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# Determination of Total Polyphenols and Antioxidant Activity of Leaf and Bark Extracts of *Combretumglutinosum*

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**Abstract:** The emergence of certain diseases such as cardiovascular diseases, diabetes, cancer, resulting from diet or environmental pollution makes relevant researches for therapeutic solutions among which, medicinal plants. It is in this perspective that we must place this study which is about the evaluation of the total polyphenol contents and the determination of the antioxidant activity of leaf and bark extracts of Combretum glutinosum. The results has shown the presence of polyphenols in all the extracts with a higher content in the extract by infusion of the leaves, which is 258, 8mg gallic acid equivalent per gram. The study of the antioxidant activity has given low  $IC_{50}$  values compared to those of the reference antioxidants, namely ascorbic acid and quercetin. The lowest  $IC_{50}$  value was obtained with fractions of the leaf infusion extract and was 0.122 µg/ml with the ethyl acetate fraction and 2,370 µg/ml with the aqueous fraction for the DPPH and ABTS tests respectively.

Keywords: antioxidant, polyphenols, quercetin, Combretum

## 1. Introduction

Over the last decades, special attention has been put on the development of plants with medicinal value as sources of natural bioactive substances. So thus, many studies are increasingly interested in the therapeutic effects of naturally occurring antioxidants due to the potential toxicological risks of synthetic antioxidant molecules [1].

However, the number of plants studied for pharmacological activities remains low. Only 144 out of 6763 families of species known to the wild have been studied, resulting in 939 approved drugs, 369 clinical trials, 119 preclinical drugs and 19,721 natural bioactive products [2].

*Combretumglutinosum* (Combretaceae) selected from an ethnobotanical survey, is a plant traditionally used in medicine in Senegal and particularly in Saloum. Methanolic extracts from these leaves and bark have already shown interesting antibacterial activities *in vitro*[3],[4] and alsoantiplasmodial activities with bark extracts [5]. The leaves of this plant are consumed as herbal tea next to *Combretummicranthum* and *Vitexdoniana* especially for breakfast or breaking of fasting. Despite their widespread use, the leaves of Combretum glutinosum have not been studied to determine their antioxidant potential

The aim of this study is to measure the total polyphenols and to assess the antioxidant activity of the fractions of the aqueous and alcoholic extracts of the leaves and barks of *Combretumglutinosum*.

## 2. Materials and Methods

#### 2.1. Plant material

The plant material used is madeof leaves and bark of *Combretumglutinosum*.

#### 2.2. Preparation of plant material

The leaves and bark used in this study were harvested in Saloum (western Senegal). The plant material thus harvested were dried in the absence of light at ambient temperature and then ground into fine powder.

#### 2.3. Extraction and fractionation

#### 2.3.1. Barks

A test sample of 50 g of bark powder of *Combretumglutinosum* were macerated with 500 ml of hexane for 1 day. The mixture was filtered and the refined is dried before being re-macerated with 500 ml of methanol for 5 days [3].

A test sample of 5 g of the dried methanol extract wascompletely dissolved in 250 ml of pure water and then a liquid-liquid fractionation is carried out successively with the same volume of diethyl ether and chloroform. These three solutions are evaporated to dryness on a rotary evaporator before being used.

#### 2.3.2. leaves

A test sample of 50 g of leaf powder was infused into 500 ml of boiling water. After cooling, the mixture was filtered and evaporated to dryness on a rotary evaporator under vacuum.

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A sample of 5 g of the dry extract obtained by infusion is dissolved in 250 ml of water and then subjected to fractionation with ethyl acetate and chloroform successively.

The ethyl acetate, chloroform and aqueous fractions were evaporated to dryness and the residues obtained were used for the various tests.

