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Optimization of the Aqueous Decoction Process of *Combretum micranthum* Leaves

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Abstract

This work proposes to compare the extraction of total polyphenols and the coloring obtained after several series of decoction at 100˚C/20min, of whole or crushed leaves of *Combretum micranthum*. The total phenolic content of the extracts obtained after decoction was determined by Folin's method. The color parameters were measured using a colorimeter based on the CIELAB system. The analysis of variance (ANOVA) shows significant differences ($p < 0.05$) on different parameters evaluated. The Minitab 19 software was used to classify the extracts according to the color parameters (L, A, and B). The results showed that the maximum of polyphenols (21.67%) was extracted in the crushed leaves after three series of decoctions while with the whole leaves the maximum of 16.17% is obtained after six series of decoctions. The concentration of total polyphenols becomes low from four series of decoctions with 0.146 g·AG·100 g^{-1} MS. The red and yellow coloring drops respectively by 85 and 69% with crushed leaves and 64 and 21% with whole leaves. The conductivity and the brix of the extracts fall according to the series of decoctions. These results show that for optimal use of *C. micranthum* leaves in traditional medicine, crushed leaves and three series of decoctions are indicated and sufficient.

Keywords

Combretum micranthum, Extraction, Decoction, Polyphenols, Coloring

1. Introduction

In West Africa, *C. micranthum* is a plant widely used in traditional medicine.

Herbal tea prepared from the leaves is widely consumed for its flavoring, nutritional and medicinal properties [1]. Better known as "*kinkeliba*" in Senegal, it is a plant that grows in most countries of the Sahel. It is also found in Somalia [2] [3]. The decoction of the leaves is consumed as a drink to treat malaria (Traoré, 2010), for its diuretic action, antidiabetic, antibacterial and antifungal activities [4] [5] [6] [7] [8]. For the treatment of liver failure, constipation, bronchitis and cough, the leaves are used as a 10% infusion [5]. According to Burkill (1985), fruit powder is used to treat weeping skin diseases in children (impetigo type) [9]. In Senegal, the dried leaves are sold tied in twigs and tied [7].

The phenolic compounds in kinkeliba leaves can act as antioxidants by extinguishing biological systems with their phenolic ring and multiple hydroxyl moieties [10]. Compounds that exhibit such antioxidant activity may also exhibit anti-inflammatory activity [10]. The duration of decoction, the temperature of infusion and maceration remain parameters not controlled by the populations for the extraction of total polyphenols. In West Africa, particularly in Senegal, for the preparation of breakfast herbal tea, the same quantity of dried leaves of *Combretum micranthum* is used for several decoctions with an addition of water every day until the decoction is completely discolored. A prolonged decoction denatures the phenolic compounds and reduces the quality of the extracts obtained, not to mention the energy expenditure. Thus, the main objective of this work is to determine the optimal conditions for obtaining the decoction of *C. micranthum*.

2. Material and Methods

2.1. Plant Material

The dried leaves of *Combretum micranthum* are harvested from the Thiès region of Senegal and dried in the shade. One batch was coarsely ground with a blender (*Moulinex Blender* 5 Speeds) for testing purposes. Generally, the leaves are packaged with the stems of the plant and sold in the various markets in Dakar (**Figures 1**-**3**).

Figure 1. Tied leaf and stems.

Figure 2. Dried leaves.

Figure 3. Crushed leaves.

2.2. Method of Extraction by Series of Decoction

Decoction is a method of extracting soluble compounds by immersing crushed or whole *C. micranthum* leaves in constantly boiling water at 100˚C for 20 minutes. The leaves are first sorted manually then weighed and 25 ± 0.1 g of leaves are packaged in plastic bags for the purpose of the various tests. A volume of 1500 ml of tap water is used. The water is brought to a constant boil at 100˚C and then the 25 g of leaf are introduced. A stopwatch is started as soon as the 25 g of leaves are introduced. After 20 minutes of decoction at 100˚C, the extract is recovered and filtered on wahtman paper for the analysis of the various parameters. After this first decoction, the leaves are washed and rinsed for a new decoction with the addition of 1500 ml of water. This same operation is repeated for up to 10 decoction badges on the same leaves under the same conditions. Parameters such as the amount of total polyphenols, the color, the conductivity, the pH and the amount of soluble solids (brix) are analyzed after each series.

