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## Total polyphenols content and the antiradical activity leaves extracts of *Combretum micranthum* G. don harvested from different regions of Senegal (Diass, Sandiara and Essyl)

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**Abstract**

This study examines the total polyphenol content and the antiradical properties of extract from *Combretum micranthum* leaves harvested in Diass, Sandiara in west-central Senegal and in Essyl in the south, during the rainy season versus the after-rainy season. The total polyphenol content expressed in tannic acid equivalent per 100 g of dry matter during the rainy season and the after-rainy season are respectively: Sandiara:  $8.5 \pm 1.31\%$  vs  $10.44 \pm 2.19\%$ ; Essyl:  $10.53 \pm 4.6\%$  vs  $17.17 \pm 0.73\%$ ; Diass:  $13.57 \pm 1.7\%$  vs  $16.37 \pm 0.69\%$ . The IC<sub>50</sub> of Diass, Sandiara and Essyl leaf extracts are in rainy versus after-season periods respectively  $117.83 \pm 8.93 \mu\text{g} / \text{ml}$  vs  $59.5 \pm 4.13 \mu\text{g} / \text{ml}$ ;  $155 \pm 4.6 \mu\text{g} / \text{ml}$  vs  $119.9 \pm 10.78 \mu\text{g} / \text{ml}$ ;  $150.5 \pm 3.36 \mu\text{g} / \text{ml}$  vs  $136.17 \pm 6.54 \mu\text{g} / \text{ml}$ . The antiradical power is in favor of the after-rain season.

**Keywords:** *Combretum micranthum*, kinkéliba, antiradical activity, total polyphenols, harvest period

**Introduction**

Polyphenols are a family of organic molecules widely present in the plant kingdom. They are becoming increasingly important, particularly because of their beneficial effects on health. Indeed, their role as natural antioxidants that help fight against the damage caused by free radicals is attracting more and more interest in the prevention and treatment of certain diseases [1]. This role of shield to prevent the oxidation of cells, led us to study the relationship between the total polyphenols content and the antiradical activity of the leaves extracts of *Combretum micranthum* named locally kinkeliba, a plant widely used in traditional medicine in Senegal [2]. This plant is found throughout the country from north to south. This work aims to compare the samples collected at different sites: Diass and Sandiara (Thiés Region) in the center of the country and Essyl (Ziguinchor Region) in the extreme south. Two harvest periods were observed: in the rainy season (RS) and in the after-rain season (ARS).

**Material and Methods****Plant Material**

Series of harvests were carried out during the RS and ARS at the different sites: Sandiara: 2016 RS (July, August, September); ARS 2016-2017 (October, December, January); Diass: RS 2016 (July and September), ARS 2017 (January); Essyl: RS 2016 (August), ARS 2017 (January). The leaves are then dried in the shade and then reduced to powder. The water content is evaluated by loss on drying.

**Polyphenol Content**

The polyphenol content is estimated by the Folin-Denis method [3]. 20 g of leaf powder are extracted by decoction with three times 250 ml of ethanol for 15 min at 95 ° C. The extract thus obtained is completed to 1 liter and then diluted to 1/100. To 10 ml of this diluted solution is added 2 ml of the Folin-Denis reagent and 3 minutes later 2 ml of a saturated solution of sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>). After centrifugation of the tubes for 4 minutes at 4000 rpm, reading of the absorbance is made at 670 nm.

**DPPH Test**

The determination of the antiradical activity by the DPPH test (2, 2-diphenyl-1-picrylhydrazyl) was carried out using the method of Molyneux [4].

Prepare the DPPH<sup>•+</sup> radical by dissolving 4 mg of the DPPH reagent in 100 ml of 95 ° ethanol. After complete dissolution, the solution is stored in the dark at 4 ° C for at least 16 hours. Measure the absorbance at 517 nm of the DPPH solution thus prepared before testing. If the absorbance value is over 1, make dilutions so that to obtain values under 1. It corresponds to the value of the absorbance at time To (A0). Prepare a stock solution of 250 µg / ml of each drug then a dilution series will be made to obtain the following concentrations: 250 - 125 - 62.5 - 31.25 - 15.62 - 7.81 µg / ml. In each test tube containing 50 µl of extract at a given concentration, 950 µl of the previously diluted DPPH solution is added. Incubate the tubes for 30 minutes, protected from light and at room temperature. Measure the absorbances at 517 nm. The determination of IC50 (concentration of extract required to

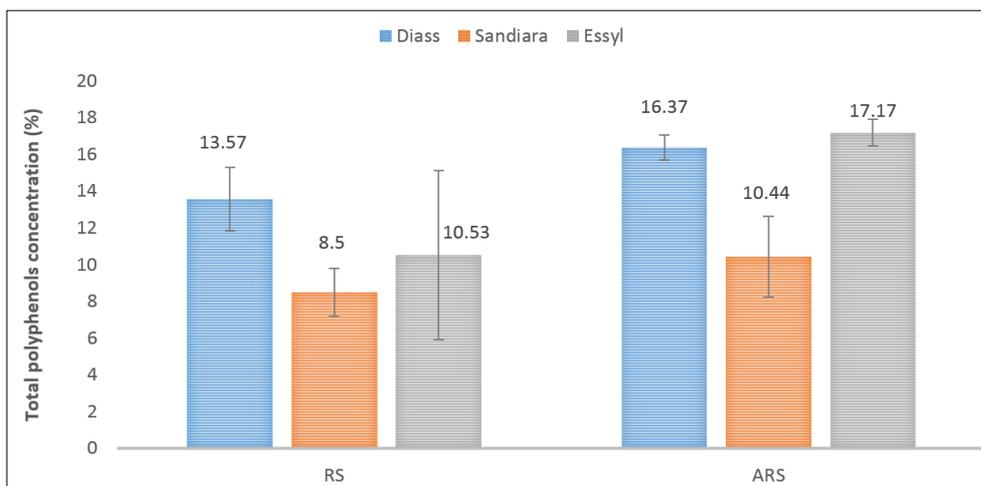
trap 50% of free radicals) is performed using the Statgraphics software.

