

Effect of Sodium Carbonate on Extraction by Aqueous Decoction of Total Polyphenols from Crushed and Whole Leaves of *Combretum micranthum*

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Abstract

Sodium bicarbonate is sometimes used to aid in the extraction of total polyphenols. Its main effect is to increase the pH of the extraction solution. Raising the pH can cause changes in the chemical structure of polyphenols. This can lead to variations in their biological properties, solubility and stability. This work studied the effect of sodium carbonate on the extraction by aqueous decoction of total polyphenols from the leaves of *Combretum micranthum*. The content of total phenolic compounds in the extracts was estimated by the Folin-Ciocalteu method. The color of the samples was measured using a colorimeter (type: KONICA MINOLTA, Japan) based on the CIELAB color system. The results obtained were subjected to a one-way ANOVA analysis of variance with R software version 3.2.4 Revised (2018) and Minitab-18 software. The results reveal a drop in the concentration of extracted polyphenols proportional to the addition of sodium carbonate, *i.e.* a drop from 3.30 to 1.04 mg-AG-100 g⁻¹ of extract on whole leaves and 3.921 to 2.551 mg-AG-100 g⁻¹ extract on crushed leaves. On the other hand, the intensity of the coloring of the extracts increases significantly with the addition of sodium carbonate from 0.0 g-L⁻¹ to 0.666 g-L⁻¹.

Keywords

Baking Soda, *Combretum micranthum*, Extraction, Decoction, Polyphenols

1. Introduction

In West Africa, *Combretum micranthum* is a plant widely used in traditional medicine. Medicinal plants have curative and preventive properties that have been recognized for centuries. They can be used to relieve various health problems, such as headaches, joint pain, infections, and digestive disorders [1]. According to UNICEF (2004), nearly 11 million children die each year in most tropical African countries, of which about 70% are due to malaria and diarrhea [2]. The decoction of this plant is consumed as a drink to treat malaria [3]. Call kinkeliba in Senegal, it is a plant that grows in most countries of the Sahel (Burkina Faso, Guinea, Senegal, Mali, Niger, Guinea-Bissau). This plant is also found in Ivory Coast and Sudan [4] [5]. Diallo *et al.* (2001) reported that the diuretic properties of *Combretum micranthum* are explained by the presence of potassium nitrate and various acid-alcohols [6]. In the treatment of liver failure, constipation, bronchitis and cough, the leaves are used as a 10% infusion. According to Burkill *et al.* (1985), the fruit powder is used to treat weeping dermatoses in children (impetigo type) [7]. Taura *et al.* (2009) demonstrated the antibacterial and antifungal activities of organ extracts from *Combretum micranthum* [8]. The antidiabetic effect of *Combretum leaves* is revealed by Chika *et al.* (2010). In Senegal, the dried leaves are sold tied in twigs and tied [9]. Several authors have reported the mechanisms of therapeutic action of phenolic compounds against cancer, inflammation, malaria and cardiovascular diseases [10] [11] [12] [13].

The phenolic compounds contained in kinkeliba leaves can act as antioxidants by quenching radicals from biological systems with their phenolic ring and multiple hydroxyl moieties. Compounds that exhibit such antioxidant activity may also exhibit anti-inflammatory activity [14] [15]. Polyphenols are the result of secondary plant metabolism through fundamental metabolic pathways [16] [17]. According to Manach *et al.* (2004), these secondary metabolites are involved in the defense of plants against ultraviolet radiation and against attack by pathogens [17]. The term “polyphenols” is used to designate all the phenolic compounds of plants [18]. These compounds include a multitude of molecules present in the plant kingdom [19] [20] [21].

