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Optimization of the extraction of phenolic compounds by decoction on the leaves and stems of Momordica charantia

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Abstract
The optimization of the extraction of phenolic compounds by decoction on the leaves and stems of *M. Charantia* was the subject of this study. Thus, a quantification of total polyphenols and flavonoids, as well as the evaluation of the color index and the pH were carried out. The total polyphenol content was determined using the folin-ciocalteu reagent and that of flavonoids using the aluminum trichloride method. The results obtained indicate that the polyphenol and flavonoid contents are higher in the leaves (PPT: 0.252 g EAG/100 g, FT: 0.098 g EC/100 g) than in the stems (PPT: 0.053 g EAG/100 g, FT: 0.015g EC/100g). The comparison of the content of the different extracts showed that the leaves and stems of the Fatick region are richer in phenolic compounds than that of Dakar. However, the optimal levels of polyphenols are obtained after 2 h of extraction for the stems of Dakar and 2 h 20 minutes for the stems of Fatick with respective concentrations of 0.024 and 0.037gEAG/100g of extract, whereas the duration optimal for the extraction of flavonoids on the stems is 2 h.

Keywords: Décoction, optimization, polyphénols, flavonoids, extraction, duration

1. Introduction
Nowadays, people are increasingly interested in the bioactive substances of plants to fight against several diseases. Thus, these bioactive substances constitute multiple interests, particularly in the cosmetic, pharmaceutical and agri-food industries (El-Haci *et al*., 2012) [1]. The numerous health-related properties of these compounds, widely described in epidemiological studies, are mainly based on their antioxidant activities (Pinelo *et al*., 2005) [2]. Among these plants we have Momordica charantia, commonly known as bitter melon or bitter gourd. It is a creeping, annual species belonging to the Cucurbitaceae family. Studies have revealed that it exhibits antioxidant, antiparasitic, antibacterial and antidiabetic activities (Rammal *et al*., 2009) [3]. Indeed, the therapeutic value of M. charantia is due to the presence of several natural secondary metabolites in the different organs of the plant. Indeed, the latter are generally phenolic acids, flavonoids and tannins, which constitute an omnipresent family in the plant kingdom. These phenolic compounds can be easily isolated from plant tissue by extraction with organic solvents (Gani & Shama, 2021) [4]. But the extraction procedure is influenced by several parameters such as: the chemical nature of these compounds, the extraction method used, and the extraction time (Naczk & Shahidi, 2004) [5]. According to some authors, time is an important parameter to optimize in order to differentiate extraction methods and reduce energy expenditure (Spigno *et al*., 2007) [6]. It is in this context that the objective of this work is to optimize the extraction of phenolic compounds on the leaves and stem of M. charantia by decoction.

2. Material and Methods
2.1 Plant material
The plant material used in this study consists of fresh leaves and stems of M. charantia. These samples were collected in October 2022 in two geographically different regions of Senegal including Dakar in the Niayes area (14°43'10" North and 17°28'21" West) and Fatick (14°22 North and 16°08 West). These samples are shown in the figure below.

Fig 1: Organs used: (a) Leaves and stems of Fatick and (b) Leaves and stems

2.2. Extraction methodology

2.2.1 Dosage of phenolic compounds

- **Principle**

Total polyphenols were determined spectrophotometrically using the Folin-Ciocalteu method. It consists of oxidizing the oxidizable groups of phenols in a basic medium (Georgé *et al*., 2005). The Folin-Ciocalteu reagent is a yellow acid made up of a mixture of two acids: phosphotungstic acid (H3PW12O40) and phosphomolybdic acid (H3PMo12O40). It is reduced during the oxidation of phenols to form a stable blue complex of tungsten and molybdenum oxides. The coloring produced, whose maximum absorption is around 760 nm, is proportional to the quantity of phenolic compounds present in the plant extracts. The quantification of total polyphenols was carried out using a solution of gallic acid taken as reference polyphenols.

