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Phytochemical analysis, investigation of antioxidant and anti-inflammatory activities of ethanolic and aqueous extracts of roots of *Combretum glutinosum* Perr. ex DC from Côte d'Ivoire

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RESEARCH ARTICLE

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ABSTRACT

The phytochemical, antioxidant, and anti-inflammatory potential of root (ethanol and aqueous) extracts of *Combretum glutinosum* was investigated in this study. Their antioxidant activity was determined using an *in vitro* DPPH radical scavenging activity assay. The ethanol extract had the lowest IC_{50} (0.055 mg/mL), which is comparable to vitamin C. Phytochemical screening of extracts revealed the presence of sterols and polyterpenes, polyphenols, alkaloids, flavonoids, catechin tannins, gallic tannins, saponosides, terpenoids, mucilages, anthocyanins, volatile oils, and cardiac glycosides. The extracts significantly inhibit the development of paw edema induced by carrageenan. Anti-inflammatory studies showed that the inflammation inhibition potential of 200 mg/kg body weight of all extracts was significantly lower than the standard diclofenac (20 mg/kg) in the first hours. At the third hour, the inflammation inhibition potential of ethanolic and aqueous extracts was significantly higher than that of the standard. This study revealed that *Combretum glutinosum* extracts have anti-inflammatory effects and can act as an effective antioxidant.

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1. Introduction

Oxidative stress is implicated in a wide spectrum of diseases that have a huge impact on the health of populations [1]. Aerobic metabolism in mammals generates substances called reactive oxygen species (ROS) that are involved in physiological processes. However, the excess production of ROS can become toxic for the major components of the cell, lipids, proteins, and nucleic acid and therefore give rise to oxidative stress [2]. The latter is implicated in various pathologies such as cardiovascular diseases, cancers, diabetes, *etc*. An antioxidant is defined as any substance capable, at a relatively low concentration, of competing with other oxidizable substrates and thus delaying or preventing the oxidation of these substrates [3]. Inflammation is the body's defense mechanism against attacks of physical, chemical, biological, or infectious origin, essential to its integrity [4]. This protective immune response can sometimes be harmful due to the aggressiveness of the pathogen, its persistence, and abnormalities in the regulation and production of cells involved in inflammation [5]. These inflammatory processes are involved in the appearance of a large number of human pathologies such as arthritis, diabetes, asthma, allergies, and cancer. Inflammation and its associated pathologies are becoming an increasingly a major health problem for both the majority of people who suffer from it and the different forms in which they manifest themselves [6]. Their treatment is often based on the intake of nonsteroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids. These molecules have harmful side effects on the body, especially in the event of long-term use, particularly in the treatment of chronic inflammation. Taking anti-inflammatories often presents gastrointestinal risks (peptic ulcer, perforation, stenosis), renal risks such as acute renal failure, and sometimes cardiac complications. The search for new medical molecules without the risk of side effects is essential for the treatment of subjects. This is why the emphasis is increasingly placed on the search for new molecules endowed with anti-inflammatory activities in medicinal plants [4-6]. Furthermore, very few studies have been carried out on the anti-inflammatory activity of *Combretum glutinosum*. Therefore, this research work aims to assess the antioxidant power, anti-inflammatory, and phytochemical activities of aqueous and ethanolic extracts. Previous work carried out on various organs of *Combretum glutinosum* showed that they contained mostly phenolic compounds with a high antioxidant potential, corroborating their traditional use.

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Table 1. Usual phytochemical screening methods.

