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Research Article

In vivo anti-inflammatory and healing activities of the methanolic fraction of *Combretum glutinosum* Perr. bark

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Abstract

Combretum glutinosum Perr. is a shrub from the Sahelian belt. The different parts of the plant (leaves, bark, roots) are used in traditional medicine as an antidiarrheal, anti-infective, anti-tussive, healing, anti-parasitic and anti-inflammatory. The objective of this study was to evaluate the anti-inflammatory and healing activities of the methanolic fraction of *C. glutinosum* bark (MFCGB). *C. glutinosum* bark powder was extracted in solvents of different polarity, Hexane/Ethyl acetate/Methanol and characterized. Experiments were carried out on in vivo models of carrageenan-induced inflammatory edema and experimental deep second-degree burns in rats. Phytochemical characterization of MFCGB revealed the presence of phenolic compounds such as flavonoids and tannins. At doses of 1, 3 and 10 mg/kg *per os*, this fraction significantly inhibits carrageenan-induced edema in all phases. However, the greatest activity was observed during the late phase of inflammatory edema. Percentage changes in inflammatory edema were 20.09±1.80, 15.75±2.12 and 24.19±2.76 respectively after 5 hours, compared with untreated control 92.72±6.05 and aspirin-treated 30.95±7.25. Complete healing was observed after 20 days of daily application of MFCGB-based ointments in Vaseline. *C. glutinosum* bark is anti-inflammatory and healing in in vivo models. These effects may be due to the flavonoids and tannins present in this fraction.

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Combretum glutinosum, flavonoids, tannins, inflammation and healing.

1. Introduction

Wound healing is a dynamic, interactive and delicate process involving blood cells, soluble mediators, the extracellular matrix and parenchymal cells. It takes place in several overlapping phases [1]: haemostasis, inflammation, proliferation and tissue remodeling [2]. Inflammatory phasis plays a positive role. In fact, it is essential for fighting pathogens and ridding the wound site of dead tissue. However, when it is main-tained, it becomes damaging and can lead to deregulation of the healing stages, which can lead to excessive scarring [3]. The use of extracts or bioactive fractions of plants with anti-inflammatory activity could be a good compromise for rapid and correct healing.

Previous studies have highlighted the anti-inflammatory activity of the aqueous extract of

Combretum glutinosum Perr. bark, which is thought to be due to the presence of phenolic compounds such as flavonoids and tannins [4]. *C. glutinosum* is a shrub or small tree that is widespread throughout West Africa and extends as far as Sudan. It is a drought-resistant species, one of the most common in the shrub and tree savannahs of the Sudanian and South-Sahelian regions. It colonises almost all types of soil and climate [5, 6]. In Senegal, it forms monospecific stands in the fixed dunes of the Sahel (Djolof) and forms the basis of many cultivated coppices (Sine-Saloum, Casamance, Tambacounda) [7].

Ethnobotanical studies show that all parts of the plant are used in traditional medicine in Africa to treat many human diseases [8-10]. Also, many pharmacological studies have reported the therapeutic potential of *C. glutinosum* extracts. These studies mostly focus on antioxidant [11], antiparasitics [12-14], antidiarrheal [15], antihyperglycemic and cytotoxic [16], antibacterial [17] and anti-inflammatory [18] activities.

This study participates to the valorization of traditional medicinal plants and aims to highlight the anti-inflammatory and healing activities of the methanolic fraction of the bark of *C. glutinosum*.

2. Materials and methods

The study was conducted at the Pharmacology Laboratory at the Faculty of Medicine, Pharmacy and Dentistry (FMPD) of Cheikh Anta Diop University in Dakar (CADU), Senegal.

2.1 Drugs, chemicals and solvents

Vaseline (Valdafrique Laboratory, Rufisque, Senegal), Sulfadiazine (pharmacy), sodium benzoate, carrageenan, acetylsalicylic acid, acetic acid and extraction solvents (hexane, ethyl acetate, methanol) were obtained from Sigma/BES (Dakar, Senegal).

2.2 Plant material

The *C. glutinosum* bark used was harvested in the Tambacounda region (eastern Senegal) on 6 March 2022. It was identified in the pharmacognosy and botany laboratory of FMPO of CADU where the voucher specimen (DPB-16-10) was deposited.

2.3 Animals

Rats from Pharmacology Laboratory, FMPO were used. The weights of the rats varied between 132 and 245 g. The animals were housed in a cage under conditions of 25 ± 2°C temperature, 12 h light cycle and provided with food and water *ad libitum*. The experimental protocols were conducted in accordance with the guidelines of the Institutional Ethics Committee (Research Ethics Committee of CADU).