- **Operating mode**

To do this, 50 µl of extract are measured using the Folin-Ciocalteu reagent according to the method developed by Georgé *et al*, 2005. In practice, the method is carried out as follows:

1. Place 50 µl of the extract in a screw tube.
2. Add 450 µl of distilled water.
3. Add 2.5 ml of Folin-Ciocalteu reagent diluted 1/10.
4. Vortex and incubate for 2 min at room temperature.
5. Add 2.5 ml of a 75 g/l sodium carbonate solution.
6. Vortex and incubate at 50°C in a water bath for 15 min.

The absorbance of the polyphenols contained in the sample was measured at 760 nm against distilled water with an 'Agilent Technologies' brand spectrophotometer manufactured in New York. The concentration of total polyphenols is given by the following formulas:

- **Liquid products**

$$C_P = \frac{(A - b)}{a} * Fd$$

- **Solid products**

$$C_P = \frac{(A - b)}{a} * fd * \frac{10}{1000} * \frac{100}{m}$$

Cp: Total polyphenol content expressed in g gallic acid equivalent/L.
A: Absorbance of the sample; a: Direction coefficient of the calibration line = 3.12.
b: Y-intercept of the calibration line = 0.0696; Fd: Dilution factor.

2.2.2 Dosage of flavonoids

- **Principle**

The determination of total flavonoids is based on a colorimetric test using aluminum trichloride AlCl3 with which they form stable acid complexes either with the carbonyl (C=O) in the C-4 position or with the hydroxyl group at C-3 or C-5 of flavones and flavanols (Kim *et al*., 2003). Furthermore, AlCl3 can also form labile acid complexes with the orthodihydroxyl groups possibly present on the A and/or B core of the flavonoids. The absorbances are read at 510 nm. The results are expressed in g catechin equivalent per 100g of product.

- **Protocol**

1. Take 2 ml of extract.
2. Add 400 µl of distilled water.
3. Add 30 µl of a 5% NaNO2 sodium nitrite solution.
4. Vortex and incubate for 5 min at room temperature.
5. Add 20 µl of a 10% AlCl3 solution.
6. Add 200 µl of 1 M Na2CO3 solution.
7. Vortex and incubate for 5 min room temperature.

The absorbance reading was carried out at 510 nm against distilled water and the results are expressed in milligram catechin equivalent (mg EC) per gram of dry matter according to the following formula:

$$C_P = \frac{A * Pm}{\varepsilon} * fd * \frac{v}{1000} * \frac{100}{m}$$

C: Total flavonoid content expressed in g catechin equivalent/100 g.
A: Absorbance of the sample; Pm: Molar mass of catechin = 290.26 g/mol.
ε: Molar extinction coefficient = 10,332 l/mol. Cm; Fd: Dilution factor.
v: Extraction volume (ml); m: Test portion (g).

2.3 Color determination

The color of the extracts obtained was measured using a colorimeter (type: KONICA MINOLTA. Japan) based on the CIELAB color system (L*, a*, b* and L*, C*, h, YI). The color parameters (L*, a*, b* and L*, C*, h, YI) were measured 3 times for each sample. L*, a*, b* describe the colors black-white, Green-Red and Blue-Yellow respectively: L* (0 = Black, 100 = White); a* (- a = Green, + a = Red); b* (- b = Blue, + b = Yellow) (Figure 3).

Fig 2: Colorimeter (a) and Color Parameters (L*, a*, b*) (b)

2.4 Static analyzes

For statistical analysis of the data, Microsoft EXCEL and STATISTICAT software were used. These two software programs allowed better analysis and interpretation of the results in order to make a good comparison between the samples on the one hand and on the other hand between the sampling areas of these samples.

3. Results and discussions

3.1 Total polyphenol content of leaves and stems

- **At leaf level**

The results illustrated in Figure 3 present the polyphenol content of leaf extracts from Fatick and Dakar. Thus, the evolution of the polyphenol content of the extracts shows an increase in the concentration of total polyphenols as a function of the decoction duration. For an extraction time of between 1 h to 3 h 20 min, the polyphenol concentration varies from 0.075 to 0.25 g EAG/100 g of extract for Dakar leaves and from 0.091 to 0.286 g EAG/100 g of extract. extract for Fatick leaves. The quantity of polyphenols obtained on the Dakar leaves after 3 h 20 min of extraction (0.25 g EAG/100 g of extract) is 3 times greater than the value recorded in 1 h (0.075 g EAG of extract) . The same observation is made on the Fatick sheets.