Secondary metabolite	Reagent of identification	Indicator (positive reaction)
Sterols and polyterpenes	Acetic anhydride acid and H_2SO_4	Colors from purple to blue or green
Polyphenols	$FeCl_3$ (2%)	Dark blue or greenish color
Flavonoids	Hydrochloric alcohol, magnesium shavings, and iso-amyl alcohol	Pink-orange or purplish color
Catechin tannins	Formalin and HCl	Gelatinous precipitate
Gallic tannins	Sodium acetate and $FeCl_3$	Blue-black color
Free quinones	NH_4OH	Red to purple color
Saponosides	Foam index	Persistent foam
Alkaloids	$HgCl_2$ and KI (Mayer)	Reddish-brown precipitate
	Picric acid (Hager), I_2, and KI (Wagner)	Creamy-white precipitate
Coumarins	KOH and HCl	Precipitate
Anthraquinones	NH_4OH	Yellow color
Terpenoids	$CHCl_3$, H_2SO_4	Brown color
Mucilage	Absolute ethanol	Flocculent precipitate
Anthocyanin	H_2SO_4 and NH_4OH	Black color
Volatile oils	NaOH and HCl	Black color
Cardiac glycosides	$CHCl_3$, H_2SO_4	Brown color

This plant is used in traditional medicine against skin, oral and urogenital infections, inflammatory diseases such as hepatitis, rheumatism, bronchitis, and blood disorders [7-9]. To our knowledge, no study has been carried out on the antioxidant potential of *Combretum glutinosum* from Côte d'Ivoire as well as its anti-inflammatory activity.

2. Experimental

2.1. Plant material collection and identification

The plant material includes roots of *Combretum glutinosum* (Combretaceae) harvested at Abatta in the city of Abidjan, Côte d'Ivoire. The roots were harvested in October 2019 and identified at the Botanical Garden of Felix Houphouët Boigny University in Côte d'Ivoire. This specimen has been listed in the herbarium index of the Floristic Center of Côte d'Ivoire under the number CNF 6127.

2.2. Animals

Albino rats of the Wistar strain, male and female, were raised at the Animal Facility of the University Jean Lorougnon Guédé (UJLoG) where the average temperature varies between 25-26 °C, with a photoperiodic cycle of 12 hours of light/ darkness. The rats are housed in plastic cages and were fed with pellets provided by Ivorian Compound Food Manufacturing Company added to dry bread from surrounding bakeries. They received tap water in baby bottles as drinking water. The litter used is sawdust, renewed twice a week to ensure the good hygienic condition of the animals.

2.3. Preparation and extraction of plant material

The roots of *Combretum glutinosum* were cleaned and dried in the shade for four weeks. They were pulverized using an electric grinder. The aqueous or ethanolic extract of the plant was obtained from 50 g of powder dissolved in 500 mL of distilled water or ethanol and homogenized using a blender for 20 minutes at room temperature at a speed of 2500 rpm. After a period of settling for 5 minutes, the supernatant was filtered through a white cloth and then three times through cotton wool. The filtrate was dried in a Med Center Venticell oven at 55 °C for 48 hours to obtain the dry extract [10].

2.4. Study of phytochemical screening

Phytochemical analyzes were performed at the Laboratory of Environmental Sciences and Technology. Preliminary phyto-chemical tests were carried out on the aqueous and ethanolic extracts of the roots of *Combretum glutinosum* according to the tube staining method (Table 1) [11].

2.5. DPPH radical reduction test

The method for measuring antioxidant power with 2,2-diphenyl-1-picrylhydrazyl (DPPH) is based on the ability of a compound to reduce the DPPH· radical. The reduction results in a changed in color of the solution which turns from purple to yellow in the presence of an antiradical compound. The reaction is then quantified by measuring the absorbance of the solution by spectrophotometry at 520 nm. The change in color from purple to yellow is proportional to antioxidant power [12]. The ability of the extracts to reduce DPPH free radicals was determined by the spectrophotometric method described by Rakiatou [2014]. DPPH (4 mg) was dissolved in 100 mL of methanol and 50 mg of each sample in 10 mL of DMSO (5 mg/mL). A series of ten successive dilutions was carried out on a 96-well microplate from the stock solution (5 mg/mL) of samples. A test plate was prepared according to the same configuration as the plate of the dilution range of the substance to be studied. Trolox served as a control for the evaluation of antioxidant activity. After 30 minutes of incubation, the absorbance is read at 520 nm against a blank using a UV-VIS spectrophotometer and the concentration necessary to degrade 50% of the DPPH radical (IC_{50}) was determined [13,14]. The experiments were performed in triplicates and the ability to scavenge the DPPH radical was expressed as percentage inhibition and calculated using the Equation (1).

