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Determination of phenolic compound contents in fruits of *Momordica charantia* L. (Cucurbitaceae) from Chad

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ABSTRACT

Momordica charantia L. (cucurbitaceae), is a plant whose fruits are widely consumed in Chad. Seeds, green and ripe pulps of the fruits of this plant were subject of this study. The total polyphenols, antioxidants, flavonoids and anthocyanins were evaluated by the Folin - Ciocalteu, DPPH, iron trichloride and pH-differential methods respectively. The total polyphenol obtained were 9.368 mg GAE/g for the seeds extract, 9.068 mg GAE/g for the green pulp extract and 8.505 mg GAE/g for the mature pulp extract. The total antioxidant were 2.351, 2.074 and 1.365 mg QE/g for seeds, green pulp and ripe pulp respectively. The total flavonoid was 0.818 mg QE/g for seeds, 0.812 mg QE/g for green pulp and 0.808 mg EQ/g for ripe pulp. The total anthocyanin for seed, green and ripe pulp extracts were 0.360, 0.318 and 0.238 mg/g, respectively. The results of the polyphenol assays of the samples after traditional culinary transformed, show that the thermal transformation has a negative impact which is characterized by a decrease in the polyphenol contents. Percentages of total polyphenols after culinary transformation were 11.28; 8.84 and 8.26% respectively for the extracts of green pulp, seeds and ripe pulp.

Keywords: *Momordica charantia* L., total polyphenols, total antioxidants, total flavonoids, total anthocyanins, Chad.

INTRODUCTION

Momordica charantia L., commonly known as margosa, bitter melon, bitter cucumber or *Momordica* is a creeping or climbing vegetable plant on any support (Djoumoi, 2018). It is a plant of the Cucurbitaceae family, growing in hot or temperate climates. There are several species widespread in tropical or temperate regions, including Africa, Asia and southern America (Jean Michel, 2020). *Momordica charantia* L. is widely consumed and sometimes used as a medicinal plant.

The species commonly known as bitter melon or bitter cucumber, is very bitter and this bitterness is related to compounds contained in all parts of the plant but these different parts do not have the same chemical compositions (Laleye et al., 2015).

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Previous studies have shown that *Momordica charantia* L. is used as antimalarial, inflammatory, anticancer, antiparasitic, antioxidant. The leaves, fruits and roots of this plant are often used to induce abortion and/or birth control and alleviate painful menstruation or facilitate childbirth (Djoumoi, 2018; Obafèmi et al., 2015). These biological activities mentioned above can be controlled by phenolic compounds.

Polyphenols form a very diverse group of compounds. They differ from each other by the number and position of the hydroxyl functions as well as by the presence of non-phenolic substituents of various natures (Zahra et al., 2021). They have various effects on plant physiology due to their antibacterial and antifungal actions (Laurent et François, 2011 and Hafid et al., 2022). They participate in the pigmentation of flowers, vegetables and some fruits (grapes, citrus fruits). Some of them are responsible for bitterness and astringency (George et al., 2013 ; Palé et al., 2010). They absorb in the ultraviolet and/or visible light (Faustin et al., 2019).

Phenolic compounds can be found in various organs of a plant at very variable levels. They can be found in leaves, fruits (Launay, 2017), calyxes (Bindhu and Jayaraj, 2020), stems, seeds, roots, tubers, bulbs (Sambe et al., 2017 ; Lamia et al., 2016; Guy et al., 2021). Throughout the bibliography, countless polyphenols identified by appropriate study techniques, are divided into several classes, the main ones being: phenolic acids, tannins and flavonoids. In this last group, anthocyanins are responsible for the red color of some fruits and vegetables

Previous studies also showed that this plant contains the amino acids, fatty acids, glycosides, alkaloids, triterpenes, proteins (Raghavan et al., 2015; Perera et al., 2021; Nadirah, 2020). *Momordica charantia* seeds and leaves contain ascorbic acid, iron and are rich in phenolic compounds. The leaves contain magnesium, manganese, iron, copper, vitamin A and vitamin E (Jean Michel, 2020). The phytochemical study also reveals the presence of tannins, flavonoids, saponosides, potassium, sodium, calcium and zinc (Séverin et al., 2018).