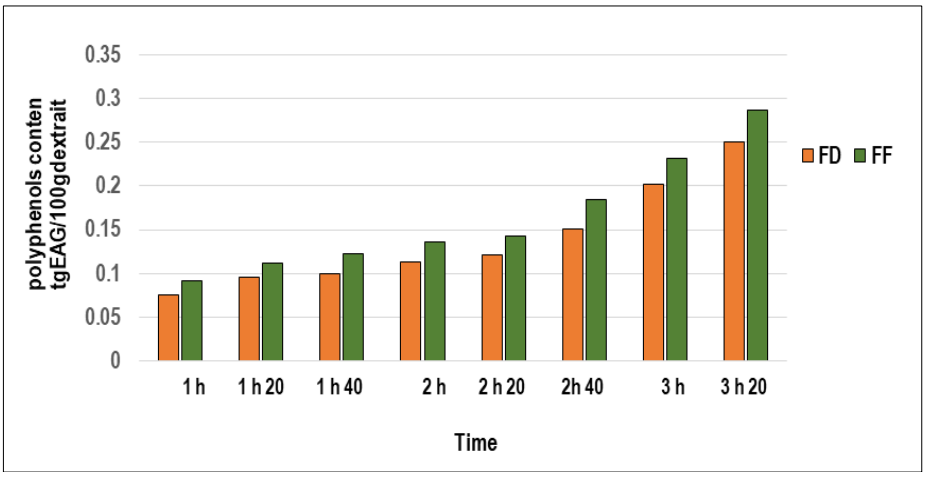

Fig 3: Evolution of the polyphenol content of the leaves depending on the extraction duration

These values also reveal that leaf extracts from Fatick contain more polyphenols than those from Dakar. This difference in concentrations between samples could be linked to pedoclimatic conditions (temperature, humidity and soil). According to some authors, the quantity of polyphenols in plants varies considerably depending on different intrinsic and extrinsic factors, such as plant genetics, soil composition and phenological stages. Studies have reported that the content of secondary metabolites in a plant's tissues varies depending on the availability of soil nutrient resources and the physiological state of the plant. Furthermore, the production of pro-anthocyanidins increases when the phosphorus content decreases. However, phenolic compounds are synthesized en masse when the plant grows on soil poor in iron.

- **At the level of the stems**

The results in Figure 4 reveal an increase in the polyphenol content between 1 h and 2 h 20 minutes, it goes from 0.0078 to 0.025 g EAG/100 g of extract for the Dakar stem and from 0.012 to 0.037 g EAG /100 g of extract for that of Fatick. The values show that the maximum extraction of polyphenols is recorded at 2 h for the Dakar stems (0.025 g EAG/100 g of extract) and 2 h 20 min for that of Fatick (0.037 g EAG/100 g of extract). However, after 2 h 20 min of extraction, a drop in the polyphenol concentration was noted for both stems. This concentration of polyphenols decreases to 0.00013g EAG/100 g for the extracts from Fatick and 0.0018 g EAG/100 g of extract for those from Dakar after 3 h 20 min of extraction.

