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Evaluation of the anti-inflammatory activity of aqueous extracts from *Parquetina nigrescens* (Afzel.) Bullock (Apocynaceae)

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Abstract

The decoction of the leaves of *Parquetina nigrescens* (Apocynaceae) is used by Ivorian traditional healers for the treatment of inflammation. Regarding this traditional practice, the present study aimed at evaluating through outa scientific approach the anti-inflammatory activity of the aqueous extracts from *Parquetinanigrescens* leaves. The extracts were tested according to the model of acute rat paw oedema induced by 1% carrageenan. Several doses of phytomedicines were administered orally to the animal sand ranging as follows: 1600, 2400, 4800 mg/kg. The results obtained with the aqueous extracts of *Parquetinanigrescens* leaves were firstly compared with those of physiological control (NaCl 0.9%) and thereafter with those of the reference (diclofenac sodium 25 mg/kg). The comparisons were performed at 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 12 hours and 15 hours after injecting carrageenan into rats. The parameters considered for this purpose arethe percentage increase of paw circumference (%AUG) and the percentage inhibition of treatment (%INH). Thus, therewas a significant difference ($p<0.001$) between the %AUG of phytomedicine treatment at different doses and those of saline (NaCl). However, for a dose of 4800 mg/kg, when administered byoralroute, the AUG of the phytomedicine are statistically the same as those of diclofenac (25 mg/kg). Comparisons of %INH leadto similar results with %AUG. This study, therefore, confirms the anti-inflammatory properties of *Parquetina nigrescens* leaves and emphasizes the empirical use of that plant species in the treatment of inflammation.

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Introduction

Current knowledge places the beginning of humanity at around seven million years ago (Ta *et al.*, 2023). Since that time, man has used plants (Fleurentin, 2007). This is explained by the use of plant in various areas of life (housing, health, food). The health field has given rise to a science called ethnomedicine. Ethnomedicine is defined as the set of beliefs and practices relating to illness in each given society (Laurence, 2021). It is also known as traditional medicine, which is highly developed in African countries. According to traditional medicine, no disease is incurable. It offers treatments for all kinds of diseases: cardiovascular diseases, metabolic diseases and especially inflammatory diseases. Related to inflammation, it is above all a defense mechanism, aiming to neutralize the attacking agent and eliminate damaged tissues (Souhel, 2010). In modern medicine, this health condition is treated with chemical compounds known as steroidal and non-steroidal anti-inflammatories (Ta-Bi *et al.*, 2018). Unfortunately, these drugs provoque other health damages in humans, particularly cardiovascular diseases (Heymonet, 2013; Ta, 2017). Therefore, the use of phytomedicines is increasingly encouraged. Previous ethnomedicinal report in Côte d'Ivoire mentioned the use of *Parquetina nigrescens* leaves extract in the treatment of anti-inflammatory condition (N'guessan, 2008). Although no scientific investigation was performed in Côte d'Ivoire, the anti-inflammatory effect of that plant species was carried out elsewhere like in Nigeria (Owoyele *et al.*, 2009). But the methodology used in this study is different and will be of interest to compare both studies or confirm scientifically the potential of *Parquetina nigrescens* leaves in inflammation removing. The objective of this study is therefore to seek scientific bases of the use of leaf decoction against inflammatory diseases by traditional medicine form from Côte d'Ivoire.

Materials and methods

Plant material

The plant material used for the anti-inflammatory activity consists of fresh leaves from *Parquetina nigrescens* (Fig. 1). The leaves were collected on the site of the University of Man (Côte d'Ivoire).

Animals used

The use of animals in this experiment was validated by the ethics committee of the pharmacognosy laboratory of Félix HOUPHOUËT-BOIGNY University. The experiments were carried out on adult rats of the Wistar breed weighing between 100 and 190 grams and aged 2 to 3 months. They were fed pellets from the company FACI (Fabrication d'Aliments de Côte d'Ivoire) and hadtap water for drink.

Technical equipment

The device used to evaluate the anti-inflammatory activity is called DIGITAL CALIPER brand electronic display caliper with a capacity of 0 to 150 mm. It is functional for measuring the circumferences of rat paws.

The preparation of the extracts required the use of hydrophilic cotton, an electronic balance, a graduated cylinder, a 2CC syringe, an insulin syringe for the injection of carrageenan, a mortar, a porcelain pestle, sterile glass jars, a refrigerator, metal cages lined with wood shavings, spatulas, indelible ink and an incubation cannula.