2.3. Analytical Tracking Parameters

The content of the compounds total phenolics—extracts of *Combretum micranthum* was estimated using the *Folin-Ciocalteu method* (Ertas, 2014) and the results expressed in grams of gallic acid per 100 grams of dry matter (g·AG·100 g^{-1} MS). The color of the extracts was measured using a colorimeter (type:

KONICA MINOLTA. Japan) based on the CIELAB color system (L*, a*, b* and L*, C*, h, YI). The parameters 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). Measurements were made 3 times for each sample.

Brix is measured according to a standardized NA 5669 method using an Abbe ATAGO universal refractometer with digital reader and temperature correction. It is expressed as a percentage by mass (g/100g of extract). The conductivity is determined by a conductivity meter integrating the measurement of the pH (Hanna *instruments*, *Germany*) at 25˚C.

2.4. Statistical Analyzes

The results were subjected to a one-way ANOVA analysis of variance with R software version 3.2.4 Revised (2017) and Minitab19 software. The X value 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% level.

3. Results and Discussion

3.1. Monitoring of Physical Parameters

The values of physical parameters such as conductivity, pH and soluble solids in decoction with whole *Combretum micranthum* leaves are represented in Table 1 and Table 2. The results show that the maximum conductivity and dry matter are reached from the first decoction carried out in 20 minutes. However, the extracts become more and more basic during the series of decoctions; from 8.76 to 8.93 for the whole leaves, and from 7.52 to 8.67 for the crushed leaves.

The results reveal for the first decoction with the use of crushed leaves a conductivity of 1138.33 µs/cm at 25˚C which gradually drops according to the series

Table 1. Monitoring of physical parameters during decoction of whole leaves

Number of decoction	Conductivity in µs/cm at 25˚C	pH at 25˚C	Soluble solids g/100g
Decoction 1 (20 mn)	$1067.22^{a} \pm (8.82)$	$8.76^{e} \pm (0.01)$	$0.39^{a} \pm (0.01)$
Decoction 2 (40 mn)	$442.09^{b} \pm (5.08)$	$8.74^{e} \pm (0.00)$	$0.32^{b} \pm (0.00)$
Decoction 3 (1H)	$429.23^{c} \pm (0.95)$	$8.80^{d} \pm (0.01)$	$0.21^{c} \pm (0.02)$
Decoction 4 (1H 20 mn)	$419.10^{c,d} \pm (2.73)$	$8.88^{c} \pm (0.01)$	$0.10^{d} \pm (0.00)$
Decoction 5 (1H 40 mn)	$413.78^{d} \pm (1.92)$	$8.91^{b,c} \pm (0.01)$	$0.10^{d} \pm (0.00)$
Decoction 6 (2H)	$413.38^{d} \pm (1.26)$	$8.92^{a,b} \pm (0.00)$	$0.10^{d} \pm (0.00)$
Decoction 7 (2H 20 mn)	$411.28^{e} \pm (1.02)$	$8.94^{a} \pm (0.01)$	$0.10^{d} \pm (0.00)$
Decoction 8 (2H 40 mn)	$402.24^{e,f} \pm (2.10)$	$8.93^{a,b} \pm (0.00)$	$0.10^{d} \pm (0.00)$
Decoction 9 (3H)	$394.98^{f,g} \pm (2.32)$	$8.93^{a,b} \pm (0.00)$	$0.10^{d} \pm (0.00)$
Decoction 10 (3H 20 mn)	$387.16^{g} \pm (5.05)$	$8.92^{a,b} \pm (0.00)$	$0.10^{d} \pm (0.00)$

Table 2. Monitoring of physical parameters during decoction of crushed leaves.

