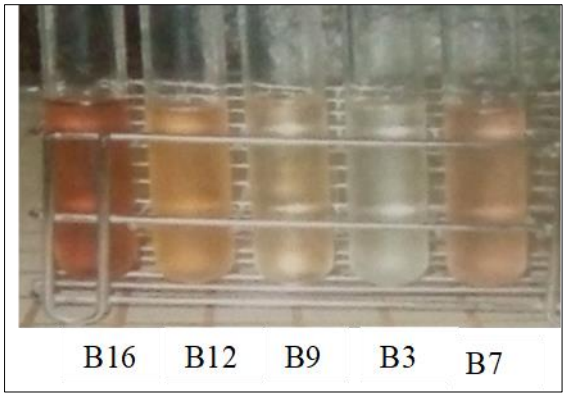

Figure 2 *Vigna subterranea* seed extracts

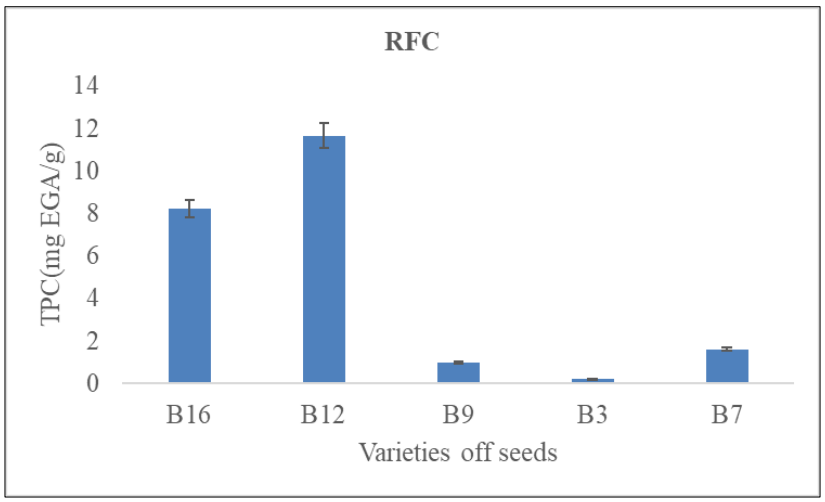

Figure 3 Total polyphenol content of five varieties of *Vigna subterranea* seeds

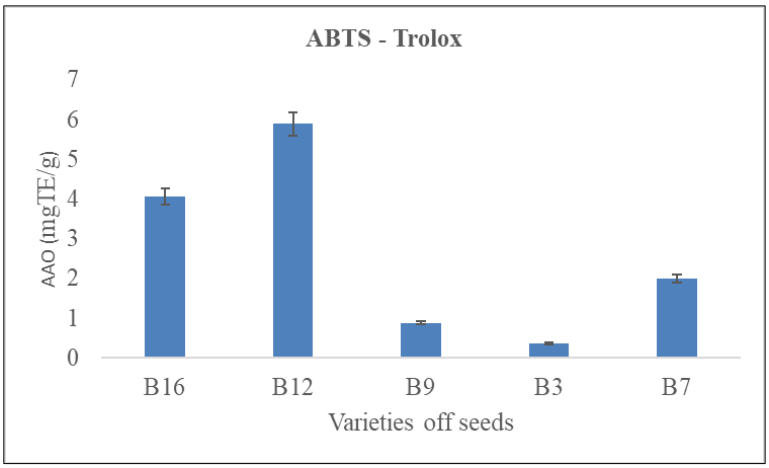

Figure 4 Total antioxidant content of five varieties of Vigna subterranea seeds (ABTS-Trolox)