#### 2.4. Determination of Total Polyphenols

The dosage of polyphenol was made according to the methoddescribed by Vermerius and Nicholson in 2006 [6]. 0.1 ml of aqueous extract solution at 1 mg / ml concentration was mixed with 2 ml of a 2% sodium carbonate solution Freshly prepared, the whole is agitated by a vortex [6]. After five minutes, 100  $\mu$ l of the Folin-Ciocalteu reagent (1 N) are added to the mixture, the whole is left for 30 minutes at room temperature and the reading is performed against a blank using a spectrophotometer (Perkin Elmer, Lambda 800 Spectrophotometer) at 700 nm. A calibration curve is carried out in parallel under the same operating conditions using gallic acid as a positive control. The results are denominated in micrograms gallic acid equivalent per milligram of the dry plant extracts ( $\mu$ g GAE / mg).

#### 2.5. Evaluation of the antioxidant power

#### 2.5.1. Experimental DPPH test protocol

Anquantity of 4 mg of DPPH powder was dissolved in 100 ml of ethanol and the solution obtained was kept away from light for 12 h.

The antioxidant activity is measured by putting 0.8 ml of the extract with 3.2 ml of the DPPH solution into the test tubes to obtain final concentrations ranging from 1 to 9  $\mu$ g/ml [7].

Ascorbic acid and quercetin, used as reference antioxidants, were also tested at the same concentrations using the same method.

The absorbance reading was made after 30 minutes at the spectrophotometer at the wavelength of 517 nm using ethanol as white. Three measurements of the absorbance were made.

#### 2.5.2. Experimental protocol of the ABTS test

Anquantity of 38.40 mg of ABTS is dissolved beforehand in 10 ml of water.

Anquantity of 6.75 mg of potassium persulfate is added thereafter. The mixture obtained was kept in the dark and at ambient temperature for 12 hours before use. This mixture is diluted with ethanol in order to obtain an absorbance of the order of 0.7 to 734 nm. The antioxidant activity was measured by mixing 0.8 ml of the extract dissolved in ethanol with 3.2 ml of the ABTS solution in order to obtain the concentrations varying from 1 to 9  $\mu$ g / ml.

Ascorbic acid and quercetin, used as reference antioxidants, were dissolved in ethanol and tested at the same concentrations. The absorbance reading was made after 2 minutes at the spectrophotometer at 734 nm using ethanol as white. Three absorbance measurements were performed for each concentration.

#### 2.5.3. Expression of results

Inboth methods described above, the antioxidant activity was denominated as the percentage inhibition (PI) of the absorbance of the radical which corresponds to:

$$PI = \frac{A0 - A1}{A0} \times 100$$

A0: absorbance of the solution of ABTS or pure DPPH A1: absorbance of the solution of ABTS or of DPPH  $^{\circ}$  after addition of the extract tested to a given concentration and after the reaction.

IC50 values are calculated from GraphPad Prism software (v5.0d, San Diego, CA) using a non-linear regression model using percent inhibition (PI) values.

## 3. Results

#### **3.1.** Quantification of total polyphenols

Table 1 shows the results obtained by a direct reading of the polyphenol contents of the extracts and fractions of the plant material. Average values are presented.

	Extracts or fractions	Quantity(mg GAE/g)
	Extract infusion	258,8
	Methanolic extract	155,2
leaves	Chloroform fraction	109
	Ethyl acetate fraction	266,4
	Aqueous fraction	267,7
	Methanolic extract	210,6
	Chloroform fraction	192,90
barks	Fraction with ethyl ether	49,4
	Aqueous fraction	232,1

## 3.2 Antioxidant activity

#### 3.2.1. Percent inhibition with the DPPH method

Table 2 shows the percent inhibition of aqueous and methanolic extracts, fractions (chloroform, ethyl acetate and water) of aqueous extracts of the leaves of *Combretumglutinosum* and the reference antioxidants (ascorbic acid and quercetin).