Number of decoction	Conductivity in µs/cm at 25˚C	pH at 25˚C	Soluble solids g/100g
Decoction 1 (20 mn)	$1138.33^{a} \pm (3.51)$	$7.52^{c} \pm (0.02)$	$0.60^{a} \pm (0.01)$
Decoction 2 (40 mn)	$437.86^{b} \pm (0.05)$	$7.68^{bc} \pm (0.06)$	$0.35^{ab} \pm (0.05)$
Decoction 3 (1H)	$426.26^{b} \pm (0.11)$	$8.16^{ab} \pm (0.04)$	$0.25^{b} \pm (0.05)$
Decoction 4 (1H 20 mn)	$425.30^{b} \pm (1.40)$	$8.37^{a} \pm (0.04)$	$0.10^{b} \pm (0.00)$
Decoction 5 (1H 40 mn)	$426.20^{b} \pm (3.00)$	$8.50^{a} \pm (0.05)$	$0.10^{b} \pm (0.00)$
Decoction 6 (2H)	$417.83^{b} \pm (1.85)$	$8.52^{a} \pm (0.09)$	$0.10^{b} \pm (0.00)$
Decoction 7 (2H 20 mn)	$408.70^{b} \pm (2.40)$	$8.60^{a} \pm (0.04)$	$0.10^{b} \pm (0.00)$
Decoction 8 (2H 40 mn)	$405.40^{b} \pm (1.80)$	$8.62^{a} \pm (0.03)$	$0.10^{b} \pm (0.00)$
Decoction 9 (3H)	$407.43^{b} \pm (4.75)$	$8.68^{a} \pm (0.05)$	$0.10^{b} \pm (0.00)$
Decoction 10 (3H 20 mn)	$406.20^{b} \pm (5.30)$	$8.67^{a} \pm (0.03)$	$0.10^{b} \pm (0.00)$

of decoctions to reach the value 406.2 µs/cm after 10 badges of decoction. This same tendency is observed for the decoction with the whole leaves of *Combretum micranthum*, the conductivity drops from 1067.22 ± (8.82) µs/cm to 387 µs/cm after 10 decoction badges. This conductivity is directly proportional to the quantity of dissolved substances, so the greater the extraction of dissolved substances, the more the conductivity increases. With *Combretum* leaf crushing *micranthum* the extraction of dissolved substances is maximum and greater than with whole leaves. This extraction is associated with the extraction of minerals and other compounds present on the leaves (calcium salt, magnesium salt, sodium salt, potassium salt, and citrate) which increases the conductivity of the decocted solution. Conductivity remains a good indicator for monitoring the extraction of soluble substances contained in the leaves.

The results show an increase in pH as a function of the number of decoction badges with the use of whole leaves or crushed leaves. The pH increases by 15% between the first and the tenth decoction with crushed leaves with a value which evolves from 7.52 to 8.67. This same trend is observed with the decoction of whole leaves, the pH increases from 8.77 to 8.93 or 1.8% more. The increase in pH with crushed leaves after ten decoction badges is 11.5 times greater than the increase in pH with the use of whole leaves. This significant increase in pH will be associated with the faster extraction of organic compounds from crushed leaves than from whole leaves. With the renewal of the liquid phase, the diffusion continues until exhaustion of the extraction of the solid phase and the pH remains constant this same phenomenon is reported by Dibert in 1989 [11].

3.2. Monitoring of Total Polyphenolic Compounds

The concentrations of total polyphenols extracted with the decoction of crushed leaves (**Figure 4**) or with whole leaves (**Figure 5**) are presented below.