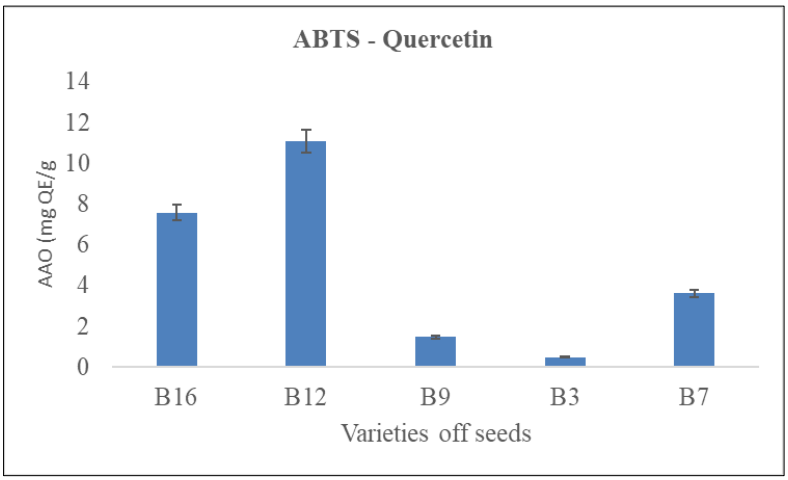

Figure 5 Total antioxidant content of five varieties of *Vigna subterranea* seeds (ABTS - Quercetin)

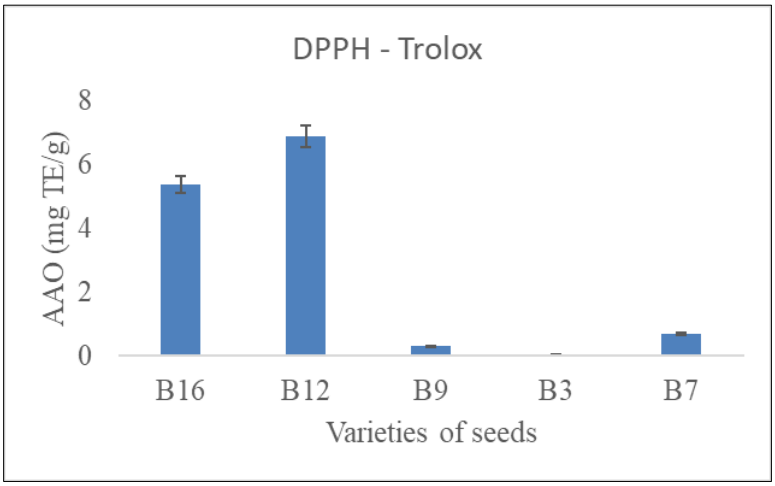

Figure 6 Total antioxidant content of five varieties of Vigna subterranea seeds (DPPH - Trolox)

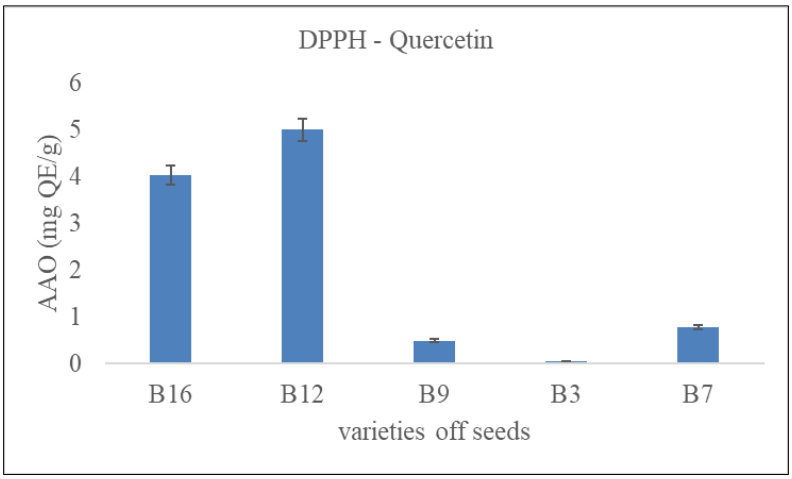

Figure 7 Total antioxidant content of five varieties of *Vigna subterranea* seeds (DPPH-Quercetin)

