International Journal of Innovation and Scientific Research
ISSN 2351-8014 Vol. 74 No. 2 Sep. 2024, pp. 269-278

http://www.ijisr.issr-journals.org/

Extraction and comparison of phenolic compounds obtained using three different methods on Momordica leaves and stems charantia linn

Papa Guedel FAYE[1-2], Samba BALDE[1], Claudine Stéphanie Cocote DOS REIS[1], Khadim Niane[1], Alioune Sow[3], Omar Ben Khatab Cissé[4], Omar TOURE[1-2], Nicolas Cyrille AYESSOU[1-2], and Mady CISSE[1-2]

[1]Cheikh Anta Diop University, Ecole Supérieure Polytechnique de Dakar, Department of Chemical Engineering and Applied Biology, Water, Energy, Environment and Industrial Processes Laboratory (LE3PI), BP 5080 Dakar Fann, Senegal

[2]Center for Studies on Food Safety and Functional Molecules (CESAM), Dakar, Senegal

[3]Gaston BERGER University, UFR Agronomic Sciences, Aquaculture and Agrifood Technologies, Saint Louis, Senegal

[4]Iba Der THIAM University, National Higher School of Agriculture, BP A296 Thiès, Senegal

ABSTRACT: The objective of this work is to evaluate the content of total polyphenols in the leaves and stem of *M. charantia.* Three extraction methods (decoction, infusion and maceration) were used for a period of 60 minutes with a sample every 20 minutes. The conductivity and the coloring were evaluated on these different extracts. The total phenolic content of the extracts was estimated by *Folin's* method. The color of the samples was measured based on the *CIELAB* color system using a colorimeter (*KONICA MINOLTA-Japan*). The results were processed with R software version 3.2.4 Revised (*2018-03-16, R-70336*) and *Minitab 18* software. The results showed that decoction is the best method for extracting total polyphenols with 1.69 gAG.100.g^{-1} of extract, followed by infusion and maceration respectively 0.61 gAG.100.g^{-1} and 0.32 gAG.100.g$^{-1.}$ The analysis of the color of the extracts reveals that the intensity of the yellow color is more accentuated in decoction. The comparison of the content of phenolic compounds in the leaves and stems showed that the leaves and stems of M. *charantia* from the Fatick region are richer in total polyphenols than of M. *charantia* from the Dakar region.

KEYWORDS: decoction, infusion, maceration, polyphenols, extraction, M. charantia.

1 INTRODUCTION

The plant kingdom constitutes a diverse source of edible materials but also of remedies [1]. According to the World Health Organization (WHO), approximately 80 % of the population in developing countries rely primarily on traditional medicinal plants for their primary health care [2]. Despite the remarkable progress in organic chemistry currently, more than 25% of drugs prescribed in industrialized countries have their origins directly or indirectly in plants [3]. These medicinal plants can also have nutritional uses by providing nutrients for the body's vitality and natural defenses. Among these plants, *Momordica charantia* is used for its traditional medicinal and food uses. It is a valuable plant used in Asia, Africa and South America for thousands of years. It is cultivated both for human food and for its traditional use in the treatment of various diseases. It is used as an antimalarial, against inflammations, particularly hemorrhoids [4], [5]. It reveals antioxidant, antiparasitic, antibacterial, anti- ulcerogenic and even antidiabetic activities because its consumption leads to a reduction in blood sugar levels [6]. Indeed, the therapeutic power of *M. charantia* is due to the presence of more than 100,000 natural bioactive compounds present in all organs of the plant called "secondary metabolites" [7]. Among these metabolites, we distinguish phenolic compounds, alkaloids and terpenoids. Furthermore the wide range of therapeutic properties of *M.charantia* is linked to the concentration of their phenolic compounds which are the most abundant molecules in plants [8]. Their concentration varies from one organ to another. They include many classes ranging from simple phenolic acids to complex flavonoids. Phenolic compounds constitute a wealth widely exploited by the food, cosmetic and pharmaceutical industries. The extraction of these secondary metabolites is a very important step in their isolation as well as their identification [9]. The food or therapeutic quality of an extract is linked to the effectiveness and selectivity of the extraction process used [9]. The decoction is the most used method, either in

the preparation of tea or in the treatment of patients without mastering the technique which gives the maximum phenols. It is in this context that this work aims to evaluate the phenolic compound content in the leaves and stem of *M. charantia* by different extraction methods. The total phenolic content of the extracts will be estimated by the ***Folin method***. The color of the samples will be measured based on the ***CIELAB color system, the*** **R** software version **3.2.4** Revised (**2018-03-16, R-70336**) and the **Minitab 18 software** will be used for data processing.

2 MATERIAL AND METHODS

2.1 MATERIAL

The plant material used (leaves and stems) was harvested in August 2022 during the fruiting period, in two 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) and are shown in **Figure 1.**

Fig. 1. Samples of respective leaves and stems (Fatick and Dakar)

2.2 METHODS

2.2.1 SEPARATION OF STEMS AND LEAVES

The stems and leaves were separated using a scissor and then the stems were cut into small pieces for ease of use.