Momordica charantia L. is a widespread in Chad whose fruits are widely consumed. Therefore, it is important that a phytochemical study be done on these fruits to determine its secondary metabolite composition. The hypothesis on which this study is based stipulates that the fruits of *Momordica charantia* L. from Chad contain phenolic compounds. The aim of this study is to valorize the fruits of *Momordica charantia* L. as a source of antioxidants.

MATERIALS AND METHODS

Plant material

The study focuses on *Momordica charantia* L. fruits harvested in October 2020 in N'Djamena and identified in Chadian Institute of Agronomic Research for Development (CIARD). The fruit parts submitted to this study are seeds, green pulp and ripe pulp.

Extraction of phenolic compounds from pulps and raw seeds

Seeds, green pulps and dry mature pulps of *Momordica charantia* L. were ground in a mortar. Three (3) grams of powder of each sample were weighed and put in 20 mL of methanol - water - acetic acid system (70 : 29.5 : 0.5) for 24 h then filtered. The residues are extracted twice with 15 mL in the same solvent system for 24 h, then filtered. The filtrates are stored in the refrigerator for subsequent determination of polyphenols and measurement of antioxidant activity (Hacer EK and Özgül UH. 2019).

Extraction of phenolic compounds after cooking

To extract phenolic compounds in *Momordica charantia* L. pulps and seeds that have undergone culinary processing in order to evaluate their antiradical activities, we proceeded as follows: five (5) grams of pulps and seeds are cooked with 100 mL of a potash solution at pH 8 for 1h 30 min and then crushed into a paste. Three (3) grams of paste from each sample is subjected to maceration for 24 h at

4°C in 20 mL of methanol - water - acetic acid mixture (70: 29.5: 0.5). After filtration, the residues were extracted twice with 10 mL of same solvent for 24 hours at 4 °C (Abel, 2015).

Determination of total polyphenols

The phenolic compound contents of *Momordica charantia* L. were determined by the Folin - Ciocalteu method (Andzi and Feuya, 2014). 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.

Measurement of antioxidant

To evaluate the antioxidant activities, we used the 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 QE/g dry material (Natividad et al., 2020).

Determination of total flavonoids

The flavonoid contents of the extracts are estimated by the aluminium trichloride ($AlCl_3$) method. It is based on the formation of a flavonoid - aluminium complex. The quantification of flavonoids was done using a calibration curve performed by a standard (quercetin) at different concentrations under the same conditions as the sample (Table 1). Results are expressed as mg quercetin equivalent per gram (mg QE/g) of dry material (Habouche et Mimoune Somia, 2019).

4 ml of 2% aluminum trichloride ($AlCl_3$) is added to 4 ml of the sample. The mixture is allowed to react for 15 min at room temperature and protected from light. Total flavonoids are measured at 430 nm. All experiments were performed in triplicate (Habouche Hadjer & Mimoune, 2019).

Determination of total anthocyanins

The total anthocyanin contents of the extracts are 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 min later. The 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 was calculated as cyanidin-3-glucoside $\frac{mg}{L} = (\frac{A \times MW \times DF \times 1000}{\varepsilon \times l})$ (Eliseu et al., 2011) where A: absorbance; MW: molecular weight; (449.2); DF: dilution factor; ε: molar absorptivity (26900). Total anthocyanin contents are expressed as micrograms of cyanidin-3-glucoside per gram of dry material.

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 (Athamena et al., 2010).

Table 1: Establishment of the curves – standards.