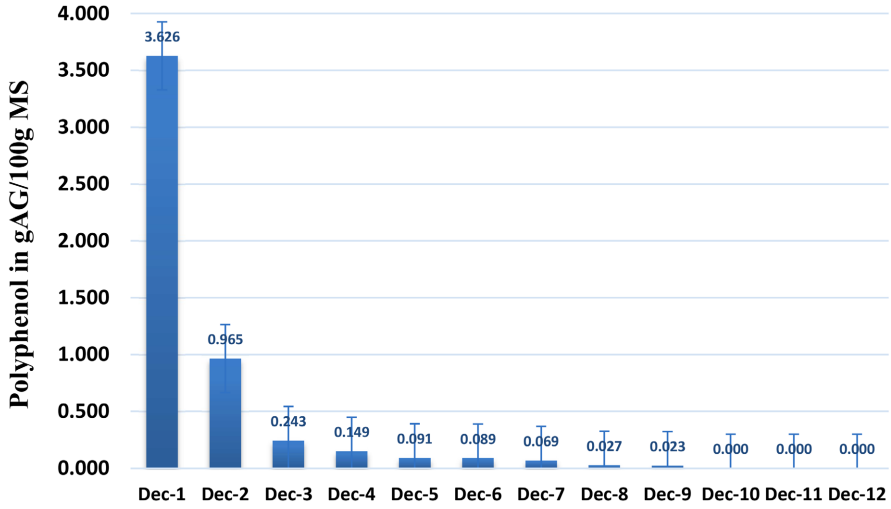

Figure 4. Evolution of polyphenolic compounds according to the number of decoctions with crushed leaves.

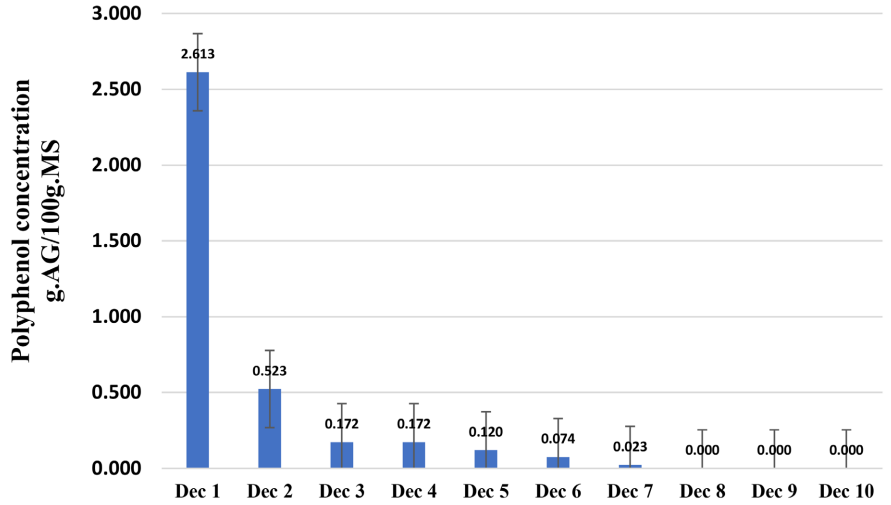

Figure 5. Evolution of polyphenolic compounds according to the number of decoctions with whole leaves.

The results show that the content of total polyphenols decreases according to the number of aqueous decoctions whatever the nature of the leaves, going from 3.626 to 0.027 g·AG·100 g^{-1} MS for the crushed leaves and from 2.613 to 0.023 g·AG·100 g^{-1} MS for whole leaves. It emerges from its results that the content of total polyphenols obtained in the first decoction at 100˚C for 20 min is always maximum. Moreover, it is 1.38 times higher with crushed leaves than with whole leaves. After ten series of extraction with crushed leaves, the decoction extracts 5.282 g·AG·100 g^{-1} MS or 23.68% of the total polyphenols extracted compared to 22.3 g·AG·100 g^{-1} MS reported by Ousmane in 2017 and 3.697 g·AG·100 g^{-1} MS with whole leaves or 16.57% [12]. The extraction is more optimized with crushed leaves. Indeed, the crushing of the leaves makes it possible to intensify the solvent transfer phenomena through the increase in the specific exchange surface and the reduction of the distance of penetration through the crushed leaves [13].