Fig. 2. Separation of organs (stem and leaves): (a) Fatick and (b) Dakar

2.2.2 EXTRACTION METHODS

Three different extraction methods were used to extract the phenolic compounds:

2.2.2.1 EXTRACTION BY DECOCTION

Decoction is a method of extracting soluble compounds by introducing the leaves or stems of *M.charantia* into water constantly boiling at 100 °C at atmospheric pressure. The leaves and stems are first weighed (16 g ± 0.1 g). Then, a volume of 1000 ml is brought to a constant boil at 100 °C and then the 16 g of leaves or stems are introduced. A timer is started as soon as 16 g of leaves or stems are introduced. A sample is taken every 20 minutes up to 60 minutes at constant temperature (100 °C) for the analysis of the different parameters.

2.2.2.2 EXTRACTION BY INFUSION

Infusion is a method of extracting the active ingredients of *M.charantia* in an initially boiling liquid which is allowed to cool.

The same quantity (16 g) of leaves and stems was added to 1000 ml of boiling water (100 °C). The mixture is sealed, the stopwatch is started and left to cool. A sample is taken every 20 minutes up to 60 minutes.

2.2.2.3 EXTRACTION BY MACERATION

Maceration is the prolonged cold contact (25 °C) of the fresh leaves and stems of *M.charantia* in order to extract the soluble compounds.

A quantity of 16 g of the stems and leaves is macerated in a volume of 1000 ml of water at room temperature in the laboratory. The stopwatch is started and a volume is taken every 20 minutes and analyzed for up to 60 minutes and the remainder is kept at room temperature for a period of 72 hours.

2.2.3 DOSAGE OF PHENOLIC COMPOUNDS

The content of these total phenolic compounds in the extracts was estimated by the Folin - Ciocalteu method which is based on the reduction in an alkaline medium of the *phosphotungstic mixture. phosphomolybdic acid of* Folin 's reagent by the oxidizable groups of phenolic compounds. The results are expressed in grams of gallic acid equivalent per 100 g of extract (g AG/100 g) used as a standard, using the calibration curve.

2.2.4 COLOR DETERMINATION

The color of the samples of the products 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*) 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) [10].

2.2.5 CONDUCTIVITY MEASUREMENT

The conductivity is determined by a conductivity meter integrating pH measurement (Hanna instruments, Germany) at 25°C.

2.2.6 STATISTICAL ANALYZES

Statistical analyzes were performed using one-way ANOVA with **R** software version **3.2.4** Revised (***2018-03-16, R-70336) and Minitab*** software. **18**. The value X of each sample is assigned a superscript letter ($X^{(i)}$ where i = a, b, c). Samples with the same letter are not statistically different at the 5 % threshold.

3 RESULTS AND DISCUSSION

3.1 DETERMINATION OF TOTAL POLYPHENOL CONCENTRATION

The results obtained after 60 minutes of decoction of leaves and stems are reported in Figure 3.