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Abs. of the control} - \text{Abs. of the sample}}{\text{Abs. of the control}} \times 100 \quad (1)$$

2.6. In vivo anti-inflammatory activity determination of the extracts (Carrageenan-induced paw edema model)

The rats were divided into eight groups (n = 6), each receiving distilled water (control), diclofenac sodium 20 mg/kg p.o. (reference standard) and 50, 100, 200 mg/kg p.o. dose of the aqueous and ethanolic extracts of *Combretum glutinosum*, respectively. Carrageenan (0.1 mL of 1%) was injected into the subplantar tissue of the right hind-paw of each rat. The volume of carrageenan injected into the foot was measured at 0, 30, 60, 120, and 180 minutes using a plethysmometer. The method of Kajal *et al.* was employed [15]. The percentage inhibition (PI) at each time interval was calculated:

$$\% \text{ Inhibition (PI)} = \frac{(Vt-Vo)\text{control} - (Vt-Vo)\text{treated}}{(Vt-Vo)\text{control}} \times 100 \quad (2)$$

where Vt = Volume of the paw edema in a particular time interval (t), Vo = Volume of the paw before induction of inflammation (0 hour), (Vt-Vo)control = Volume of the edema of the control group of rats, (Vt-Vo)treated = Volume of the edema in the group of treated rats.

Table 2. Percentage yield of the aqueous root extract of *Combretum glutinosum* (AECG) and the ethanolic root extract of *Combretum glutinosum* (EECG).

Extract	Mass (g)	Yield (%)
EECG	5.25	10.50
AECG	7.65	15.30

Table 3. The qualitative determination of phytochemicals in ethanolic and aqueous extracts of *Combretum glutinosum.*

Secondary metabolite	Ethanolic extract	Aqueous extract
Sterols and polyterpenes	+	+
Polyphenols	+	+
Flavonoids	+	+
Catechin tannins	+	+
Gallic tannins	+	+
Free quinones	+	+
Saponosides	+	+
Alkaloids	+	+
Coumarins	–	–
Anthraquinones	+	+
Terpenoids	+	+
Mucilages	+	+
Anthocyanins	+	+
Volatile oils	+	+
Cardiac glycosides	+	+

(+) = Present, (–) = Absent.

Table 4. DPPH inhibitory concentration (IC_{50}) of the extracts of *Combretum glutinosum.*

Extract	IC_{50} values (mg/mL)	Scavenging activity at 0.10 mg/mL (%)
Ethanolic (EECG)	0.055	76.55±1.67
Aqueous (AECG)	0.064	64.25±4.78
Vitamin C	0.043	80.25±1.94

2.7. Statistical analysis

The results were expressed as a mean accompanied by standard errors on the mean (Mean±SEM). The graphical representation of the data was carried out using the software Graph Pad Prism 7.0 (Microsoft USA). The statistical analysis of the results was carried out using the analysis of variance (ANOVA ONE WAY). Differences between means were determined according to Dunnet's comparison test, $p < 0.05$ is considered significant.

3. Results and discussion

3.1. The percentage yield of Combretum glutinosum extract

The percentage yield of ethanolic and aqueous extracts of *Combretum glutinosum* is presented in Table 2. Aqueous extract had the highest yield (15.30%), ethanolic extract had a yield of 10.50%. This showed that the percentage yield of crude *Combretum glutinosum* root extracts increased as the polarity of the solvent of extraction used increased.