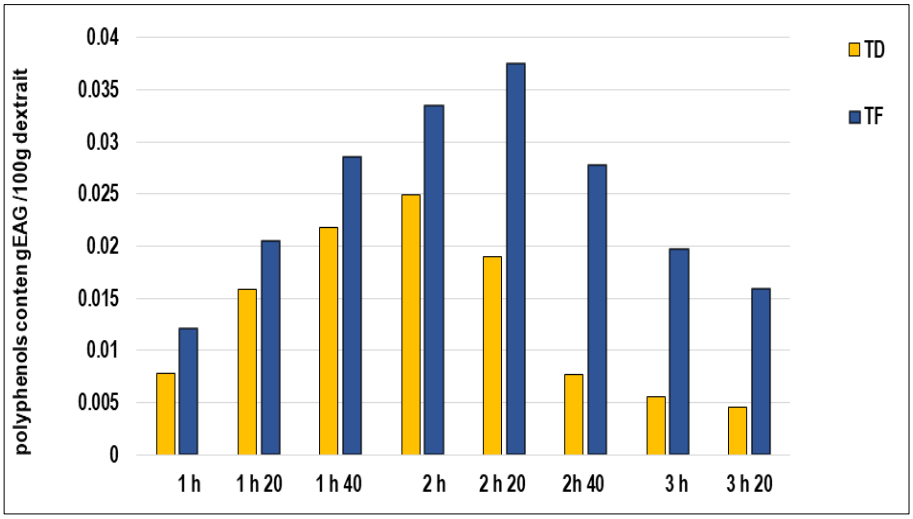

Fig 4: Evolution of the concentration of polyphenols on the Dakar and Fatick stem

These results can be explained by the extension of the extraction time which improves the solubility of the polyphenols. On the other hand, prolonged extraction can induce undesirable reactions such as enzymatic degradation and oxidation of phenolic compounds, resulting in a reduction in the polyphenol content (Stévigny *et al*., 2007) [10]. These results agree with those found by Guédel *et al*, (2022) on Combretum micranthum. They reported that the total polyphenol contents of aqueous leaf extracts by decoction increase with time and that the maximum value is obtained at 70 min, while a decrease is observed at 80 min (Faye *et al*., 2022) [11].

- **Comparison of total polyphenol contents on leaves and stems**

The results in Figure 5 show that the polyphenol content in the leaf extracts (0.286 EAG/100 g of extract) is higher than that obtained in the stem extracts. This content is approximately 4 times higher than that recorded at the stem level (0.0375 EAG/100 g of extract).