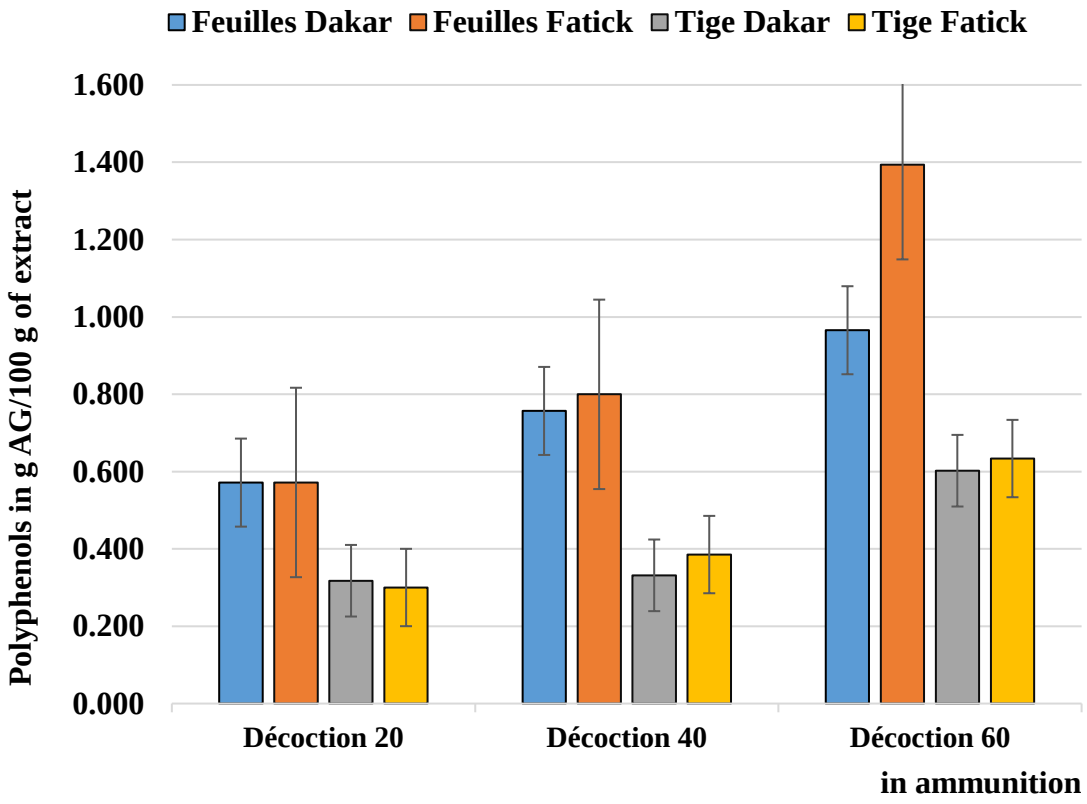

Fig. 3. Polyphenol content as a function of decoction time

The results of the polyphenol concentration on the leaf and stem extracts obtained after 60 min of infusion are presented in Figure 4.

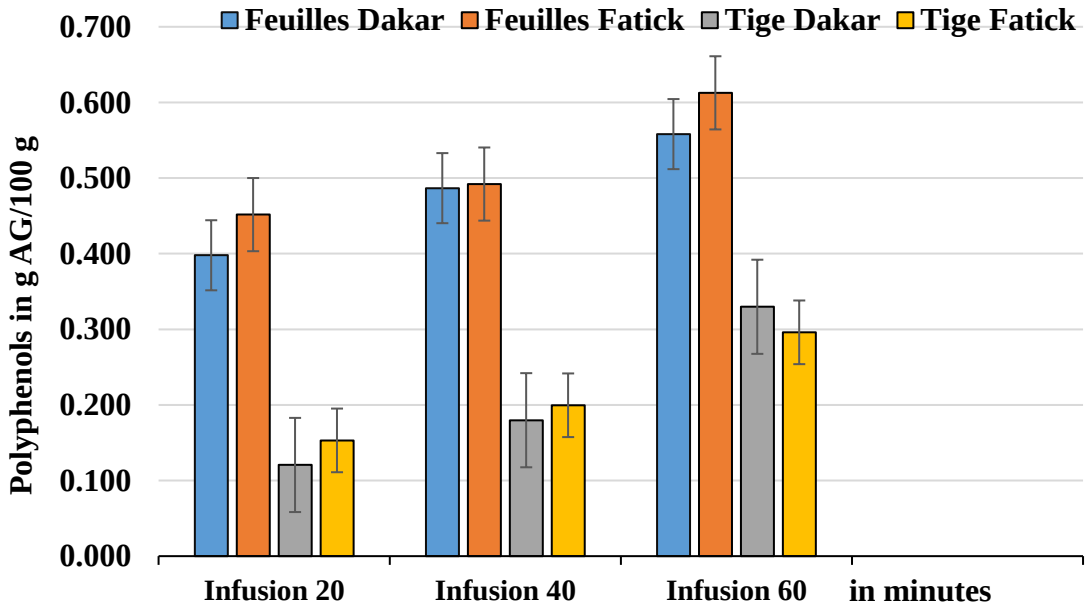

Fig. 4. Polyphenol content during infusion of leaves and stems

The results of the total polyphenol content obtained on leaf and stem extracts from the Dakar and Fatick region after maceration are shown in Figure 5.