3.2. Qualitative determination of phytochemicals in aqueous and ethanolic extracts of Combretum glutinosum

The qualitative determination of phytochemicals presents in the ethanolic and aqueous extracts of *Combretum glutinosum* is presented in Table 3. Result showed sterols and polyterpenes, polyphenols, flavonoids, catechin tannins, gallic tannins, saponosides, alkaloids, free quinone, anthraquinones terpenoids, mucilages, anthocyanins, volatile oils, and cardiac glycosides were present in all extracts. Coumarins were absent in all extracts.

3.3. The free radical scavenging ability of extracts on DDPH

The results in Table 4 present the 50% inhibitory concentration (IC_{50}) values, the equation formula, and DPPH radical scavenging activities at 1.00 mg/mL of ethanolic and aqueous extracts of *Combretum glutinosum*. The result shows that the ethanol extract has an IC_{50} value (0.055 mg/mL) and an aqueous (0.064 mg/mL) compared with vitamin C, which had an IC_{50} of 0.043 mg/mL. The percentage of free radical scavenging activity of all extracts showed that the ethanol extract had the highest percentage of scavenging activity at all concentrations, although it was significantly lower than vitamin C scavenging activity (Figure 1). The free radical scavenging activity of the different extracts is in the following order: AECG < EECG < Vitamin C.

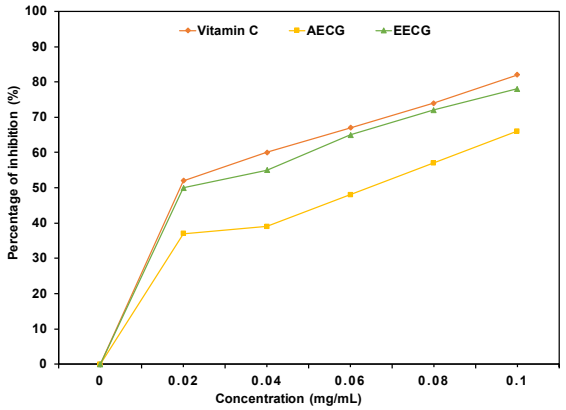

Figure 1. DPPH radical scavenging activities of ethanolic and aqueous extracts of *Combretum glutinosum.*

3.4. Anti-inflammation studies of Combretum glutinosum extracts (Carrageenan-induced paw edema model)

The aqueous and ethanolic extracts of the roots of *Combretum glutinosum* (50, 100, 200 mg/kg, p.o.) showed a dose-dependent, significant inhibition of carrageenan-induced rat paw edema from 0.5 to 3 hours following drug administration, compared to the control group. The maximum percentage inhibition (PI) of paw edema by the aqueous extract was observed as 66.67, 67.70, and 69.80% at doses of 50, 100, and 200 mg/kg p.o., respectively. The maximum PI of paw edema from the ethanolic extract was observed as 65.62, 70.83, and 72.92% at the doses of 50, 100, and 200 mg/kg p.o., respectively. Diclofenac sodium 20 mg/kg p.o. showed a maximum PI of 64.58% at 3 hours after its administration (Table 5). Aqueous and ethanolic extracts (200 mg/kg) of *Combretum glutinosum* inhibit carrageenan-induced acute inflammation (Figure 2).

Table 5. Effect of AE and EE of the roots of *Combretum glutinosum* on carrageenan-induced paw edema in rats.