2.4 Experimental procedures

2.4.1 Extractions

C. glutinosum bark was dried in the laboratory at room temperature for 3 weeks before being pulverised. 500 g of bark powder was macerated for 3 days in hexane. The pomace from the hexane maceration was drained twice with 500 ml of hexane. The hexane solution was filtered and the filtrate evaporated using a rotary evaporator at 60°C. The concentrated hexane filtrate obtained was placed in a drying oven at 60°C to obtain a dry hexane extract. The pomace from the hexane extraction was then mixed with 1L of ethyl acetate using the same procedure. The result was an ethyl-acetate fraction. The methanolic fraction of *C. glutinosum* bark (MFCGB) was obtained from the pomace of the ethyl acetate fraction with methanol as the solvent using the same process [19].

2.4.2 Phytochemical characterizations

The characterisation test used the classic methods for identifying the major phytochemical families present in *C. glutinosum* bark. Tannins were characterized using Stiasny reaction, Flavonoids by Shibata reaction, Alkaloids with Bouchardat, Dragendorff and Valser-Mayer Reactions, Sterols and triterpenes by the Liebermann Reaction [20].

2.4.3 Ointment formulation

From the methanolic fraction powder of *C. glutinosum* three (3) ointments at 1, 3 and 10% in vaseline were prepared qsp 50g in the proportions shown in Table 1. The powder of the methanolic fraction and the sodium benzoate were triturated in a mortar. Vaseline was gradually added to the mixture with gentle stirring until homogenization [4].

Table 1: Composition of ointments

Composition (g)	Ointment (1%)	Ointment (3%)	Ointment (10%)
MFCGB	0.5	1.5	5
Vaseline	49.425	48.425	44.925
Sodium benzoate	0.075	0.075	0.075
Total (g)	50	50	50

MFCGB: methanolic fraction of *C. glutinosum* bark

2.4.4 Pharmacological tests

2.4.4.1 Anti-inflammatory activity

The anti-inflammatory activity was evaluated according to the method described by Winter et al. (1962), carrageenan-induced rat paw edema (Winter, et al., 1962). The rats were divided into groups of 5 and fasted for 12 h before the test, with free access to water. Rat paw edema was induced by subcutaneous injection of 100 µl of a 1% carrageenan solution under the plantar region of the left hind paw of rats, 1 h after oral administration of the different solutions. The initial diameter (D0) of the left hind paw was measured using a digital caliper. The evolution of edema was measured at 1, 3 and 5 hours after carrageenan injection and the percentage increase (%INC) was determined by the following formula:

$$\%INC = \frac{Dt - D0}{D0} \times 100$$

Dt = Paw diameter at t time

D0 = Initial paw diameter

The anti-inflammatory activity is evaluated by calculating the mean percentage inhibition (%INH) of edema by the following formula:

$$\%INH = \frac{\%INCctrl - \%INCtested}{\%INCctrl} \times 100$$

% INCctrl = percentage inhibition control

% INCtested = percentage inhibition solution to be testing

2.4.4.2 Burn induction and evaluation of the healing activity

An experimental deep second-degree burn model in rats was used for this test [21]. Twenty-five rats were divided into 5 lots of 5: untreated rats (Lot 1), treated with sulfadiazine cream (Lot 2), rats treated with ointment of 1%, 3% and 10% methanolic fraction of C. glutinosum bark in vaseline (MFCGB) (Lot 3, 4 and 5). Sulfadiazine, topically is often used in preclinical and clinical studies to demonstrate the healing activity of a new product. It is an antibacterial sulfonamide [22, 23]. The rats were then anesthetized with a 3% chloral solution by intra-peritoneal injection (1 mL/100 g). The dorsal flanks of the rats were shaved and cleaned. Experimental burns have been induced using a 3 cm diameter metal cylinder and heated for 5 min. The cylinder was applied for 20 s by slightly pressing on the surface of the shaved skin of the rats to cause second degree burns [24].

Healing activity was assessed using the Kamoshida method, which assigns scores from 1 to 5 according to the extent of the burn (Table 2). It was carried out daily for 28 days [24].

Table 2. Scores of the evolution of experimental burns

Score	Cicatrization process evaluation
0	Healing is complete and tissue repair is complete
1	Tissue healing is almost complete
2	Remnants of the crust remain the size of the lesion decreases (skin reconstruction)
3	All dead tissues (scabs) are removed, wounds and oozing
4	Necrotic skin is partially removed, ulcerated and oozing
5	Necrotic skin completely covers the burned area

2.5 Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM). They were analyzed by GraphPad 6.0 software. A one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test against the control group. P-values <0.05 were considered significantly different.