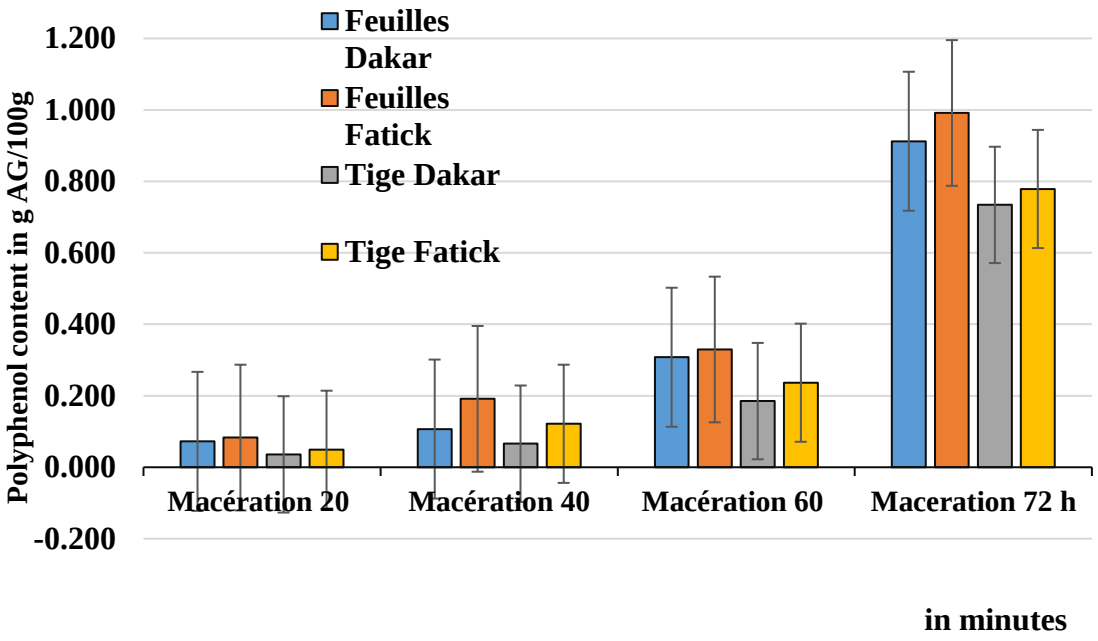

Fig. 5. Evolution of the polyphenol concentration as a function of maceration duration

The results show that the concentration of total polyphenols increases as a function of the decoction duration. It goes from 0.571 to 0.966 g AG/100 g of extract for Dakar leaves and from 0.572 to 1.394 g AG/100 g of extract for Fatick leaves (figure 3).

The stems are also rich in polyphenols with concentrations reaching 0.634 g AG/100 g of extract for Fatick and 0.602 g AG/100 g of Dakar extract. The results reveal that the polyphenol content in the leaf extracts is higher than those obtained from the stems after 60 min of extraction. Samples from the Fatick region contain more polyphenols than those from the Dakar region. This same trend is confirmed for infusion and maceration (***Figure 4, 5)***. The difference in polyphenol content on the leaves and stems is due to a heterogeneous distribution of polyphenols in the different organs of *M.charantia* [11]. This uneven distribution could be explained by the fact that the biosynthesis process of these secondary metabolisms is more important in the leaves [12]. Chloroplasts are regularly supplied with initial compounds for the formation of polyphenols, with energy (ATP) and a reducing factor (NaDPH $_{2)}$, which encourages them to be considered as a privileged site for this biosynthesis [13]. The difference in concentration between samples can be linked to environmental conditions (climate, soil). *M.charantia* is a chilly plant that needs heat (24 and 35°C) for its growth and it is noted that during harvest, the Fatick region has a warmer climate than that of Dakar. It also appreciates cool, moist soil but cannot tolerate waterlogged soils, whereas the one from Dakar is found in water.

3.2 COMPARISON OF EXTRACTION TECHNIQUES FOR PHENOLIC COMPOUNDS

The extraction technique is a very important step in the recovery of phenolic compounds. Figure 6 represents the synthesis of the three extraction methods.

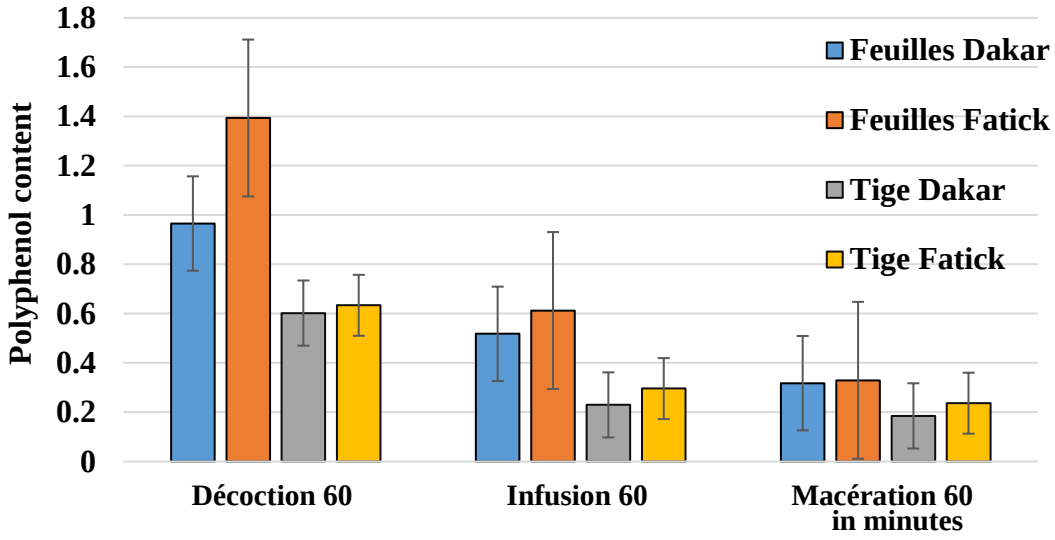

Fig. 6. Comparison of the three extraction techniques for total polyphenols

By combining the results obtained by the 3 extraction techniques after 60 minutes, we found that the decoction is the most effective method for the extraction of total polyphenols. Our results are consistent with those obtained by Guedel et al, 2022 on *Combretum Micranthum* [14]. The quantity of polyphenols obtained in decoction on Fatick leaves (1.69 g AG/100 g of extract) is 2.77 times greater than the value obtained in infusion (0.61 g AG/100 g of extract) and 5 times the value obtained in maceration (0.32 g AG/100 g of extract). In fact, the temperature facilitates extraction by permeabilizing the cell walls through denaturation, which increases the solubility and diffusion of the materials to be extracted [15]. On the other hand, maceration is not very effective because it is done at low temperature. Several studies have shown the positive effect of increasing temperature on the diffusivity of phenolic compounds [16], [17].