Chemical products

Carrageenan 1% solution was used to induce rat paw edema. The reference anti-inflammatory was diclofenac sodium 25 mg/kg (Olfen-75 SR). We also needed physiological saline (NaCl) as control during the experiments. The preparation of the extracts required distilled water.

Preparation of aqueous extracts

The aqueous extracts were chosen, in this experiment, to remain closer to the traditional therapists' model. We boiled 1 kg of fresh leaves of *Parquetina nigrescens* in 10 liters of tap water during 30 minutes. A liter and half (1.5 l) of the decocted fresh leaves were first drained throughout a square-shaped clean cloth, filtered successively twice on hydrophilic cotton

then on 3 mm thickness Watt man paper. The filtrate (one liler) was evaporated with a Rota vapor then from an oven at 60°C. After 2 days, the crystals obtained were ground into powder, using a porcelain mortar and pestle. The fine powder collected weighed 15.29 g. From this powder, we obtained the saturation concentration or maximum concentration at 160 mg/ml. The given solution was diluted successively 1/2, 1/3, 1/4 and 1/5 which brought to the respective concentrations of: 80; 53.33; 40; 32 mg/ml. From these concentrations, we calculated the doses according to the pharmacological standard which is the proportion 0.6 ml per 20 grams of body weight (Ake-Assi *et al.*, 2015). The corresponding doses in mg/kg of body weight at these different concentrations are respectively: 4800; 2400; 1600; 1200; 960 mg/kg by oral route. We used 3 doses for the evaluation of anti-inflammatory activity (high, medium and low). These 3 doses are coded: EPN1 for 4800 mg/kg, EPN2 for 1600 mg/kg and EPN3 for 960 mg/kg.

Conditioning, batch creation and force-feeding of rats

The animals were first conditioned, then fasted for 16 hours before treatment and divided into 5 groups of 6 rats. The batches were created according to the treatments as follows:

Lot 01: control rats receiving NaCl at 10 ml/kg
Lot 02: rats treated with EPN1 at 4800 mg/kg
Lot 03: rats treated with EPN2 at 1600 mg/kg
Lot 04: rats treated with EPN3 at 960 mg/kg
Lot 05: rats treated with diclofenac at 25 mg

Induction of inflammation

The experiment was carried out on the model of acute rat paw edema induced by carrageenan. Thus, 0.2 ml of 1% carrageenan solution is injected under the plantar pad of the right paw of each rat 1 hour after the administration of the different treatments. Measurements of the circumference of the hind paw are then performed with a caliper 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 12 hours and 24 hours after the injection of carrageenan.

Parameters evaluated

In this section, the parameters evaluated are the percentage increase in edema paw circumference (%AUG) and the percentage inhibition of edema (%INH). They are calculated as follows:

$$\%AUG = \frac{\text{Paw circumference} - \text{Initial circumference}}{\text{Initial circumference}} \times 100$$

$$\%INH = \frac{\%\ AUG\ control - \%AUG\ treated}{\%\ AUG\ control} \times 100$$ (Kouamé, 2016)

Statistical analysis

In this study, values of % AUG of plant extracts, control and reference are expressed as mean± standard error of mean (S.E.M.). Statistical significance was determined using the one-way ANOVA analysis coupled with the test of Tukey. Values with $p<0.001$ are significantly different. The same statistical test was used for the determination of % INH. In this last case, the comparison concerned the plant extracts and the reference solution. Graphs resulting from these inhibition percentages were produced using Excel 2016 software.

Results

Percentage increase in infected paw volume (%AUG)

The injection of carrageenan leads to an increase in the circumference of the paw (Fig. 2). The means relating to its increases (% AUG) and the results of the statistical tests carried out are recorded in Table 1. This table shows that there is a significant difference ($P<0.001$) between the percentages of increase in paw volumes (AUG) of the animals having undergone the control treatment (Nacl) and those of the animals receiving EPN1, EPN2 and diclofenac treatments. However, there is no significant difference between the AUG percentages of this control treatment and those of the lowerdose EPN3.According to Table 1, the AUG of this control treatment are similar to those ofEPN3. There is no significant difference between the AUG percentages of the animals which have received the EPN1 treatment (higher dose) and those of diclofenac, a pharmaceutical anti-inflammatory used as reference in our experiments. However, there is a significant difference between the AUG of EPN1 and those of EPN2 (medium dose) and EPN3 (lower dose).

Table 1. Evolution of the percentage increase in paw circumference (% AUG) for the control treatments and plant extracts (EPN).