Laurent showed in 1983 that in addition to diuretic and cholagogue properties, the leaves and the fluid extract of *Combretum* possess antibiotic activity against *Staphylococcus*, *Streptococcus* and *Entamoeba coli* [22]. This action has also been shown on *Shigella sp*, *Salmonella paratyphi B*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Klebsiella ozaenae*. Aqueous extracts from the roots of the Nigerian species also show significant antibiotic power against Gram + and Gram - organisms [23] [24]. The duration of decoction, the temperature of infusion and maceration remain parameters not controlled by the populations for the extraction of total polyphenols. The main objective of this work is to reveal the effect of sodium carbonate on the extraction of total polyphenols and on the color intensity of the extract obtained using aqueous decoction extraction. Dried leaves and crushed leaves of kinkékiba will be used, Folin’s method will be used for the determination of total polyphenols, and the results will be processed by R

software version 3.2.4 (2018) and Minitab-18.

2. Material and Methods

2.1. Plant Material

The dried leaves of *Combretum micranthum* come from the Thiès region of Senegal harvested and dried. They are packaged with the stems of the plant and sold on the various markets in Dakar (Figure 1). Figure 2 presents the whole leaves of *Combretum micranthum* (A) and (B) the crushed leaves. Figure 3 presents the soda ash.



Figure 1. Leaves packaged with stems.



Figure 2. Dried (a) and crushed (b) leaves.



Figure 3. Edible soda ash.

2.2. Methods

2.2.1. Decoction Extraction Method

Decoction is a method of extracting soluble compounds by introducing the leaves of *Combretum micranthum* in constantly boiling water at 100°C. The leaves are first sorted by hand and weighed (Figure 4, Figure 5), 25 ± 0.1 g of leaf is packaged in plastic bags (Figure 6).

A volume of 1500 ml of water is used. The water is brought to constant boiling at 100°C, and then the 25 g of ground leaf are introduced with 0.1 g of sodium carbonate. A stopwatch is started as soon as the 25 g of leaves are introduced and is stopped after 20 min. Filtration is carried out, and the extract is analyzed. This cyclic operation is carried out 10 times with the addition of sodium carbonate at variable doses ranging from 0.1 g to 1 g for the same quantity of 25 g sheets.



Figure 4. Sorting of leaves.



Figure 5. 25 g weight.



Figure 6. 25 g sachets.

2.2.2. Determination of Total Polyphenols

The content of these compounds total phenolic extracts of *Combretum* was estimated by the **Folin—Ciocalteu method** which is based on the reduction in environment alkaline of their mixture *phosphotungstic phosphomolybdic* of reagent of Folin by THE groups oxidizable phenolic compounds, leading to the formation of reduction products blue in color. These have an absorption maximum at 760 nm. Whose intensity is proportional to the quantity of total polyphenols present in the extract [25].

2.2.3. Determination of Color Parameters

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^* 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 7** [26].

2.2.4. Determination of Soluble Dry Matter (Brix)

Brix is defined as the concentration of soluble solids in an aqueous solution. This concentration measured at 25°C by the refractive index is then expressed by the percentage by mass (g/100 g), is measured according to a standardized method (NA 5669) using a universal refractometer. Abbe ATAGO type refractometer with digital reader and temperature correction.

2.2.5. Determination of Conductivity and pH

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

2.2.6. Statistical Analyzes

The results were subjected to a one-way **ANOVA analysis of variance with R** software version **3.2.4** Revised (2018) and **Minitab-18** software. The X value of each sample is assigned a superscript letter ($X^{(i)}$ where $i = a, b, c \dots$). Samples with the same letter are not statistically different at the 5% level.

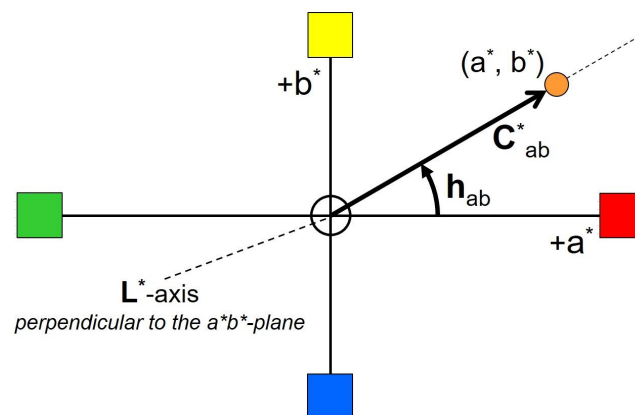


Figure 7. Color settings (a, b, L).