3.3 Color Setting For The Three Extraction Methods

The color parameters (L*, a*, b*) were measured 3 times for each sample. The results of the coloring intensity of the leaf and stem extracts after 60 min of decoction are shown in Table 1 and Figure 7.

Table 1. Color parameters (a, b*, L*) after 60 minutes of decoction*

Decoction	settings	Dakar Leaves	Fatick Leaves	Dakar Stem	Fatick rod
	L*	96.23 ^{bc} ±0.05	96.03c $^{\pm}$ 0.02	99.88 ^{b} ±0.05	99.25 ^{to} ±0.01
20 mins	has*	-2.445 ^{b} ±0.007	-2.970 ^{to} ±0.00	-0.410 ^{b} ±0.113	-0.68c $^{\pm}$ 0.00
	b*	11.37 ^{b} ±0.10	143 ^{to} ±0.02	4.55c $^{\pm}$ 0.33	2.57d $^{\pm}$ 0.00
	L	96.19 ^{b} ±0.01	96.36 ^{b} ±0.01	99.97 ^{to} ±0.02	99.94 ^{to} ±0.05
40 mins	has*	-1.95 ^{b} ±0.02	-2.85 ^{to} ±0.17	-0.31d $^{\pm}$ 0.00	-0.67c $^{\pm}$ 0.00
	b*	12.14 ^{b} ±0.26	15.63 ^{to} ±0.16	1.71 ^{d} ±0.02	2.23 ^{c} ±0.01
	L*	89.38c $^{\pm}$ 0.07	66.91 ^{d} ±0.70	96.79 ^{to} ±0.05	94.50 ^{b} ±0.81
60 mins	has*	-2.96c $^{\pm}$ 0.03	-2.99 ^{to} ±0.07	-0.87c $^{\pm}$ 0.02	-1.74 ^{b} ±0.11
	b*	23.37 ^{b} ±0.02	54.07 ^{to} ±0.52	8.65 ^{d} ±0.09	13.23c $^{\pm}$ 0.13

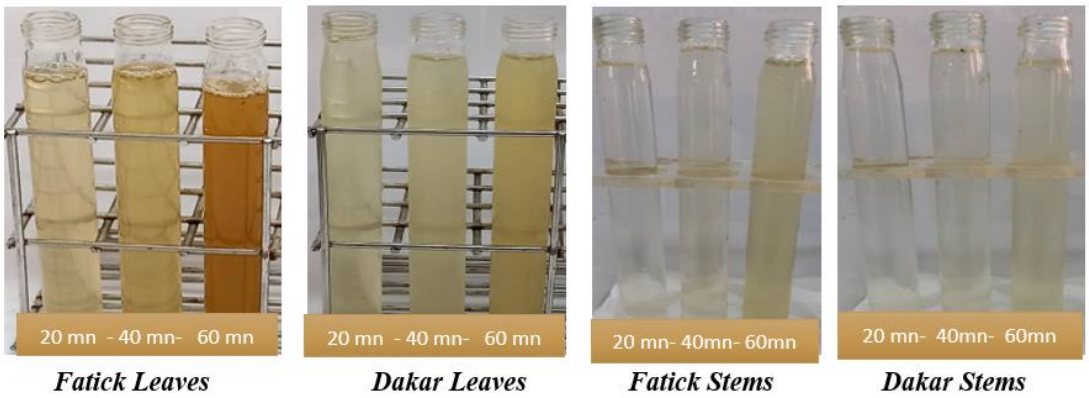

Fig. 7. Extracts obtained after successive decoction of 20, 40 and 60 minutes on the leaves and stems of M. charantia

Table 2 and Figure 8 present the results of the different color parameters and the coloring of the leaf and stem extracts obtained after 60 min of infusion.