Calibration curves	Standard	Equations	Correlation coefficients
RFC	Gallic Acid	Y = 36.233x + 0.2911	$R^2 = 0.9956$
DPPH	Quercetin	Y = -25.45x + 0.670	$R^2 = 0.9955$
TFC		Y = 14.185x + 0.4992	$R^2 = 0.9883$

RESULTS

Quantitative analysis

The results of total polyphenols, total flavonoids, total anthocyanins and total antioxidants contents are shown in Table 2.

Total polyphenol contents range from 8.505 for ripe pulp to 9.368 mg GAE/g for seeds. And the contents of total flavonoids go from 0,808 for the ripe pulps to 0,818 mg EQ/g for the seeds, then the contents of total anthocyanins from 0,238 for the ripe pulps to 0,360 mg/g for the seeds. Total antioxidant levels ranged from 1.365 for ripe pulp to 2.351 mg EQ/g for seeds.

The results in Table 2 were used to draw the histograms in the figures (Figures 1, 2, 3 and 4). The results in Table 2 were also used to plot the correlation between total polyphenol and total antioxidant levels (Figure 5).

Table 3 shows the results of the percentage reduction of total polyphenols under the culinary conditions. For culinary processing, the reduction rates of total polyphenols are 8.26%, 8.84% and 11.28% for ripe pulp, seed and green pulp respectively.

Table 2: Total polyphenols contents (TPC), total flavonoids contents (TFC), total anthocyanins contents (TAC) and total antioxidants (TAO) of extracts.

Study parts	TPC (mg EGA/g)	TFC (mg EQ/g)	TAC (mg/g)	TAO (mg EQ/g)
	RFC	**$AlCl_3$**	**pH - diff**	**DPPH**
Green pulp	9.068 ± 0.002^b	0.812 ± 0.0013^a	0.318 ± 0.0026^b	2.074 ± 0.0013^b
Seeds	9.368 ± 0.003^a	0.818 ± 0.002^a	0.360 ± 0.0066^a	2.351 ± 0.0013^a
Mature pulp	8.505 ± 0.0007^c	0.808 ± 0.0013^b	0.238 ± 0.0013^c	1.365 ± 0.0007^c

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

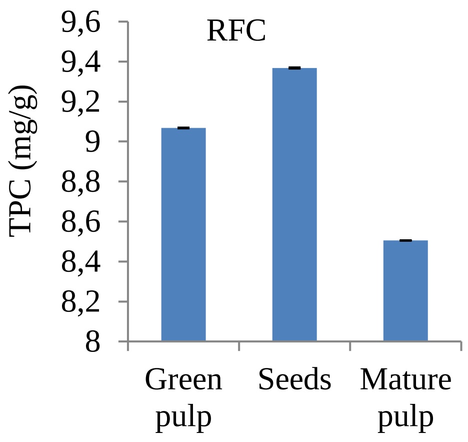

Figure 1 : Total polyphenol contents of different fruit parts.

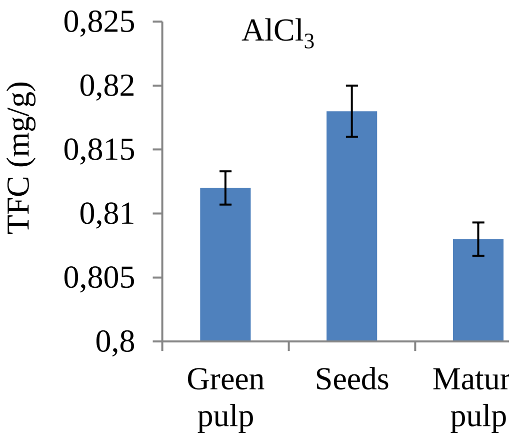

Figure 2 : Total flavonoids contents of the different parts of the fruits.