Similar results have been recorded by several authors [14] [15]. Bernaud in 2014 reported contents of 20% to 30% DM in green tea leaves. Three decoction badges using crushed leaves extract 21.67% of total polyphenols and 16.17% for whole leaves [16].

3.3. Tracking of Color Parameters According to the Number of Decoctions

The evolutions of the color parameters (A, B and L) are given in **Table 3** and **Table 4**.

The results reveal a drop in color parameters (A) and (B) and an increase in clarity (L) depending on the decoction badges (**Table 2** & **Table 3**). The color

Table 3. Color parameters depending on the number of decoction with crushed leaves.

Color settings	A	B	L
Decoction No. 1	$17.07^{a} \pm (0.47)$	$50.69^{a} \pm (0.44)$	$64.97^{g} \pm (1.01)$
Decoction No. 2	$13.70^{b} \pm (0.03)$	$37.56^{b} \pm (0.01)$	$78.23^{f} \pm (0.15)$
Decoction No. 3	$7.14^{c} \pm (0.04)$	$26.40^{c} \pm (0.07)$	$87.10^{e} \pm (0.19)$
Decoction No. 4	$2.54^{d} \pm (0.09)$	$15.42^{e} \pm (1.81)$	$92.39^{d} \pm (0.04)$
Decoction No. 5	$2.29^{d} \pm (0.04)$	$17.16^{d} \pm (0.22)$	$93.15^{d} \pm (0.19)$
Decoction No. 6	$0.87^{e} \pm (0.01)$	$11.75^{f} \pm (0.07)$	$95.21^{c} \pm (0.11)$
Decoction No. 7	$0.70^{e} \pm (0.03)$	$9.72^{g} \pm (0.17)$	$96.13^{c} \pm (0.17)$
Decoction No. 8	$0.17^{f} \pm (0.00)$	$6.84^{h} \pm (0.03)$	$97.23^{b} \pm (0.10)$
Decoction No. 9	$0.03^{g} \pm (0.01)$	$5.89^{h} \pm (0.05)$	$97.67^{ab} \pm (0.05)$
Decoction No. 10	$0.00 \pm (0.00)$	$5.51^{hj} \pm (0.06)$	$97.77^{ab} \pm (0.03)$
Decoction No. 11	$0.00 \pm (0.00)$	$4.73^{ij} \pm (0.07)$	$98.19^{ab} \pm (0.12)$
Decoction No. 12	$0.00 \pm (0.00)$	$4.01^{d} \pm (0.04)$	$98.47^{a} \pm (0.00)$

Table 4. Color parameters depending on the number of decoctions with whole leaves.

Color settings	A	B	L
Decoction No. 1	$24.20^{a} \pm (0.02)$	$48.07^{a} \pm (0.09)$	$69.15^{i} \pm (0.12)$
Decoction No. 2	$16.71^{b} \pm (0.10)$	$41.36^{b} \pm (0.19)$	$78.23^{h} \pm (0.12)$
Decoction No. 3	$9.66^{c} \pm (0.04)$	$37.13^{c} \pm (0.10)$	$85.43^{g} \pm (0.09)$
Decoction No. 4	$8.56^{d} \pm (0.04)$	$37.83^{d} \pm (0.12)$	$85.83^{f} \pm (0.09)$
Decoction No. 5	$4.46^{e} \pm (0.01)$	$26.31^{e} \pm (0.04)$	$90.00^{e} \pm (0.04)$
Decoction No. 6	$0.66^{f} \pm (0.04)$	$15.12^{f} \pm (0.06)$	$94.42^{d} \pm (0.11)$
Decoction No. 7	$0.46^{g} \pm (0.10)$	$7.78^{g} \pm (0.02)$	$96.84^{c} \pm (0.04)$
Decoction No. 8	$0.37^{gh} \pm (0.01)$	$5.56^{h} \pm (0.08)$	$97.82^{b} \pm (0.06)$
Decoction No. 9	$0.30^{gh} \pm (0.05)$	$4.60^{i} \pm (0.11)$	$98.26^{a} \pm (0.07)$
Decoction No. 10	$0.42^{h} \pm (0.03)$	$4.20^{d} \pm (0.09)$	$98.51^{a} \pm (0.07)$