Table 2. Color parameters (a*, b*, L*) of the extracts obtained after 60 minutes of infusion

Infusion	settings	Dakar Leaves	Fatick Leaves	Dakar Stem	Fatick rod
20 mins	L*	96.66 ^{b} ±0.17	96.50 ^{b} ±0.49	100.00 ^{to} ±0.00	100.00 ^{to} ±0.00
	has*	-2.38 ^{to} ±0.02	-2.14 ^{to} ±0.13	-0.38b $^{\pm}$0.00	-0.52 ^{b} ±0.01
	b*	11.99 ^{b} ±0.08	12.70 ^{to} ±0.31	1.75c $^{\pm}$0.00	2.20c $^{\pm}$0.03
40 mins	L*	96.21 ^{b} ±0.04	96.36 ^{b} ±0.23	99.97 ^{to} ±0.02	99.87 ^{to} ±0.00
	has*	-1.95b $^{\pm}$0.01	-2.85 ^{to} ±0.17	-0.31d $^{\pm}$0.00	-0.67c $^{\pm}$0.00
	b*	12.11 ^{b} ±0.11	14.53 ^{to} ±0.17	1.71 ^{d} ±0.02	2.23 ^{c} ±0.01
60 mins	L*	95.37 ^{c} ±0.12	96.05 ^{b} ±0.02	99.79 ^{to} ±0.02	99.86 ^{to} ±0.01
	has*	-2.33 ^{to} ±0.00	-2.48 ^{to} ±0.01	-0.35b $^{\pm}$0.00	-0.64 ^{b} ±0.00
	b*	12.67 ^{b} ±0.09	14.92 ^{to} ±0.39	1.81 ^{d} ±0.02	2.46c $^{\pm}$0.01

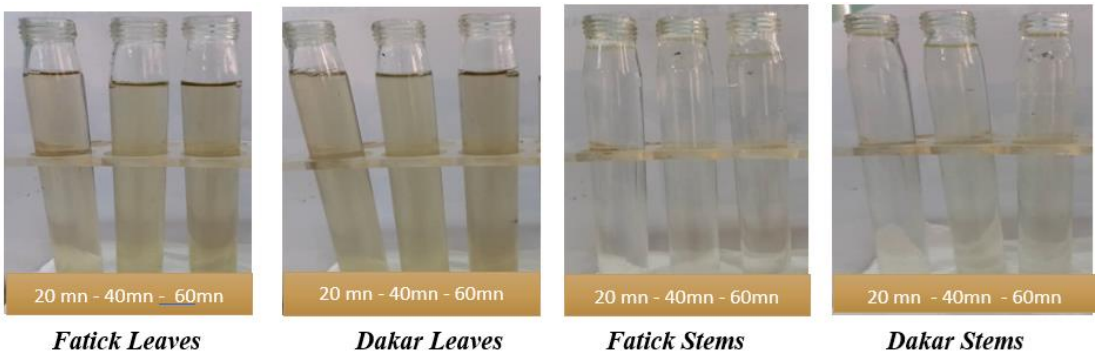

Fig. 8. Extracts obtained after successive infusion of 20, 40 and 60 minutes on the leaves and stems of M. charantia

The results of color parameters of leaf and stem extracts after maceration are shown in Table 3 and Figure 9.

Table 3. Color parameters (a, b*, L*) after 60 minutes of maceration*

Maceration	settings	Dakar Leaves	Fatick Leaves	Dakar Stem	Fatick rod
	L*	98.90 ^{b} ±0.07	98.98 ^{b} ±0.05	100.00 ^{to} ±0.00	100.00 ^{to} ±0.00
20 mins	has*	-1.02 ^{to} ±0.00	-0.83 ^{b} ±0.00	- 0.13c $^{\pm}$ 0.00	- 0.12c $^{\pm}$ 0.00
	b*	4.55 ^{to} ±0.04	3.78 ^{b} ±0.09	0.73c $^{\pm}$ 0.00	0.60 ^{c} ±0.02
	L*	98.65 ^{c} ±0.03	98.98 ^{b} ±0.03	100.00 ^{to} ±0.00	100.00 ^{to} ±0.00
40 mins	has*	-1.22 ^{to} ±0.00	-1.10 ^{b} ±0.00	-0.14c $^{\pm}$ 0.00	-0.14c $^{\pm}$ 0.00
	b*	5.53 ^{to} ±0.03	4.69 ^{b} ±0.05	0.85 ^{to} ±0.02	0.69 ^{d} ±0.01
	L*	98.46 ^{to} ±0.06	98.65 ^{to} ±0.05	99.98 ^{b} ±0.01	99.99 ^{b} ±0.00
60 mins	has*	-1.43 ^{to} ±0.00	-1.29 ^{to} ±0.00	-0.15c $^{\pm}$ 0.00	-0.15c $^{\pm}$ 0.00
	b*	6.41 ^{to} ±0.05	5.74 ^{b} ±0.03	0.86 ^{d} ±0.02	1.03 ^{c} ±0.03