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[µg/ml]	MeOH	E.I.	Fr.CHCl <sub>3</sub>	Fr.AE	Fr. H <sub>2</sub> O	Asc. Ac.	Quercétine
1	9,16	0,30	70,68	58,92	10,60	32,22	46,36
2	16,85	3,95	81,69	86,05	16,52	45,79	66,79
3	27,06	11,66	88,21	92,50	28,04	61,78	83,16
4	37,75	30,84	93,60	92,82	53,67	72,92	90,02
5	46,45	58,25	94,08	92,99	74,55	85,45	90,05
6	54,53	73,69	94,29	93,10	84,85	89,33	90,55
7	63,00	84,32	94,72	97,01	87,46	91,69	90,63
8	72,04	87,06	98,24	97,85	88,24	93,24	90,67
9	80.4	87 50	98.45	98 25	88 58	94 78	90.73

Table 2: Percentages of inhibition of extracts and fractions of the extract by infusion of leaves tested with DPPH

MeOH: methanolextract; E.I: infusion extract; Fr CHCl3: chloroform fraction; Fr. AE: ethylacetate fraction; Fr. H2O: water fraction; AscAc: ascorbicacid

Table 3 represents the  $IC_{50}s$  corresponding to the various extracts.

**Table 3:**  $IC_{50}$  of extracts and fractions of infusate of leavestested with DPPH

Extracts	MeOH	E.I.	Fr.CHCl <sub>3</sub>	Fr.EA	Fr. H <sub>2</sub> O	Ac. Asc.	Quercétine
IC <sub>50</sub>	5,557	4,537	1,733	0,122	3,882	3,161	2,044
[µg/ml]							

MeOH: methanolextract; E.I: infusion extract; Fr  $CHCl_3$ : chloroform fraction; Fr. E.A: ethylacetate fraction; Fr.  $H_2O$ : water fraction; AscAc: ascorbicacid

Table 4 shows the percent inhibition of methanol extract, fractions (chloroform, ethyl acetate and water) of methanolic extracts from the bark of *Combretumglutinosum* and the reference antioxidants (ascorbic acid and quercetin).

**Table 4:** Percentage of inhibition of the methanol extract of the bark and its fractions tested with DPPH

[µg/ml]	MeOH	Fr.CHCl <sub>3</sub>	Fr.E.A	Fr.	Asc.	Quercétine
				$H_2O$	Ac.	
1	11,09	0,92	3,59	14,33	32,22	46,36
2	18,34	31,11	11,62	34,19	45,79	66,79
3	27,06	50,12	14,33	74,39	61,78	83,16
4	35,65	76,21	36,05	88,11	72,92	90,02
5	46,67	87,12	50,00	89,12	85,45	90,05

-	32,87	87,79	63,46	89,26	89,33	90,55
7	60,00	88,63	76,33	89,28	91,69	90,63
8	67,17	88,77	82,60	89,29	93,24	90,67
9 '	74,40	89,11	85,09	89,32	94,78	90,73

MeOH: methanol extract;  $CHCl_3$ : chloroform fraction; Fr. AE: ethyl acetate fraction; Fr. H<sub>2</sub>O: water fraction; Ac. Asc: ascorbic acid

Table 5 represents the  $IC_{50}s$  corresponding to the various extracts.

<b>Table 5:</b> IC <sub>50</sub> of the methanolic extract of the bark and its
fractions tested by DPPH

Extracts	MeOH	Fr.CHCl <sub>3</sub>	Fr.AE	Fr. H <sub>2</sub> O	Ac. Asc.	Quercétine
$IC_{50}\left[\mu g/ml\right]$	5,493	2,567	4,980	2,370	3,161	2,044

MeOH: methanol extract;  $CHCl_3$ : chloroform fraction; Fr. AE: ethyl acetate fraction; Fr.  $H_2O$ : water fraction; Ac. Asc: ascorbic acid

#### 3.2.2. Percent inhibition with the ABTS method

Table 6 shows the percentage inhibition of aqueous and methanolic extracts, fractions (chloroform, ethyl acetate and water) of aqueous extracts of the leaves of *Combretumglutinosum* and the reference antioxidants (ascorbic acid and quercetin).