3. Results and Discussion

3.1. Polyphenols

The contents of total polyphenols obtained by decoction with addition of sodium carbonate on whole leaves and on crushed leaves are presented in **Figure 8** and **Figure 9**.

Figure 8 shows a drop in the concentration of extracted polyphenols which is proportional to the amount of sodium carbonate added. This same tendency is noted with the decoction on crushed leaves (**Figure 9**). The quantity of polyphenols extracted in decoction with whole leaves drops from 3.30 to 1.04 mg·AG·100 g⁻¹ of extract, and on crushed leaves from 3.921 to 2.551 mg·AG·100 g⁻¹ of extract for the same carbonate concentrations of soda from 0.0 g·L⁻¹ to 0.666 g·L⁻¹. The amount of polyphenols extracted with crushed leaves is 2.45 times greater than the amount extracted with whole leaves, confirming the work of Guedel *et al.*, 2022 [27].

The sodium carbonate reacts with the phenolic acids present in the extract, which can modify the chemical properties of the extracted polyphenols [28].

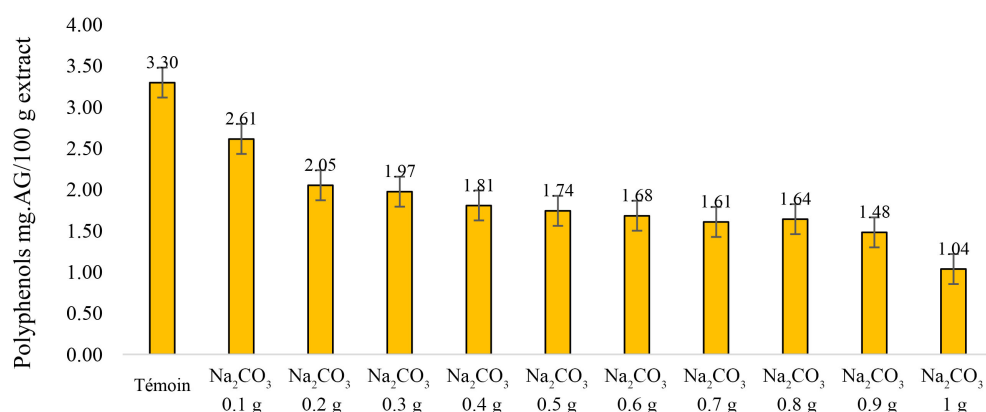


Figure 8. Quantity of polyphenols extracted according to the addition of sodium carbonate on whole leaves in decoction.

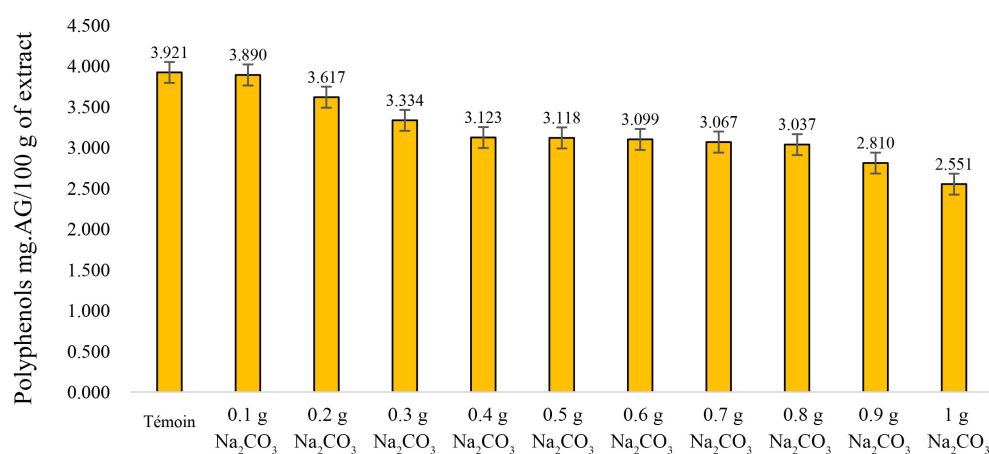


Figure 9. Concentration of polyphenols according to the mass of Na₂CO₃ added to leaves ground in decoction.