Paw volume (mL)	Percentage inhibition (%)					
Groups	**Before**	**0 h**	**0.5 h**	**1 h**	**2 h**	**3 h**
Distilled water	0.99±0.01	1.03±0.02	1.72±0.02	1.90±0.01	2.53±0.03	1.96±0.01
Diclofenac, 20 mg/kg	1.00±0.01	1.02±0.01	1.47±0.03 (34.78)	1.53±0.03 (41.37)	1.62±0.02 (60.00)	1.36±0.01 (64.58)
AECG, 50 mg/kg	0.99±0.02	1.01±0.03	1.62±0.03 (10.14)	1.74±0.01 (16.09)	1.87±0.03 (42.66)	1.33±0.03 (66.67)
AECG, 100 mg/kg	0.99±0.02	1.02±0.03	1.55±0.02 (23.18)	1.64±0.03 (28.73)	1.77±0.03 (50.00)	1.33±0.01 (67.70)
AECG, 200 mg/kg	0.99±0.01	1.03±0.02	1.67±0.03 (07.24)	1.65±0.02 (27.58)	1.84±0.02 (46.00)	1.32±0.01 (69.80)
EECG, 50 mg/kg	0.99±0.01	1.02±0.03	1.56±0.01 (21.73)	1.64±0.03 (28.73)	1.73±0.03 (50.00)	1.35±0.01 (65.62)
EECG, 100 mg/kg	0.99±0.01	1.02±0.01	1.65±0.02 (08.69)	1.67±0.03 (25.29)	1.76±0.03 (52.66)	1.30±0.01 (70.83)
EECG, 200 mg/kg	0.99±0.01	1.03±0.02	1.64±0.03 (11.59)	1.64±0.02 (29.88)	1.76±0.01 (51.33)	1.29±0.01 (72.92)

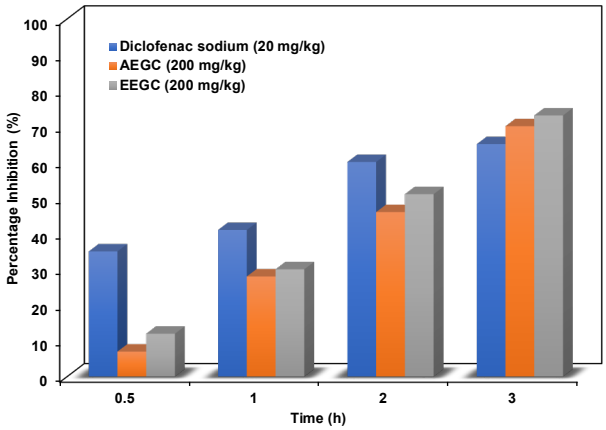

Figure 2. Effect of AECG and EECG of the roots of *Combretum glutinosum* on inflammation induced by carrageenan in rats.

This inhibition is much pronounced 0.5 hours after plantar injection of carrageenan with maximum values observed 3 hours after administration (Figure 2). However, these effects are more important than those observed with diclofenac, a non-steroidal anti-inflammatory used in the study as a reference drug. Indeed, carrageenan is a monosaccharide whose administration intraplantar to rats causes acute inflammation that induces edema, all under the influence of vasoactive mediators. The EACG and EECG (200 mg/kg) inhibit the progression of edema to varying degrees. This suggests that it interferes with the effects which inhibit the release of mediators involved in these phases of inflammation.

4. Discussion

The phytochemical study revealed that the aqueous and ethanolic extract of *Combretum glutinosum* contains most of the chemical groups sought, namely, polyphenols, flavonoids, tannins, saponins, steroids, and alkaloids. These results agree with those of Dimobe *et al*. [7] and Sene *et al*. [8], which in addition to these compounds, highlighted other compounds such as anthraquinones and anthocyanins which were not sought in this study. These molecules are involved in the management of several diseases such as tumors, cancer, diabetes, inflammation, sickle cell disease, and oxidative stress [12].

Many plant antioxidant potentials are related to their therapeutic potentials [13]. A higher DPPH radical scavenging activity is associated with a lower IC_{50} value. Therefore, the ethanolic extract had the highest DPPH-reducing activity based on its relatively low IC_{50} values which was comparable with vitamin C, a difference was observed between its IC_{50} (EEGC, 0.055; AEGC, 0.064 and Vitamin C, 0.043 mg/mL). A positive result by the aqueous and ethanolic extracts in this test indicates that they contain antioxidants that can scavenge free radicals. Therefore, it can be used as a source of natural antioxidants and in drug formulations for the treatment of diseases resulting from oxidative stress [14].