Table 6: Percentage of inhibition of the extracts and fractions of the extract by infusion of leaves tested with ABTS

[µg/ml]	MeOH	E.I.	Fr.CHCl <sub>3</sub>	Fr. E.A	Fr. $H_2O$	Asc. Ac.	Quercétine
1	36,77	64,30	58,29	66,62	66,68	76,96	93,49
2	53,67	77,30	64,84	77,60	76,09	74,27	99,41
3	72,58	87,00	69,53	89,88	86,34	76,63	99,28
4	87,12	94,64	76,25	95,05	93,46	87,02	99,36
5	99,09	98,66	83,28	99,49	97,83	93,85	99,50
6		99,24	87,28	99,50	99,35	95,17	99,61
7		99,30	92,90	99,52	99,39	99,63	99,62
8		99,38	94,55	99,55	99,41	99,77	99,64
9		99,58	95,07	99,81	99,43	99,83	99,75

MeOH: methanolextract; E.I: infusion extract; Fr  $CHCl_3$ : chloroform fraction; Fr. E.A: ethylacetate fraction; Fr.  $H_2O$ : water fraction; AscAc: ascorbicacid

Table 7 represents the  $IC_{50}s$  corresponding to the various extracts.

 Table 7: IC<sub>50</sub> of extracts and fractions of infusion extract of leaves tested by ABTS

	[µg/ml]	MeOH	I.T	Fr.CHCl <sub>3</sub>	Fr.E.A	Fr. H <sub>2</sub> O	Ac. Asc.	Quercétine			
	IC <sub>50</sub>	3,683	2,349	4,638	2,371	2,624	4,164	0,3351			
ľ	MeOH: methanolextract; E.I: infusion extract; Fr CHCl <sub>3</sub> :										
(	hlorof	orm fra	action	; Fr. E.A	ethy!	lacetate	fraction	; Fr. H <sub>2</sub> O:			

chloroform fraction; Fr. E.A: ethylacetate fraction; Fr.  $H_2O$ : water fraction; AscAc: ascorbicacid

Table 8 shows the percentage inhibition of methanol extract, chloroform, ethyl acetate and water fractions of methanolic

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extracts from the bark of *Combretunglutinosum* and the reference antioxidants (ascorbic acid and quercetin).

$[\mu g/ml]$	MeOH	Fr.CHCl <sub>3</sub>	Fr.EA	Fr. H <sub>2</sub> O	Asc. Ac.	Quercétine
1	59,38	65,06	55,82	68,73	76,96	93,49
2	67,49	74,57	59,01	80,74	74,27	99,41
3	74,48	83,14	61,55	91,56	76,63	99,28
4	81,56	91,11	65,45	97,66	87,02	99,36
5	85,47	96,17	67,88	99,49	93,85	99,50
6	90,79	98,94	68,98	99,51	95,17	99,61
7	95,43	99,32	72,56	99,53	99,63	99,62
8	97,47	99,34	75,34	99,58	99,77	99,64
9	99,04	99,68	76,00	99,94	99,83	99,75

**Table 8:** Percentage inhibition of the methanol extract of the bark and its fractions tested with ABTS

MeOH: methanol extract;  $CHCl_3$ : chloroform fraction; Fr. AE: ethyl acetate fraction; Fr.  $H_2O$ : water fraction; Ac. Asc: ascorbic acid

Table 9 shows the  $\mathrm{IC}_{50}\mathrm{s}$  corresponding to the various extracts.

**Table 9:**  $IC_{50}$  of the methanolic extract of the barks and its<br/>fractions tested by ABTS

Extraits	MeOH	Fr.CHCl <sub>3</sub>	Fr.AE	Fr. H <sub>2</sub> O	Ac. Asc.	Quercétine
$IC_{50}[\mu g/ml]$	4,851	2,842	12,11	2,224	4,164	0,3351

MeOH: methanol extract;  $CHCl_3$ : chloroform fraction; Fr. AE: ethyl acetate fraction; Fr.  $H_2O$ : water fraction; Ac. Asc: ascorbic acid

## 4. Discussion

Numerous scientific studies have attributed the antioxidant power of plant extracts to the presence of polyphenols in plant matter [8], highlighting the importance of quantifying these secondary metabolites.