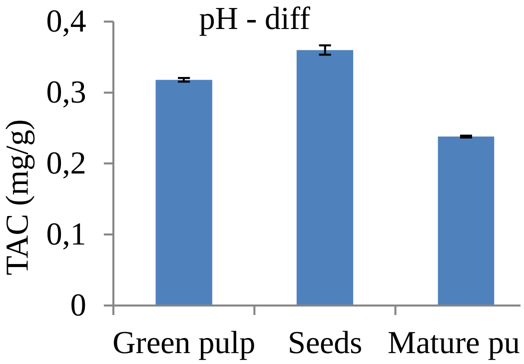

Figure 3 : Total antioxidant contents of the different parts of the fruits.

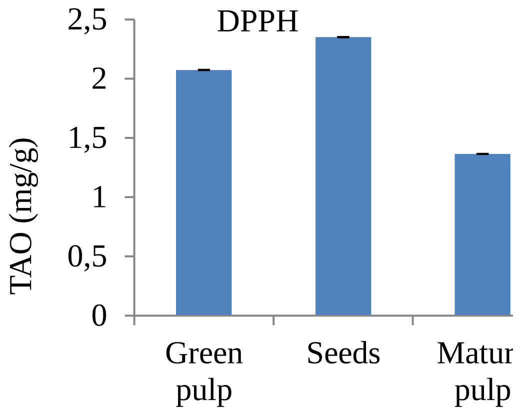

Figure 4: Total antioxidant contents of the different parts of the fruits.

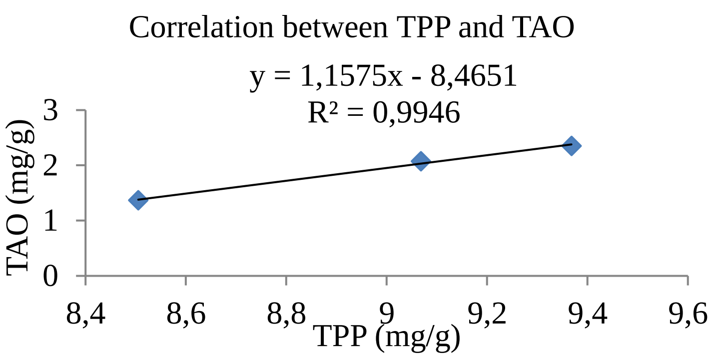

Figure 5: Correlation between total polyphenol and total antioxidant contents.

Table 3: Polyphenol contents and their degradation rates after cooking.

	TPC (mg EGA/ g)		
Parts studied	**Raw sample**	**Cooked sample**	**% reduction TPC**
Green pulp	9.068 ± 0.002[b]	8.045 ± 0.002[b]	11.28
Seeds	9.368 ± 0.003[a]	8.539 ± 0.0013[a]	8.84
Mature pulp	8.505 ± 0.0007[c]	7.849 ± 0.0006[c]	8.26

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

DISCUSSION

Total polyphenol contents

The folin method show that (table 2) the seed extract is richest in total polyphenols (9.368 mg GAE/g). It is followed by the green pulp extract with a content of 9.068 mg GAE /g. The least rich is the extract of ripe pulp (8.505 mg GAE/g). The total polyphenol contents of these extracts obtained are higher than those found by Yan et al. (2020) on the methanolic extracts of *Momordica charantia* L. (5.00 mg GAE/g to 6.00 mg GAE/g) in China. However, these total polyphenol contents are lower than those obtained by Balde et al. (2020) in pulp extracts: 19.1 mg GAE/g and 10.4 mg GAE/g respectively for the two batches of fruits from Senegal (Dakar and Fatick).

Total flavonoid content (TFC)

The total flavonoid contents measured by aluminum trichloride method are recorded in Table 2. *Momordica charantia* L. seed extract is richest in total flavonoids (0.818 mg QE/g). It is followed by the extracts of green and ripe pulps respectively (0.812 mg QE/g and 0.808 mg QE/g). These results corroborate with those of Johnson et al. (2016) who revealed the presence of flavonoids in *Momordica charantia* L. from Cotonou in Benin and also to those of Djoumoi (2018) in Madagascar proving the excessive presence of flavonoids. Flavonoids are recognized mainly for their anti-oxidant, anti-inflammatory and anti-diabetic actions (Djoumoi, 2018).