### Statistical Analyzes

Significance tests are performed by the Fisher test using the Stat View software. A value of  $p < 0.0001$  is considered statistically significant.

### Results

The total polyphenol contents expressed in tannic acid equivalent per 100 g of dry matter (TDS / 100 g) during the RS versus the ARS are respectively: Sandiara  $8.5 \pm 1.31\%$  vs  $10.44 \pm 2.19\%$ ; Essyl  $10.53 \pm 4.6\%$  vs  $17.17 \pm 0.73\%$ ; Diass  $13.57 \pm 1.7\%$  vs  $16.37 \pm 0.69\%$  (see Figure 1).



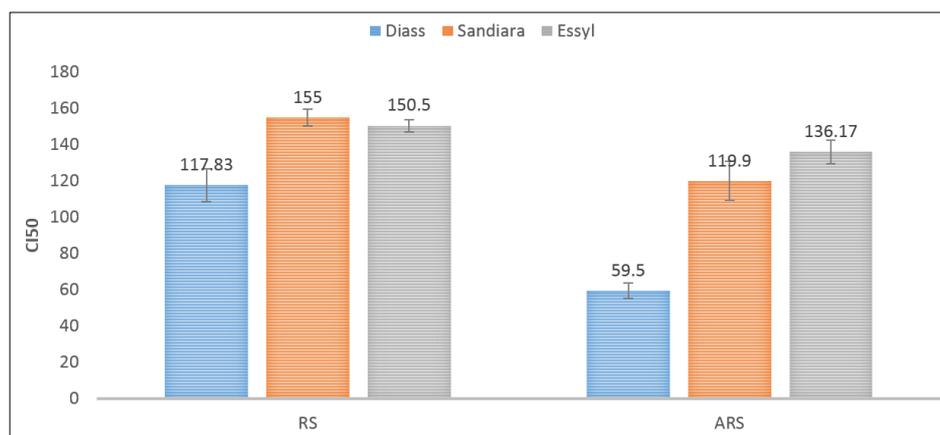
RS: Rainy season; ARS: After Rainy Season

Fig 1: Variation of total polyphenol content according season in Diass, Sandiara and Essyl.

The polyphenol concentrations of the different samples is obtained from the equation of the regression line of the tannic acid standard curve:  $y = 47.433x - 0.0158$ ;  $R^2 = 0.9911$ .

The IC50 of the leaves harvested in Diass, Sandiara and Essyl

during the RS versus the ARS are respectively  $117.83 \pm 8.93$  µg / ml vs  $59.5 \pm 4.13$  µg / ml;  $155 \pm 4.6$  µg / ml vs  $119.9 \pm 10.78$  µl / ml;  $150.5 \pm 3.36$  µg / ml vs  $136.17 \pm 6.54$  µg / ml (see Figure 2).



RS: Rainy Season; ARS: After Rainy Season.

Fig 2: Evaluation of the IC50 of DPPH on *C. micranthum* leaves extracts according to season in Diass, Sandiara and Essyl.

### Discussions

According to Bidie *et al.* [5], any biological activity is a function of the presence of certain metabolites within the tissues of the plant. Thus they showed that *Distemonanthus benthamianus*, *Chrysophyllum perpulchrum*, *Mitragyna ciliata*, *Trichilia prieriana*, *Sherboumia bignoniifolia*, *Parquetina nigrescens*, *Ageratum conyzoides* and *Milletia*

*zechiana* contain polyphenols and flavonoids, metabolites responsible for antioxidant and antiradical activity. They found that the two plants with the highest levels of total phenols: *Chrysophyllum perpulchrum* ( $74.08 \pm 1.8$  mg / g) and *Distemonanthus benthamianus* ( $70.17 \pm 0.84$  mg / g) had the strongest antiradical activities expressed in IC50 which are respectively  $4.00 \pm 0.288$  µg / ml and  $4.50 \pm 0.288$  µg /

ml). The results obtained with *Millettia zechiana* and *Ageratum conyzoides* with IC50 respectively equal to  $96 \pm 0.577 \mu\text{g} / \text{ml}$  and  $76 \pm 0.577 \mu\text{g} / \text{ml}$ , are closer to those obtained for kinkeliba leaves; with total polyphenol contents below 30 mg / gms. Similarly, a correlation between total phenol contents and antiradical activity has been shown by Hatano *et al.* [6], and later by Chen and Ho [7]. According to the latter the functional groups present in phenolic compounds in general can easily give up an electron or a proton to neutralize free radicals.

### Conclusion

This study will allow us to choose the best period of harvesting kinkeliba leaves so to enjoy the benefits of antioxidant properties of their high polyphenols level.

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