3. Results

1.1 Phytochemical characterizations

The results of the characterization of major phytochemical groups revealed the presence of phenolic compounds: tannins and flavonoids (Table 3).

Table 3. Summary of major phytochemical constituents of MFCGB

Phytochemical groups		MFCGB
Polyphenols		+++
Tannins	Condensed	+++
	Hydrolyzable	++
Flavonoids		+++
Alkaloids		-
Sterols and triterpenes		-

(+): Presence (-): Non detected

1.2 Pharmacological tests

1.2.1 Anti-inflammatory activity

1.2.1.1 Induction of rat paw inflammatory edema in the control group

Administration of the 1% carrageenan solution into

Table 4. Percentage increase of paw edema after administration of the different solutions

Lot	INC% (1h) (Mean ± SEM)	INC% (3h) (Mean ± SEM)	INC% (5h) (Mean ± SEM)
Control (10ml/kg)	34,39±8,81	67,77±6,79	92,72±6,05
ASA (10mg/kg)	21,79±2,27**	33,77±7,08**	30,96±7,25***
MFCGB (1mg/kg)	24,94±3	31,83±4,24**	20,08±1,8****
MFCGB 3mg/kg	20,36±1,44	43,98±4,24	15,74±2,12****
MFCGB 10mg/kg	9,56±3,1**	31,14±2,61**	24,18±2,75****

**=p<0.01; ***=p<0.001; ****=p<0.0001 vs Control group

the plantar foot pad of the rat paw, after gavage with physiological water (vehicle), leads to an inflammatory edema which increases the volume of the paw. The percentage increases in paw edema are 34.39±8.81; 67.77±6.79 and 92.72±6.05 (n=5) respectively at T1h, T3h and T5h.

1.2.1.2 Effect of acetylsalicylic acid (ASA) and MFCGB on carrageenan induced inflammatory edema in rat

The MFCGB at doses of 1, 3 and 10 mg/kg *per os* significantly (p<0.01, p<0.001, p<0.0001) prevented the development of inflammatory edema induced by carrageenan compared to the control group. However, at 1h, doses of 1 and 3 mg/kg did not significantly reduce inflammatory edema. The same applies to the 3 mg/kg dose at 3h. Prevention of inflammatory edema is better and more consistent with the 10 mg/kg dose every hour. The results are listed in Table 4.

The percentage inhibition evaluation shows that MFCGB at dose of 10 mg/kg *per os*, were 48.97±26.24, 51.54±7.63 and 74.07±2.23, respectively at 1, 3 and 5h. These results show a similar edema inhibition than ASA at the same dose (Fig. 1).

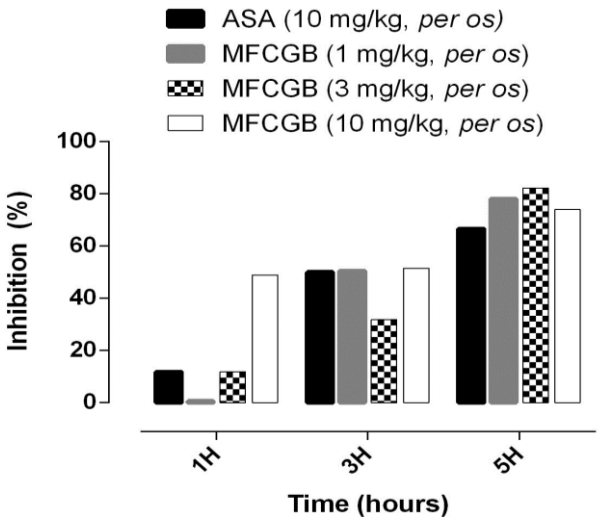

Figure 1: Inhibition of paw edema after oral administration of MFCGB.

1.2.2 Healing activity

1.2.2.1 Evolution of deep second-degree experimental burn scores in rats without treatment and sulfadiazine application

Eight days after induction of the experimental burn, the burned area was still covered with necrotic skin, corresponding to score 5. After 3 weeks, in the absence of treatment, the burned skin showed ulceration that was still oozing (score 4). After 4 weeks, the experimental burn consists of an open, oozing wound (score 2), corresponding to a total absence of healing. Daily application of sulfadiazine significantly improves wound healing compared with control (Fig. 2).