This drop in the concentration of polyphenols could be explained by the fact that sodium bicarbonate can also have undesirable effects on the extraction of polyphenols. Indeed, increasing the pH can favor the extraction of certain undesirable compounds, such as amino acids or polysaccharides, which can interfere with the analysis or subsequent use of the extracted polyphenols [29] [30] [31].

It should be noted that decoction extraction conditions, including temperature, extraction time and concentration of soda ash, can also influence the effect of this method on the extraction of polyphenols from *Combretum micranthum*.

3.2. Extract Obtained and Colorations Depending on the Amount of Sodium Carbonate Added

Figure 10 shows the staining of the control extract. **Figures 11-13** show the evolution of the color according to the doses of sodium carbonate.

When the soda ash mixed with the extract rich in polyphenols, acts as a base and can cause a change in the pH of the solution. By increasing the pH, soda ash can influence the chemical structure of polyphenols and alter their ability to absorb light, which can cause the solution to change color [32] [33].

However, it is important to note that the effect of soda ash on the staining of polyphenols can vary depending on the concentration of soda ash, the specific nature of the polyphenols present and the other compounds present in the solution [34].



Picture 10. Control extract without bicarbonate.



Figure 11. Extract obtained after decoction and filtration of crushed leaves with baking soda at variable doses (0.1 to 1.0 g).

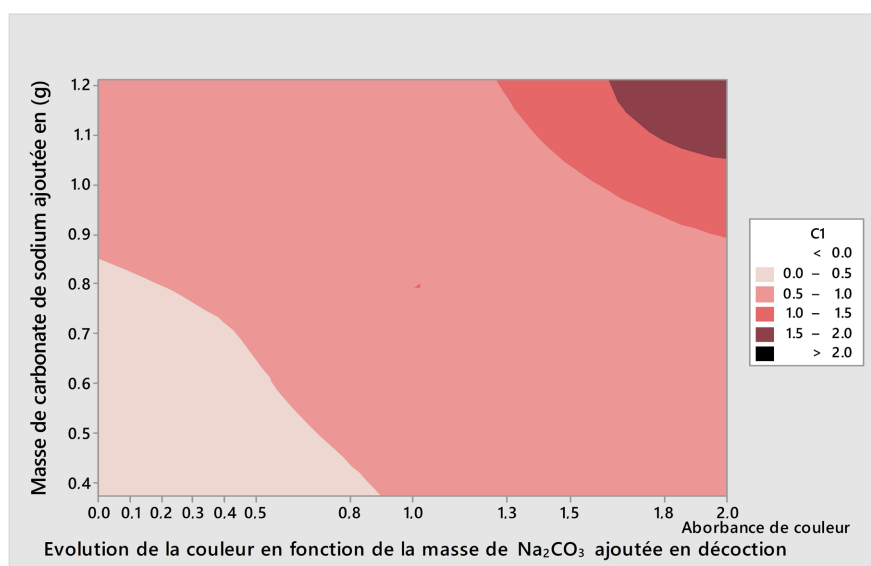


Figure 12. Coloring (whole leaves).