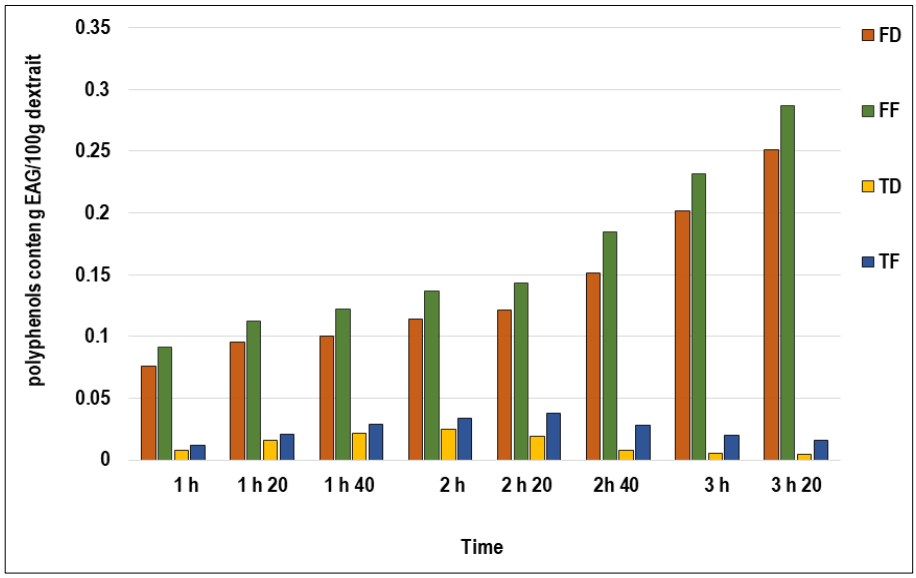

Fig 5: Comparison of polyphenol contents between leaves and stems

However, leaf and stem extracts from the Fatick region contain more polyphenols than those from Dakar. Our values match those obtained by Enneb *et al*, (2015) [12] on Lawsonia inermes L who found that the leaves have the greatest quantity of polyphenols followed by the stems (Enneb *et al*., 2015) [12]. The difference in polyphenol content between the two organs could be due to a heterogeneous distribution of polyphenols in the different organs during the biosynthesis process which is more important in the leaves than the stems (Rajaei *et al*., 2010) [13]. Although various organs and tissues are capable of synthesizing certain phenolic compounds, leaves are considered the privileged site of this biosynthesis with the presence of chloroplasts (Rajaei *et al*., 2010) [13]. Consequently, the latter are regularly supplied with initial compounds for the formation of polyphenols, energy (ATP) and reducing power (Rajaei *et al*., 2010) [13]. In addition, the analysis of these results shows that extending the extraction time allows greater extraction of total polyphenols. Indeed, the extraction time is determined solely by the quantity and chemical structure of the phenolic compounds in the sample (Chirinos *et al*., 2007) [14].

3.2. Total flavonoid content

3.2.1. Flavonoid concentration of leaves

The flavonoid assay results shown in Figure 6 indicate an increase in flavonoid content with time. It varies from 0.051 to 0.113 g EC/100 g of extract for Fatick leaves and from 0.034 to 0.098 g EC/100 g of extract for Dakar leaves

between 1 h and 3 h 20 min. however, the quantity of flavonoids (0.113 g EC/100 g of extract) obtained in 3 h 20 min is approximately 2 times higher than the value (0.03 g EC/100 g of extract) obtained in 1 h.

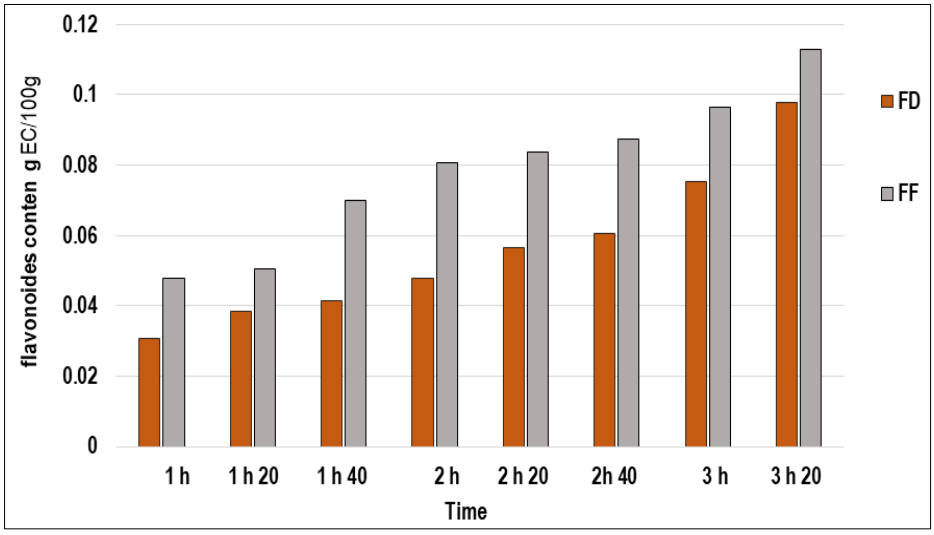

Fig 6: Evolution of the flavonoid content of leaves from Fatick and Dakar

Analysis of the results also reveals that leaf extracts from Fatick are richer in flavonoids than those from Dakar flavonoids. It is of the order of 0.113 g EC/100 g of extract for Fatick leaves and 0.098 g EC/100 g of extract for Dakar leaves. Furthermore, we note that the evolution of the concentration of flavonoids follows the same trend as that of total polyphenols.

- **Concentration of flavonoids on the stems**

The profile observed for flavonoid contents is almost the same as that of total polyphenols with some differences. The results of the evolution of the flavonoid content of the stem extracts presented in Figure 7 reveal a slight increase in the total flavonoid content of the different extracts as a function of the extraction duration. In fact, the evolution of the flavonoid content is significant between 1 h and 2 h, it is of the order of 0.01 to 0.016 g EC/100 g of extract for the Fatick stems and of 0.009 to 0.014 g EC/ 100 g of extract for those from Dakar. However, the maximum flavonoid content was obtained after 2 h for the stems of Fatick and Dakar with respective concentrations 0.016 and 0.014 g EC/100 g of extract, while the lowest content was revealed after 3 h 20 min of extraction. When the extraction time exceeds 2 h, a decrease in the quantity of flavonoids is noted.