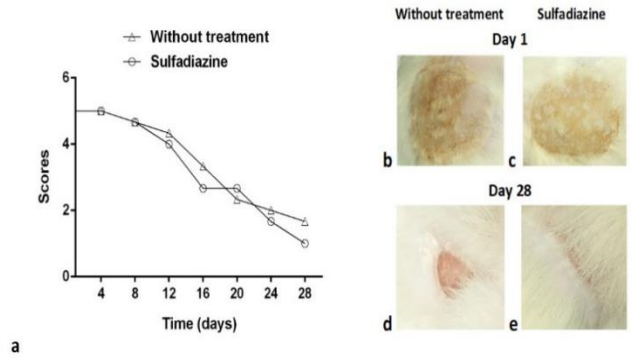

Figure 2: Evolution of deep second-degree experimental burn scores in rats without treatment and sulfadiazine application; (a) Score evolution curve, (b to e) Photographs of burned surfaces without treatment and with sulfadiazine application on day 1 and day 28.

1.2.2.2 Evolution of deep second-degree experimental burn scores after treatment with an ointment of 1, 3 and 10% MFCGB in vaseline

Daily application of MFCGB-based ointments shows almost complete tissue repair after 20 days. The scores obtained are 1. At 28 days of treatment, healing is complete with hair regrowth, particularly with the groups having received 1 and 10% ointments.

4. Discussion

In previous work, we had shown the topical anti-inflammatory and healing activities of the total aqueous extract of *C. glutinosum* bark [4]. The present study aimed to highlight the anti-inflammatory and healing activities of MFCGB.

Phytochemical screening revealed the presence in MFCGB of polyphenolic compounds, mainly flavonoids and tannins. However, the absence of alkaloids, sterols and triterpenes is noted. Screening work has also highlighted the presence of these phytochemical groups in the hydromethanolic extract of the bark [17, 26]. Other studies have shown the presence of triterpenes on stem bark [27] and alkaloids in the aqueous extract of *C. glutinosum* leaves [28]. With our work, fractionation with solvents of different polarity made it possible to concentrate phenolic compounds in the MFCGB and to get rid of the apolar constituents sterols and triterpenes, previously found in the total aqueous extract [4]. These apolar molecules could be eliminated by hexane which is used to characterize them [29].

This study shows that MFCGB exhibits anti-inflammatory activity at doses of 1, 3 and 10 mg/kg in the carrageenan-induced rat paw inflammatory edema model in rats. On the same model, studies relate the anti-inflammatory activity of the bark of plants of the *Combretaceae* family. This is the case of the ethanolic extract of the stem bark of *Anogeissus latifolia* Roxb, a plant of the *Combretaceae* family found in Asia [30], the aqueous extract of the bark of *Terminalia paniculata*, a tropical plant widely present in India [31], methanolic extracts rich in flavonoids and tannins of *Combretum micranthum* and *Combretum bauchiense*, widely found in Africa [32].

The inhibitory activity of MFCGB on inflammatory edema is greater at the dose of 10 mg/kg. It is observed every hour. It is more pronounced 5 h after induction. These elements suggest that the inhibition is greater in the late phase of carrageenan-induced inflammation.

Carrageenan-induced rat paw edema model has been used widely for the discovery and evaluation of anti-inflammatory drugs [33]. Indeed, carrageenan induces edema in 2 phases. An initial phase that occurs 2 to 3 hours after induction, characterized by the secretion of pro-inflammatory mediators such as bradykinin, histamine, nitric oxide and a late phase with an overproduction of prostaglandins [34].