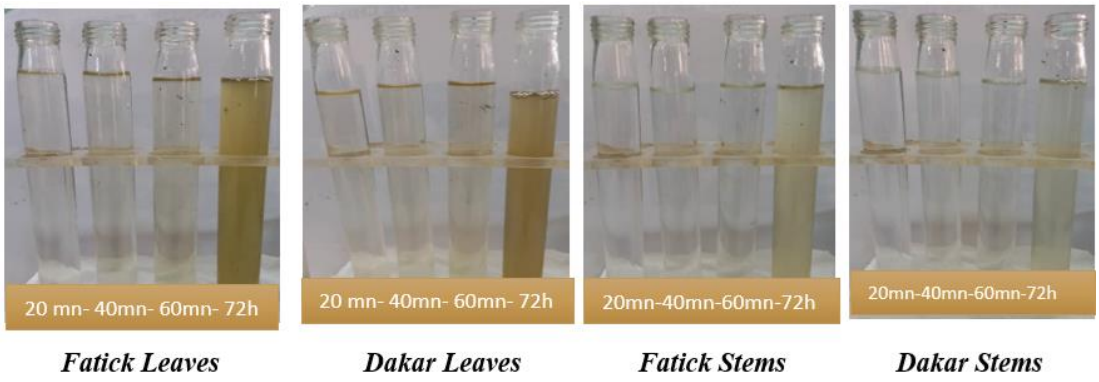

Fig. 9. Extracts obtained after successive maceration of 20, 40 and 60 minutes of the leaves and stems of M. charantia

Figure **10** shows the classification of the intensity of the yellow color (b*) depending on the extraction method and duration.

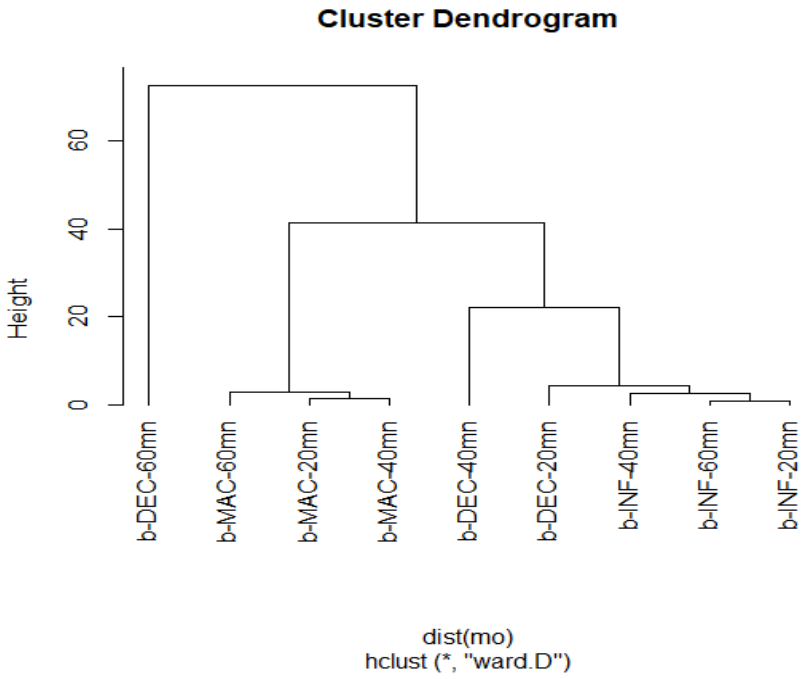

Fig. 10. Classification of yellow color intensity based on extraction method and duration

The results reveal 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 (figures 7, 8 and 9). The results reveal after 60 minutes of

decoction that the clarity of the stem extracts (Fatick) is higher (94.50) than the clarity of the leaf extracts (66.92) (table 1). After 60 minutes of extraction, the clarity obtained by maceration on the Dakar leaves is higher (98.49) than that obtained by decoction (89.39) and infusion (95.37). The intensity of the yellow coloring is more accentuated in decoction, it is of the order of 54.07 for the Fatick leaves and 23.36 for the Dakar leaves (table 1). In decoction, the intensity of the yellow coloring on the Fatick leaves is 3.6 times greater than that obtained in infusion and 8.89 times for maceration after 60 minutes. After 72 hours of maceration, the intensity of the yellow coloring is significant compared to the infusion.

The decrease in clarity (L) is explained by Maillard reactions which results in the synthesis of brown polymers (*melanoidins)*.

The reaction is strongly stimulated at high temperatures but heat is not an essential condition for the development of Maillard rection which can also occur during the storage period [18]. This confirms that the extracts obtained by maceration have a very weak brown color compared to the decoction. The difference in clarity on the two (2) plant parts used is explained by the fact that non-enzymatic rustling reactions are more intense on the leaves than on the stems. The increase in the intensity of the yellow coloring is due to the degradation of chlorophyll which is the pigment responsible for the green coloring of plants. It allows plants to produce oxygen using sunlight and water. However, it is very sensitive to heat and begins to degrade above 60 °C and high extraction time [19]. The results obtained on the intensity of the yellow color (b+ figure 7, 8 and 9), show that the decoction (100 °C/60 min) on Fatick leaf, has a b+ value of 54.07 ± 0.52 and 3.6 times higher in infusion and 9 times in maceration. The classification shows only 5 classes (figure 10). The parameter representing the intensity of the yellow coloring (b+) is higher in 60 minutes of decoction (54.07) followed by 40 minutes of decoction (15.63), 20 minutes of decoction (14.53) followed by infusion and finally maceration.