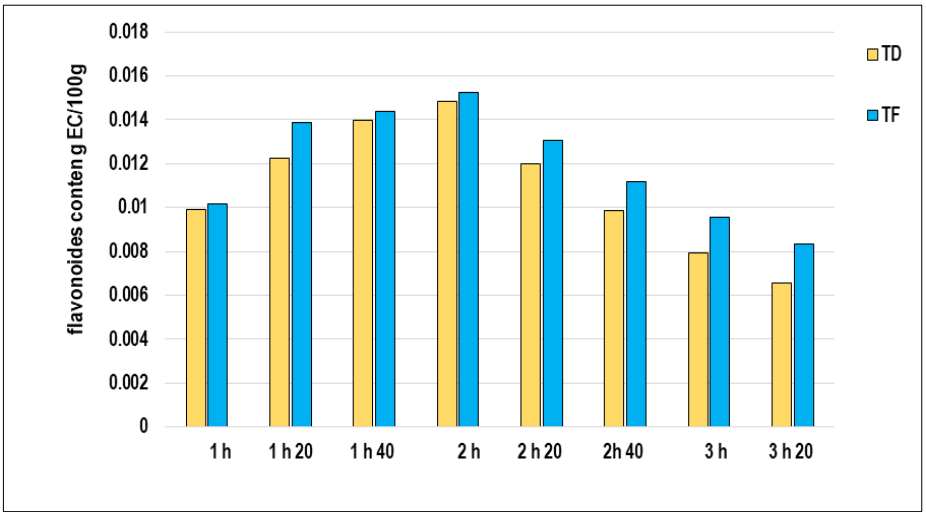

Fig 7: Evolution of the flavonoid content of Dakar and Fatick stem extracts

- **Comparison of flavonoid concentrations in leaves and stems**

The results reported in Figure 8 show that the samples from Fatick are richer in flavonoids than those from Dakar. Comparison of the flavonoid contents of the different extracts showed that the leaves contain more flavonoids than the stems, respectively 0.11 and 0.015 g EC/100 g of extract. These values obtained on the leaves and stems can be justified by the fact that the leaves are much more exposed to solar radiation than the stems. in fact, flavonoids are produced to ensure the protection of plant tissues against the harmful effects of solar radiation (Gehin *et al*., 2006) [15].