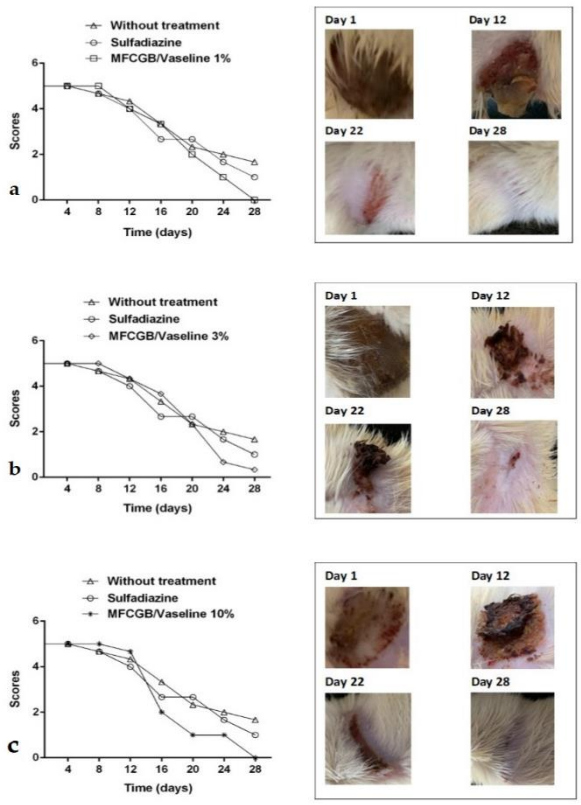

Figure 3. Healing effect of MFCGB experimental deep second-degree burns, (**a**) Treated with MFCGB 1%, (**b**) Treated with MFCGB 3% and (**c**) Treated with HMEANP 10%.

Fig. 3 shows the score evolution curves for each group, accompanied by illustrative images of the healing process.

The phenolic compounds, flavonoids and tannins highlighted in the MFCGB would promote the inhibition of edema. Polyphenols are reported in numerous studies to exhibit anti-inflammatory effects through inhibition of COX-2, inflammatory cytokines, IL-1β and TNF-α [35, 36]. Also, the presence of flavanols (quercetin and rutin) as well as gallic acid in a methanolic extract of *C. glutinosum* leaves were demonstrated [37]. Quercetin has anti-inflammatory activity by inhibiting prostaglandin synthesis and inflammatory cytokine production [38].

The MFCGB of the barks of *C. glutinosum*, like aspirin, the leading non-steroidal anti-inflammatory drug used as a reference, would reduce, in this *in vivo* model, the production of pro-inflammatory mediators in the early phase of inflammation as well as overproduction of late-phase prostaglandins due to cyclooxygenase activity.

Inflammation is important in wound healing. During an acute wound, it prepares for healing by eliminating necrotic tissue, debris, bacteria and promotes the recruitment and activation of fibroblasts [39]. However, when maintained, it can lead to excessive scarring [3]. The healing activity of plants with anti-inflammatory activity is reported in numerous studies [40-42].

On the deep second-degree experimental burn model in the Wistar rat, the MFCGB-based ointments have a healing activity. Tissue repair is almost complete after 20 days. Healing is complete with hair regrowth after 28 days of treatment. The healing effect is superior to that of the sulfadiazine used as a reference. Sulfadiazine has been a standard topical antimicrobial for burns for decades [43]. It is used all over the world, even for second degree burns [44]. In the present study, the absence of healing after 28 days of treatment with sulfadiazine suggests that the experimental burn induced is identified with a deep second-degree burn.

Healing is a complex biological and molecular process of restoring normal structure and function to injured tissue [45]. It evolves in several successive phases to achieve as complete a repair as possible. There is a vascular and inflammatory phase induced by the necrosis which follows the break-in, a proliferative phase which leads to the formation of granulation tissue and finally a scar maturation phase which remodels the tissues and eliminates excess cells [46].
This healing effect could be related to the concentration of phenolic compounds in the MFCGB. Several studies have shown the action of phenolic compounds in the healing mechanisms of wounds and burns in different animal models. Indeed, flavonoids reduce inflammation and increase angiogenesis, re-epithelialization and keratinocyte migration [47]. Tannins, with their astringent properties, promote wound healing by chelating free radicals, contracting damaged tissues and increasing the formation of capillaries and fibroblasts [48]. The tannin-rich fraction of *Terminalia chebula* and *Terminalia arjuna* (*Combretaceae*) promotes wound healing in rats due to its potent antibacterial, epithelialization and angiogenic activities [49-50].

5. Conclusions

MFCGB therefore exhibits anti-inflammatory activity at low doses in a model of carrageenan-induced inflammatory edema in rats. It is also healing in the deep second-degree burn model in rats. These effects could be related to the presence of this fraction of phenolic compounds such as flavonoids and tannins. MFCGB could facilitate complete healing of wounds and burns by regulating the inflammatory phase. Further research is needed to better elucidate the mechanisms involved.

Abbreviations

MFCGB: methanolic fraction of *C. glutinosum* bark

Authors' contributions

Initiated this work, M.S.; performed the manipulations, M.S.; F.K. and K.A.D.Z.; mined the data and wrote the manuscript, M.S. and Y.T.; supervised the extraction and characterization, A.S. and C.D.; contributed to the pharmacological evaluation, F.S.B., M.N. and A.N.S.; contributed to the supervision of the works, G.Y.S.

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Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

Authors have declared that no competing interests exist.

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