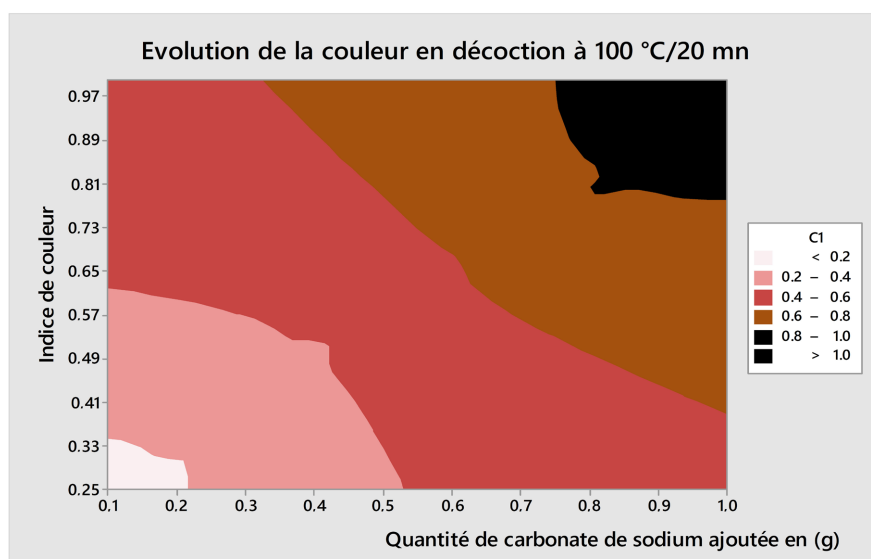


Figure 13. Coloring (crushed leaves).

3.3. Conductivity, pH and Soluble Solids

Table 1 and Table 2 show the changes in conductivity, pH and soluble solids during decoction with whole leaves and crushed leaves.

Soda ash is a chemical compound that affects electrical conductivity when dissolved in water [35]. However, this effect depends on the concentration of sodium carbonate in the solution and on the nature of the other solutes present. The conductivity increases proportionally with the addition of soda ash. It goes from $1349 \pm (4.03) \mu\text{s}/\text{cm}$ at 25°C to $3995 \pm (6.65) \mu\text{s}/\text{cm}$ with respectively 0.1 g and 1.0 g of Na_2CO_3 added.

When sodium carbonate dissolves in water, it dissociates into sodium ions (Na^+) and bicarbonate ions (HCO_3^-). These ions can conduct electricity in water

because they carry an electrical charge. Thus, the addition of sodium carbonate increases the electrical conductivity of the extracts [35] [36].

It is important to note that sodium carbonate is used to neutralize acids present in the solution, and this can lead to the formation of insoluble or precipitated products. These precipitates can add solid matter to the solution, increasing the dry matter content $0.8 \pm (0.01)$ to $1.00 \pm (0.01)$ g/100 g of extract for the addition of 0.1 g and 1 g of Na_2CO_3 . However, the solids content of the solution does not change significantly between the additions of 0.2 to 0.8 g Na_2CO_3 , because the dissolved ions do not contribute significantly to the total mass of the solution. The main modification observed will be the presence of sodium and bicarbonate ions, which can influence the chemical and physical properties of the solution, but not directly its dry matter content [37] [38].

Soda ash has a significant effect on the pH of the extracts obtained (Table 1 and Table 2). When it dissolves in water, it dissociates into bicarbonate ions. These ions can react with water to form additional hydroxide (OH^-) ions, which are bases. This reaction helps to increase the pH of the extracts [39] [40].

Table 1. Evolution of conductivity, pH and soluble solids during decoction with whole leaves.

| Whole leaves | Conductivity in $\mu\text{s}/\text{cm}$ at 25°C | pH at 25°C | Soluble solids g/100 g |
|--|---|--------------------------------|--------------------------------|
| Decoction + 0.1 g Na_2CO_3 | 1317.00 ^h \pm (6.08) | 7.71 ^d \pm (0.01) | 0.52 ^h \pm (0.00) |
| Decoction + 0.2 g Na_2CO_3 | 1374.66 ^g \pm (5.03) | 7.47 ^g \pm (0.02) | 0.56 ^g \pm (0.00) |
| Decoction + 0.3 g Na_2CO_3 | 1386.66 ^g \pm (4.93) | 7.37 ^h \pm (0.00) | 0.58 ^g \pm (0.00) |
| Decoction + 0.4 g Na_2CO_3 | 1699.66 ^f \pm (7.57) | 7.58 ^f \pm (0.01) | 0.58 ^h \pm (0.01) |
| Decoction + 0.5 g Na_2CO_3 | 1882.66 ^e \pm (23.9) | 7.62 ^e \pm (0.00) | 0.61 ^e \pm (0.01) |
| Decoction + 0.6 g Na_2CO_3 | 1971.33 ^d \pm (3.78) | 7.89 ^e \pm (0.00) | 0.63 ^d \pm (0.00) |
| Decoction + 0.7 g Na_2CO_3 | 2038.00 ^c \pm (2.64) | 7.91 ^c \pm (0.00) | 0.63 ^d \pm (0.00) |
| Decoction + 0.8 g Na_2CO_3 | 2212.00 ^b \pm (4.35) | 7.93 ^c \pm (0.01) | 0.65 ^c \pm (0.01) |
| Decoction + 0.9 g Na_2CO_3 | 2385.66 ^a \pm (7.76) | 7.98 ^b \pm (0.01) | 0.68 ^b \pm (0.01) |
| Decoction + 1.0 g Na_2CO_3 | 2650.00 ^d \pm (4.04) | 7.98 ^b \pm (0.01) | 0.73 ^a \pm (0.01) |