The edema induced by the ingestion of carrageenan at the level of the plantar aponeurosis of the hind paw is the examination most frequently adopted to evaluate the anti-inflammatory activity of natural products [15]. Carrageenan causes inflammation typically related to the activation of cyclo-oxygenase [16]. The development of edema after its injection is a biphasic process [17]. The first phase occurs immediately after its administration, resulting in the release of histamine and serotonin from mast cells. These factors cause vascular changes that lead to plasma exudation [6]. In the second, swelling occurs caused by increased release of arachidonic acid metabolites such as prostaglandins and leukotrienes in the inflammatory zone [4]. This step is sensitive to prostaglandin synthesis antagonists and natural or synthetic anti-inflammatory drugs such as glucocorticoids [18]. Non-steroidal anti-inflammatory drugs also inhibit the cyclooxygenase enzymes involved in the synthesis of prostaglandins [15].

The maximum activity of the plant was observed at the third hour of experimentation at the time of the release of prostaglandins in the inflammatory site, which could be explained by the presence within this plant of cyclooxygenase inhibitors which would lead to the inhibition of prostaglandin synthesis. This result gives the plant an anti-inflammatory mechanism of action similar to that of nonsteroidal anti-inflammatory drugs and could be explained by the presence of bioactive compounds such as phenolic compounds and saponins [19]. Numerous studies have shown that polyphenols and their metabolites inhibit the enzymatic activities of arachidonic acid metabolism and reduce the production of inflammation mediators such as arachidonic acid, nitric oxides, prostaglandins and leukotrienes [20]. Polyphenols are therefore responsible for anti-inflammatory activities, hence their use as potential chemo-preventive agents [18]. The anti-inflammatory effect of sterols, terpenes, and saponins could be due to inhibition of cyclooxygenase and release of pro-inflammatory cytokines [16]. The results obtained in this test also make it possible to confirm that the anti-inflammatory activity of the extracts of *Combretum glutinosum* is largely linked to the effect of the extracts in the

infiltration of inflammatory cells. For this, cell migration and the production of certain key mediators of inflammation are verified in this study.

5. Conclusions

This study is part of the development of medicinal plants and especially the search for new molecules from plant extracts. *Combretum glutinosum* is a plant that represents a potential source of bioactive molecules. The aqueous and ethanolic root extracts of *Combretum glutinosum* have *in vitro* DPPH radical scavenging. Therefore, it can be used as a source of natural antioxidants and used in drug formulations for the treatment of diseases resulting from oxidative stress. In addition, the anti-edematous activity of the plant has confirmed the anti-inflammatory properties of the plant since it significantly reduces the edema of the paws of rats. This activity is comparable to that of diclofenac, which is a reference anti-inflammatory. The results obtained during this study are interesting; however, additional studies will be necessary to understand the cellular and molecular mechanisms linked to these antioxidant and anti-inflammatory activities.

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Disclosure statement

Conflict of interest: The authors declare that they have no conflict of interest.
Ethical approval: All ethical guidelines have been adhered.
Sample availability: Samples of the compounds are available from the author.

CRediT authorship contribution statement

Conceptualization: Sylla Tahiri; Methodology: Sylla Tahiri; Software: Sylla Tahiri; Validation: Sylla Tahiri; Formal Analysis: Sylla Tahiri, M'bra Kouassi Fulgence; Investigation: Sylla Tahiri, M'bra Kouassi Fulgence; Resources: Sylla Tahiri, M'bra Kouassi Fulgence; Data Curation: Sylla Tahiri, M'bra Kouassi Fulgence; Writing - Original Draft: Sylla Tahiri; Writing – Review and Editing: Sylla Tahiri; Visualization: Sylla Tahiri; Funding acquisition: Sylla Tahiri; Supervision: Sylla Tahiri, Dongui Bini Kouamé; Project Administration: Sylla Tahiri, Dongui Bini Kouamé.

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