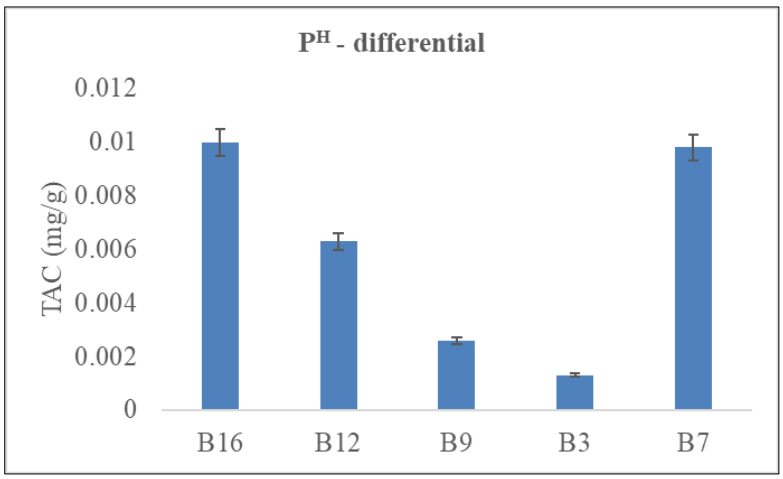

Figure 8 Total anthocyanin content of five varieties of *Vigna subterranea* seeds

In the DPPH method, with Trolox as standard, B12 seed extract (6.8893 mgTE/g) is richest in total antioxidants. Next comes B7 seed extract (0.6778 mgTE/g), followed by B9 seed extract (0.310 mgTE/g). According to the results (Table 3, Figure 7), B3 variety extract (0.0403 mgTE/g) is less rich in antioxidants. Burkina Faso varieties KVS97 (2.038 mgTE/g), KVS312 (2.006 mgTE/g), KVS67 (2.006 mgTE/g), KVS67 (1.839 mgTE/g) have lower antioxidant contents than Chad varieties B12 and B16, but higher than B7, B9 and B3[31].

Finally, using the same method and Quercetin as a reference, the B12 seed extract is consistently higher in total antioxidants, at 5.004 mg QE/g (Table 3, Figure). This is followed by B16 seed extract at 4.041 mg QE/g, B7 at 0.7802 mg QE/g, B9 at 0.4933 mg QE/g and B3 at 0.049 mg QE/g /g. The ranking of the total antioxidant content of these extracts in decreasing order is the same as above: B3< B9<B7<B16<B12.

Many scientific works contribute to show that the antioxidant activity of plant extracts is largely due to the presence of polyphenols [24]. The good correlation (R = 0.9825) between total polyphenol and total antioxidant contents evaluated by the two methods ABTS and DPPH confirmed the data in the literature [24]. This correlation means that the antioxidant activity of the extracts mostly depends of the total polyphenol content. Polyphenols contributed 98.25% to the antioxidant activity of *Vigna subterranea* extracts.

3.3. Total anthocyanin content (TAC)

A well-known subgroup of polyphenols is anthocyanins, characterized by their red color in acidic media and blue color in basic media [28], illustrating the effect of pericarp color on polyphenol levels and consequently on total antioxidant levels. The total anthocyanin contents assessed are very low in the extracts of the *Vigna subterranea* varieties studied. Their content is certainly linked to the color of the pericarp, some being more colorful than others. Thus, the three varieties distinguished by the purple or black color of their pericarp have the highest levels of total anthocyanins (Table 3, Figure 8). These are B16, B7 and B12, with contents of 0.100, 0.0098 and 0.063mg/g of dry seeds respectively. Varieties B16, B7 and B12 have lower total anthocyanin levels than KVS97 (0.331 mg/g) from Burkina Faso [24].

4. Conclusion

The study carried out on the different *Vigna subterranea* seed extracts showed that these extracts contain phenolic compounds. This study also revealed that these different seed extracts do not have the same phenolic compound content. Thus, the results obtained showed that seed extracts from colored varieties B12 (11.6476 mg GAE/g), B16 (8.2348 mg GAE/g), B7 (1.6258 mg GAE/g) are richer in total polyphenols. The total anthocyanin content of seed extracts is low. The three colored seed varieties B16 (0.10 mg/g), B7 (0.0098 mg/g), B12 (0.063 mg/g) have the highest total anthocyanin content. Results for antioxidant power revealed that the B12 seed extract was the richest in antioxidants. This was followed by B16 extract, B7 extract, B9 extract and B3 extract. With either method using Trolox or Quercetin as standards, the R coefficient is quite high (around 0.9825). This shows that antioxidant activity is a function of total polyphenols in *Vigna subterranea* seed varieties.

Compliance with ethical standards

Acknowledgments

The authors thank the University of N'Djaména and the Chadian Institute of Agricultural Research for Development for their efforts in conducting this study.

Disclosure of conflict of interest

The authors declare no conflicts of interest.

Declaration of Funding

This research did not receive any specific funding.

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