Table 2. Evolution of conductivity, pH and soluble solids during decoction with crushed leaves.

| Crushed Leaves | Conductivity in $\mu\text{s}/\text{cm}$ at 25°C | pH at 25°C | Soluble solids g/100 g |
|--|---|--------------------------------|--------------------------------|
| Decoction + 0.1 g Na_2CO_3 | 1349 ^j \pm (4.03) | 7.97 ⁱ \pm (0.01) | 0.8 ^c \pm (0.01) |
| Decoction + 0.2 g Na_2CO_3 | 1645 ⁱ \pm (5.11) | 8.34 ^h \pm (0.01) | 0.9 ^b \pm (0.01) |
| Decoction + 0.3 g Na_2CO_3 | 1179 ^h \pm (2.08) | 8.49 ^g \pm (0.01) | 0.9 ^b \pm (0.01) |
| Decoction + 0.4 g Na_2CO_3 | 2094 ^g \pm (4.34) | 8.71 ^f \pm (0.00) | 0.9 ^b \pm (0.01) |
| Decoction + 0.5 g Na_2CO_3 | 2240 ^f \pm (6.76) | 8.79 ^e \pm (0.00) | 0.9 ^b \pm (0.01) |
| Decoction + 0.6 g Na_2CO_3 | 2737 ^e \pm (6.67) | 8.91 ^d \pm (0.01) | 0.9 ^b \pm (0.01) |
| Decoction + 0.7 g Na_2CO_3 | 3017 ^d \pm (5.53) | 9.03 ^c \pm (0.01) | 0.9 ^b \pm (0.01) |
| Decoction + 0.8 g Na_2CO_3 | 3323 ^c \pm (5.93) | 9.07 ^b \pm (0.01) | 0.9 ^b \pm (0.01) |
| Decoction + 0.9 g Na_2CO_3 | 3604 ^b \pm (5.48) | 9.13 ^a \pm (0.01) | 1.00 ^a \pm (0.01) |
| Decoction + 1.0 g Na_2CO_3 | 3995 ^a \pm (6.65) | 9.14 ^a \pm (0.01) | 1.00 ^a \pm (0.01) |

4. Conclusion

The effect of sodium carbonate on the extraction by aqueous decoction of total polyphenols from the leaves of *Combretum Micranthum* was studied. It appears from this study, that the effect of sodium bicarbonate is not favorable to the extraction by aqueous decoction of the total polyphenols of the leaves of *Micranthum*. The pH plays an important role in the extraction of total polyphenols, as it can influence their solubility, their stability and their ability to interact with other compounds present in the extract obtained. It is important to note that sodium carbonate can also have an effect on the extraction of other compounds present in the leaves, such as sugars, organic acids, etc. This can influence the overall composition of the extract obtained. However, it is essential to point out that the effect of sodium carbonate on the extraction of polyphenols can depend on several factors, such as the concentration of sodium carbonate used, the extraction temperature, the duration of the decoction, the leaf particle size, etc. Specific experimental studies should be carried out to precisely evaluate its impact on the extracted polyphenols.

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Conflicts of Interest

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

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