Total anthocyanin content (TAC)

The total anthocyanin extract richest content is that of seed, 0.360 mg/g. It is followed by the green pulp extract (0.318 mg/g) and the mature pulp extract (0.238 mg/g) respectively (Table 2, Figure 3). The work of Johnson et al. (2016), did not reveal the presence of anthocyanins in the leaves of *Momordica charantia* L. from Cotonou, Benin. Anthocyanins play a crucial role in the socio-economic life of humans. Through their antioxidant properties, anthocyanosides behave as free radical scavengers in the body (Badiaga, 2011).

Total antioxidant (TAO)

The total antioxidant contents of the extracts were determined by the DPPH method. The results are expressed as milligram of quercetin equivalent per gram (mg QE/g) of dry material. From these results, the extract of *Momordica charantia* L. seeds is the richest in antioxidants with a content of 2.351 mg QE/g. The extracts of green and ripe pulp had total antioxidant contents of 2.074 mg QE/g and 1.365 mg QE/g of dry material, respectively (Table 2, Figure 4). The work of Djoumoi (2016), gave the total antioxidant content of 1512.54 µg/mL or 1.512 mg EQ/g in *Momordica charantia* leaves. This value found by Djoumoi (2016) is lower than that of this study. This proves that *Momordica charantia* seeds are richer in antioxidants than the leaves and pulp.

Many scientific works contribute to show that the antioxidant activity of plant extracts is largely due to the presence of polyphenols (Abel et al., 2013). The good correlation (R = 0.994) between total polyphenol and total antioxidant contents evaluated by the DPPH method confirmed the data in the literature (Figure 3) (Abel et al., 2013). This correlation means that the antioxidant activity of the extracts mostly depends of the total polyphenol content. Polyphenols contributed 99.4% to the antioxidant activity of *Momordica charantia* L. extracts (Abel et al., 2013).

Culinary transformation

The Folin-Ciocalteu method, which is also one of the reference methods for evaluating antioxidants, was used to evaluate the antioxidant activity of *Momordica charantia* L. extracts that had undergone culinary processing.

Table 3 shows that there is an effective decrease of total polyphenols in the different parts of the fruits after culinary processing. The total polyphenol contents vary from 7.849 for the extract of ripe pulp to 8.539 mg GAE/g dry material for the extract of seeds. Compared to the extracts of the raw samples, there is a reduction of 11.28% for the green pulp extract, 8.84% for the seed extract and 8.26% for the ripe pulp extract.

The results obtained indicate that thermal processing has an impact on the total polyphenol content of the samples studied. This impact is negative on all samples. The percentages of decrease vary from one sample to another and can be explained by the nature and content of the different phenolic compounds specific to each sample.

Conclusion

The study conducted on *Momordica charantia* L. fruits showed that the extracts of seeds, green and ripe pulps contain phenolic compounds. This study also revealed that seeds and pulps do not have the same phenolic compound contents. Thus, the results obtained revealed that the seed extracts are richer in phenolic compounds than the pulp extracts. It also appears from this study that the seed extract is richer in antioxidants (2.351 mg EQ/g) than the green and ripe pulp extracts, with total antioxidant contents of 2.074 mg EQ/g and 1.365 mg EQ/g of dry material, respectively. The thermal transformation has an impact on the total polyphenol content of the studied samples. This impact is negative on all samples. The percentages of decrease vary from one sample to another. We note a decrease of 11.28%, 8.84% and 8.26% respectively for green pulp, seed and ripe pulp extracts. The nature of the phenolic compounds of the fruits of *Momordica charantia* L. from Chad remains poorly known and we intend in our future investigations to carry out fractionations and elucidate the structures of these phenolic compounds.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

All authors contributed to this work and to the manuscript preparation.

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