Treatments and doses used	Measuring time							
	1h	2h	3h	4h	5h	6h	12h	24h
Nacl 0.9% 10ml	56.30±6.72^{d}	78.46±6.25^{d}	83.71±6.21^{d}	88.70±5.90^{d}	87.56±5.91^{d}	85.77±6.03^{d}	82.21±0.68^{d}	78.55±5.72^{d}
Diclofenac 25mg	2.01±1.35^{a}	8.26±2.03^{a}	12.72±8.04^{a}	21.01±3.01^{a}	12.51±5.70^{a}	9.15±2.78^{a}	5.03±1.17^{a}	9.98±4.62^{a}
EPN1 4800 mg/kg	3.10±2.02^{a}	11.01±0.78^{a}	15.09±1.72^{a}	26.68±1.12^{a}	16.08±8.97^{a}	13.84±0.96^{a}	7.26±1.72^{a}	13.34±0.72^{a}
EPN2 1600 mg/kg	40.97±8.49^{b}	55.78±8.72^{b}	58.01±6.72^{b}	60.02±1.79^{b}	60.03±2.07^{b}	57.24±6.72^{b}	60.03±6.73^{b}	51.97±0.78^{b}
EPN3 960 mg/kg	53.43±7.18^{c}	66.01±0.13^{c}	76.06±1.8^{c}	77.18±8.78^{c}	73.47±4.01^{c}	72.83±8.70^{c}	90.03±6.72^{c}	73.12±5.06^{c}

Treatment inhibition percentages (%INH)

The results of the inhibition percentages are exhibited in Fig. 3. These statistical tests show that there is no significant difference between the INH percentages of the diclofenac treatment (from 82.69 ± 6.67 to 94.00 ± 3.33) and the INH percentages of EPN1 extracts (from 79.61 ± 4.12 to 91.05 ± 3.30). The INH percentages of these two treatments are significantly different from the INH percentages of EPN2 and EPN3. The INH percentages of EPN2 (27.65 ± 0.79% to 33.82 ± 2.70%) are significantly different from the INH percentages of EPN3 (11.39 ± 2.01% to 18.71 ± 1.89%).

Discussion

The outcoming results from this study show the anti-inflammatory activity of *Parquetina nigrescens* leaves. The dose of 4800 mg/kg/vo of aqueous extract of this plant's organ operates similarly to dichlofenac sodium 25 mg/kg. So, there is a dose-response effect: the higher the dose, the greater anti-inflammatory effect.

Fig. 1. Leafy branch of *Parquetina nigrescens*.

Others studies were performed on the measurements of rat paw edema induced by carrageenan. Kouamé *et al.* (2016) showed that the aqueous extract of *Xylopia villosa* has anti-inflammatory activity equivalent to that of diclofenac 25 mg/kg. Soro *et al.* (2015) also proved that *Ximenia americana* has anti-inflammatory properties comparable to those of indomethacin, a reference anti-inflammatory.

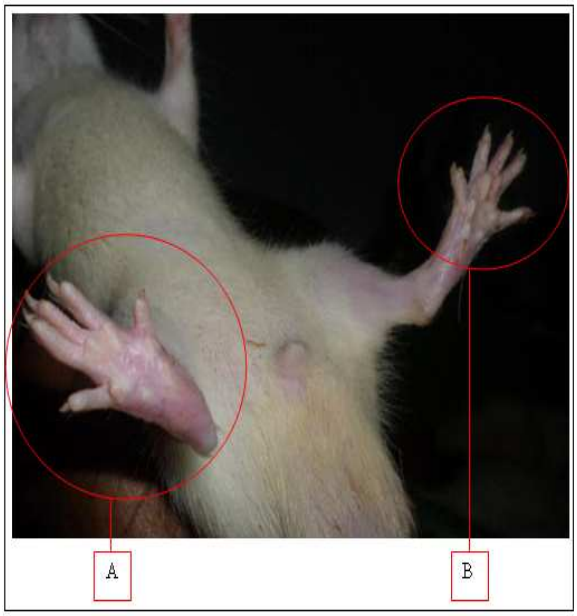

Fig. 2. Right hind leg with edema (A) compared with left hind leg without edema (B) in a rat from the NaCl batch, 2 hours after carrageenan injection.

In Congo, Epa *et al.* (2015) indicated the anti-inflammatory activity of the aqueous extract of the trunk of *Buchholzia coriaceae*. In Mali, Sanogo *et al.* (2006) also indicated the anti-inflammatory activity of three plant species in the treatment of dysmenorrhea: In Senegal, N'diaye *et al.* (2006) also showed that *Annona reticulata* has this activity.