parameter (L) corresponds to lightness or luminosity, according to a psychometric scale ranging from zero to 100. Here the values 98.51 ± (0.07) and 98.47 ± (0.00) obtained at the tenth decoction in both cases represent white or total reflection (colorless); the values 69.15 ± (0.12) and 64.97 ± (1.01), represent the partial absorption of the colors. An extraction of 64% of the red color and 21% of the initial yellow is observed from the fourth decoction with the whole leaves. These two colors disappear from decoction No. 6. For the decoction using crushed leaves, an extraction of 86% of the yellow color and 99% of the red color is observed after 8 decoctions.

Figure 6 and Figure 7 show the evolution of the coloring of the solutions obtained after decoction with crushed leaves or whole leaves.

The results reveal a degradation of the red and yellow color giving way to colorless depending on the number of decoctions (Figure 6, Figure 7). Reddening or greening is expressed by the value of the color parameter (A). Yellowing or bluing is represented by parameter (B). The R software made it possible to classify the 10 decoction badges according to the color of the extract obtained (decoction N˚ 1-2, decoction N˚ 3-4-5, decoction N˚ 6-7 and decoction N˚ 8-9-10) (Figure 8).

Figure 6. Color with whole leaves.

(a) (b)

Figure 7. Color in decoction with crushed leaves.

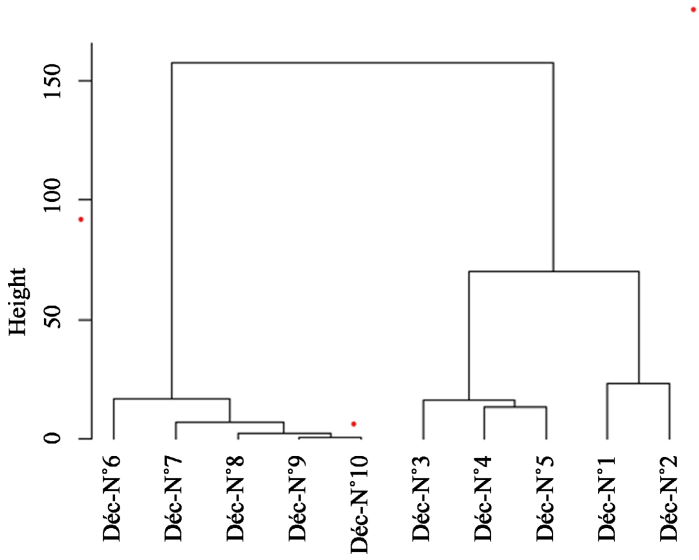

Figure 8. Classification of decoction badges according to the color of the extract obtained.

4. Conclusion

Work on the optimization of the process of decoction of the leaves of *Combretum micranthum* has shown that the aqueous decoction with the use of crushed leaves of *C. micranthum* is the best method for extracting total polyphenols. The results reveal on the one hand, that a number of three decoctions at 100˚C/20min with crushed leaves are sufficient to extract with water 21.67% of the total polyphenols against five decoctions with whole leaves to extract only 16.17% total polyphenols. On the other hand, six decoctions with the use of whole dry leaves make it possible to extract 64% of the red color and 21% of the yellow color and eight decoctions to extract 99% of the red color and 86% of the yellow color for l use of crushed leaves. The use of powdered dried leaves and crushed stems in order to quantify and determine the antioxidant power of each part of the plant is an avenue to be studied.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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