4 CONCLUSION

The objective of his work was to evaluate the polyphenol contents by three (3) different extraction techniques on the leaves and stem of *Momordica charantia.* The results showed that the leaves and stems are rich in phenolic compounds. However, extracts from the leaves are richer in polyphenols than those from the stems. In addition, the Fatick sample has the highest phenolic content than that of Dakar. The values obtained show that the decoction allows obtaining more polyphenols, followed by the infusion. The quantity of polyphenols obtained by the decoction on fresh leaves of Fatick is twice that of the infusion. Ultimately, this plant shows a good source of phenolic compounds in the aerial parts. It would be very interesting to carry out this evaluation on the dried leaves and on the powder of the leaves and stems. Then optimize the decoction to determine the maximum quantity of polyphenols that can be extracted without destroying the molecule.

ACKNOWLEDGMENT

This work was carried out at the Water, Energy, Environment and Industrial Processes Laboratory (**LE3PI)** and at the Center for Studies on Food Safety and the Development of Functional Molecules (**CESAM)** of the Ecole Supérieure Polytechnique de Dakar Cheikh Anta University Diop (Senegal). The authors warmly thank the laboratory managers, as well as all the staff.

REFERENCES

[1] «Huilier 2007 - Google Scholar». https://scholar.google.com/scholar (accessed December 16, 2022).
[2] Regional C. for Africa, «Strengthening the role of traditional medicine in health systems: A strategy for the African Region: report from the secretariat», WHO. Regional Office for Africa, 2013.
[3] FBA El Hilah Fatima, J. Dahmani, N. Belahbib, and L. Zidane, «Ethnobotanical study of medicinal plants used in the treatment of infections of the respiratory system in the central Moroccan plateau», J. Anim. *Plant Sci.,* flight. 25, no. 2, p. 3886-3897, 2015.
[4] S. Montcho, (2012). Medicinal antimalarial plants: another route of exposure to toxic metals in Cotonou (Benin). Master's thesis from the University of Benin, Specialty: Environment, Health and Development Google Search » (Accessed December 16, 2022).
[5] V. Moris, O. Dalleur, and AM Dohou, «Plants with antibiotic properties in Benin».
[6] H. Rammal, J. Bouayed, F. Desor, C. Younos, and R. Soulimani, «Validation and contribution to the study of the antihyperglycemic effect of a medicinal plant, Momordica charantia L.», *Phytotherapy,* vol. 7, no. 4, p. 191-196, 2009.
[7] «more than 100,000 metabolites have been identified - Google Search». (Accessed December 17, 2022).
[8] C. Manach, A. Scalbert, C. Morand, C. Rémésy, and L. Jiménez, «Polyphenols: food sources and bioavailability», *Am. J. Clin. Nutr.,* flight. 79, no. 5, p. 727-747, 2004.
[9] E. Nkhili, «Food polyphenols: extraction, interactions with iron and copper ions, oxidation and antioxidant power», 2009.
[10] «CIELAB or L*a*b* is a color representation model developed in 1976.
[11] H. Falleh *et al.* «Phenolic composition of Cynara cardunculus L. organs, and their biological activities», *CR Biol.,* flight. 331, no. 5, p. 372-379, May 2008, doi: 10.1016/j.crvi.2008.02.008.
[12] «A. Arnold -W., Luke, 1964 - Google Search». https://www.google.com/search (accessed December 19, 2022).

[13] «J. Brzozowsk and P. Hanower - Google Search». https://www.google.com/search (accessed December 19, 2022).
[14] PG Faye, EM Ndiaye, B. Ndiaye, OIK Cisse, NC Ayessou, and M. Cisse, «Effect of maceration, infusion and decoction on the aqueous extraction of polyphenols from dried Combretum leaves Micranthum», *Afr. Sci.,* flight. 21, no. 3, p. 114-126, 2022.
[15] B. Amor, «Control of the technological suitability of plant material in active ingredient extraction operations: texturing by controlled instantaneous relaxation (DIC)», phdthesis, University of La Rochelle, 2008.
[16] Available on: https://theses.hal.science/tel-00399131 (Accessed December 21, 2022).
[17] RA Andrianarimanana, «Extraction and characterization of proanthocyanidins from three-lobed viburnum (Viburnum trilobum L.) and ellagitannins from strawberry achenes (Fragaria X ananassa Duch.)», 2022.
[18] N. El Darra, «The phenolic compounds of grapes: study of the qualitative potential and emerging extraction processes», Doctoral thesis, Compiègne, 2013. Available at: https://www.theses.fr/ 2013COMP2060 (Accessed December 21, 2022).
[19] Journal of the Biological Society. The Society, 2007.
[20] Cédric, «How to keep the color of your green vegetables», *Cuisine Autodidacte,* 9.