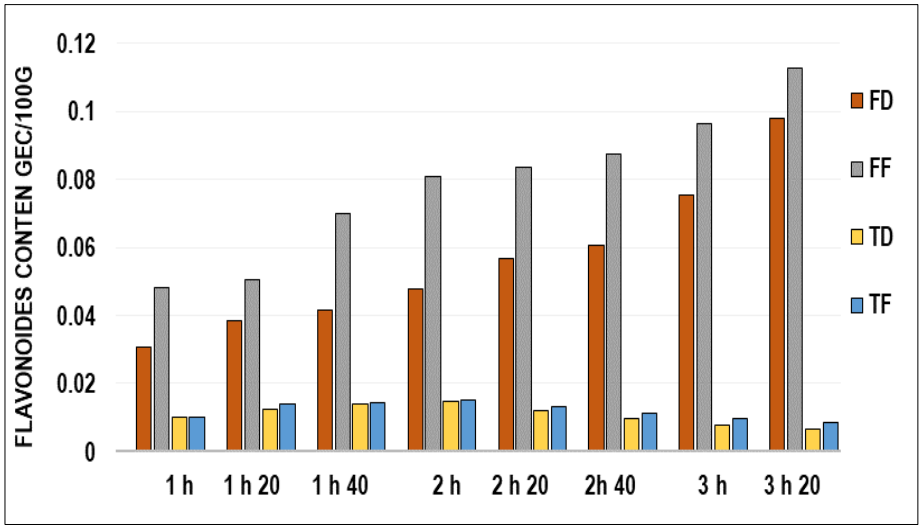

Fig 8: Comparison of flavonoid content in different organs

3.3. Color Settings

The results illustrated in Tables VIII and IX show a decrease in the clarity given by the color parameter (L*) and an increase in the intensity of the yellow coloration given by the color parameter (b*) as a function of the extraction duration. The results reveal that the clarity of stem extracts is higher than that of leaf extracts with a value varying from 99.92 to 99.45 for Fatick stems and from 95.12 to 82.44 for leaves. This was also observed in the extracts from Dakar. The results reveal a drop in color parameters (A) and (B) and an increase in clarity (L) depending on the decoction badges (Table 2-3). The color parameter (L) corresponds to clarity or brightness, following a psychometric scale ranging from zero to 100.

Table 1: Color parameters (a*, b*, L*) after leaf decoction

Ptres	Dakar leaves			Fatick leaves		
	L*	a*	b*	L*	a*	b*
1h	93,53± 0,02	-3,77±0,07	23,67±0,01	95,12±0,04	-3,11± 0,01	17,94±0,02
1h 20	92,27±0,01	-4,03±0,035	23,41±0,08	93,35± 0,01	-3,37±0,01	20,95±0,04
1h 40	92,86±0,01	-3,74±0,04	24,20± 0,00	91,9±0,00	-3,5±0,00	24,36±0,06
2h	91,07±0,03	-3,61±0,01	27,36± 0,03	91,09±0,00	-3,72±0,03	26,93±0,01
2h 20	90,6±0,00	-3,65±0,02	29,58±0,01	90,63±0,01	-3,85±0,01	30,11±0,03
2h 40	88,53±0,04	-3,63±0,011	36,22± 0,05	88,33±0,03	-3,64±0,01	36,08±0,00
3h	88,24±0,01	-3,91± 0,04	33,03± 0,01	82,44±0,08	-3,74±0,02	47,84±0,06

Fig 9: Decocted leaves of *M. charantia*

Table 2: Color parameters (a*, b*, l*) of the stems after decoction

Ptres	Dakar leaves			Fatick leaves		
	L*	a*	b*	L*	a*	b*
1h	99,83± 0,01	-1,04±0,02	6,38±0,013	99,92±0,016	-0,78±0,02	4,78± 0,02
1h 20	99,52± 0,01	-0,9±0,00	6,55±0,03	99,87±0,03	-0,81±0,03	4,84± 0,02
1h 40	98,39±0,03	-1,06±0,04	6,78±0,01	99,81±0,01	0,88±0,00	5,06± 0,008
2h	98,92±0,01	-1,23±0,03	8,51±0,05	99,75±0,05	-0,91±0,01	5,22± 0,04
2h 20	98,71± 0,04	-1,26±0,07	8,78±0,07	99,68±0,02	-0,84±0,06	5,11± 0,00
2h 40	98,52± 0,03	-1,34±0,05	8,85±0,04	99,56±0,015	-0,93±0,03	5,31± 0,014
3h	97,77± 0,03	-1,61±0,03	10,56±0,03	99,45±0,05	-0,96±0,04	5,49± 0,03

Fig 10: Decocted stems of *M. charantia*

4. Conclusion

This work focused on optimizing the extraction of phenolic compounds by decoction on the stems and leaves of Momordica charantia. The results obtained showed that the samples are rich in phenolic compounds. In fact, the leaves contain more phenolic compounds than the stems. However, the leaves and stems of Fatick contain more polyphenols and flavonoids than those of the Dakar region. Therefore, Momordica charantia constitutes a good source of phenolic compounds. The results showed that the optimal duration for the extraction of total polyphenols is 2 h and 3 h for flavonoids on the stems. However, after 3 h 20 min of extraction, an increase in the concentration of total polyphenols was still observed. It would be interesting to continue optimizing the extraction of these phenolic compounds on the leaves using other solvents such as ethanol, methanol and mixed solvents and to study these different biological activities as well as their toxicity.

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