In this study, the polyphenols are quantified using the Folin-Denis reagent consisting of a mixture of phosphomolybdic acid, sodium tungstate and phosphoric acid. It is reduced during the oxidation of the phenols to a mixture of blue oxides of tungsten and molybdenum [9].

The results showed that the aqueous extract (258.8 mg AGE/g) is richer in polyphenols than that on methanol (155.2 mg GAE/g). For the fractions of the infusate, the polyphenols are more present in the ethyl acetate fraction and in the aqueous fraction (Table 1). with regard to theextracts and bark fractions, the polyphenols are more present in the aqueous fractions (232.10 mg GAE/g) and chloroform (192.90 mg EGA/g). The lowest content was found in the ethyl ether fraction (49.4 mg GAE/g).

These high levels of polyphenols presume an interesting antioxidant activity of the extracts and fractions of barks and leaves which we sought to determine by the ABTS and DPPH methods.

As per the DPPH method, the infusion extract of leaves is more reducer than the methanol extract. In the same way, the ethyl acetate fraction was found to be more reductive than the chloroform fraction (Table 2).  $IC_{50}$  values lower than those of the reference antioxidants were obtained. In fact, they were in the order of  $0.122\mu g/ml$  for the ethyl acetate fraction and  $1.733\mu g/ml$  for the chloroform fraction, while those of the reference antioxidants, namely quercetin and ascorbic acid, were  $3.161 \mu g/ml$  and  $2.04 \mu g/ml$  (Table 3), respectively.

With the bark, the chloroform and aqueous fractions are the most inhibitory (Table 4) with respective  $IC_{50}$  values of 2.567µg/ml and 2.70µg/ml higher than those of ascorbic acid but lower than that of quercetin (Table 5).

The antioxidant power tested with the ABTS method showed that the leaf infusion extract is more antioxidant than the methanol extract (Table 6). It has also been demonstrated that among the fractions obtained from this extract by infusion that of ethyl acetate ( $IC_{50}$  of 2.37 µg/ml) is more reductive followed by the aqueous fraction (2.62µg/ml) (Table 7). These  $IC_{50}$ s are markedly lower than those of ascorbic acid (4.16 µg/ml) and quercetin (0.33 µg/ml).

As per the ABTS method, the aqueous and chloroform fractions of Combretum glutinosum peel extracts showed the most interesting antioxidant potency (Table 8) with  $IC_{50}$  values of 2.22 µg/ml and 2.84 µg/ml respectively. These values are lower than those of ascorbic acid (Table 9).

The results obtained fromall the tests has shown a proportional relationship between the antioxidant power and the total polyphenol content of the extracts and subsequently confirm previous studies [8]. Of the two parts of the plant, the leaves have an antioxidant activity greater than that of the bark, but also the extract by infusion is more antioxidant than the alcoholic extract, which is crucial since the leaves are consumed by infusion for most of the time.

Many studies have shown the importance of polyphenols in the prevention of cancer [10]. Indeed, this explains the high consumption of tea (*Camelliasinensis*) because of its polyphenols [11]. In Jo studies, tea (*Camelliasinensis*) has a much lower antioxidant power than *Combretumglutinosum* by comparing the IC<sub>50</sub> values of the same types of extracts with that of ascorbic acid used as a reference in both cases [12]using the same methods of studying antioxidant activity as those in our study.

## 5. Conclusion

*Combretumglutinosum* is a widely used plant in Senegal both for its medicinal virtues and for its qualities as food. The interesting results from this study validate the traditional use of this plant. At the same timeit highlights its therapeutic interest in the prevention of certain diseases (cancer). This study has shownthat the exploration of traditional Senegalese pharmacopoeia couldhelp to overcomemany health issuesand alsoprovide economic exploitation of local flora.

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