Thus, this study confirms the anti-inflammatory activity of *Parquetina nigrescens* leaves reported before (Owoyele *et al.*, 2009) elsewhere in Africa. However, the methodologies in both studies are a bit different. In the previous study, researchers had used rats weighing 190 -230g, leaves macerate extracts and indomethacin as reference. While herein the rats weighed from 100 to 190 g, leaves decocted extracts were used and dichlofenac was the reference. Any way this finding indicates that *Parquetina nigrescens* leaves aqueous extracts act as any type of chemical drug.

The active principle can be extracted independently from the way used (decoction or macerate). In another approach, it could mean that different phytochemical compounds from *Parquetina nigrescens* leaves act as anti-inflammatory.

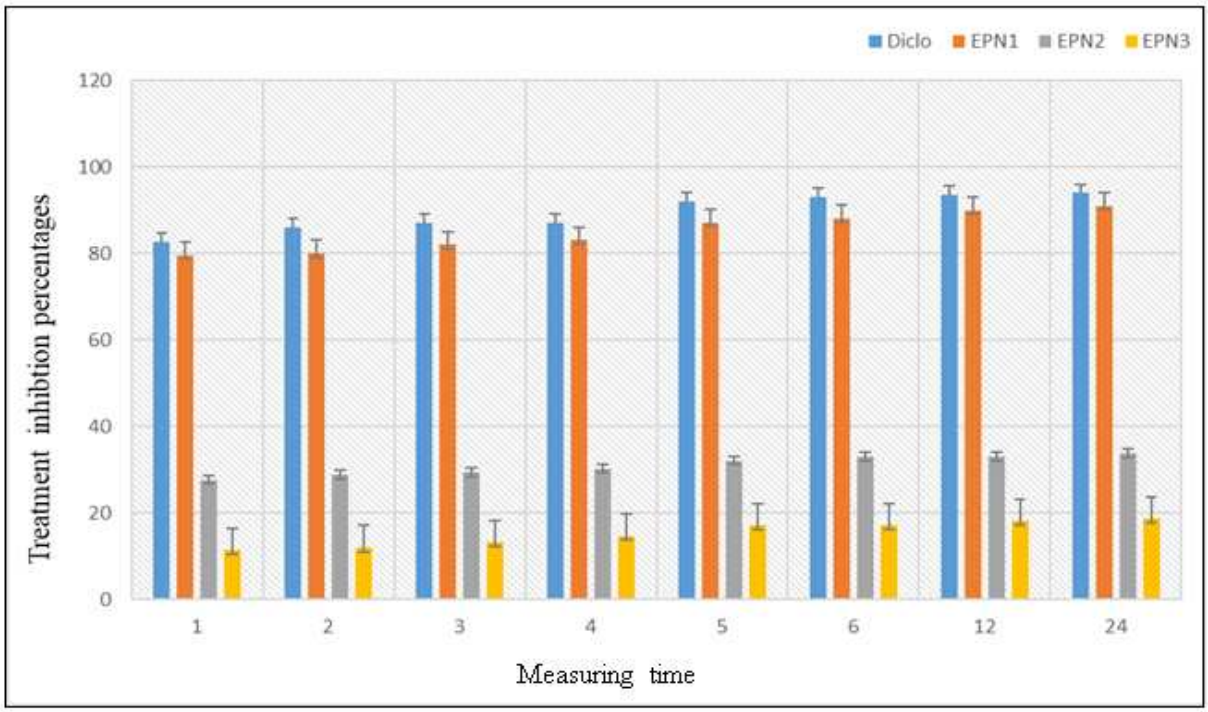

Fig. 3. Evolution of the inhibition percentages during the experiment for each treatment.

In fact, *Parquetina nigrescens* leaves contain flavonoids (Hoekou *et al.*, 2016). Which ones are known to have anti-inflammatory activity (Soro *et al.*, 2015). Moreover, plant flavonoids can be extracted either in hot or in cold condition. Furthermore, Diclofenac, used in this study as reference, has health drawbacks. According to Nurich (2015), this product increases the risk of arterial thrombosis to patients who consume it. Regarding the cardiovascular problems caused by anti-inflammatory drugs (steroidal and non-steroidal anti-inflammatories) prescribed by modern medicine (Heymonet, 2013), the use of *Parquetina nigrescens* would be desirable and encouraged, especially in Côte d'Ivoire as it is found everywhere. A particular look should therefore be put to *Parquetina nigrescens* in the search for anti-inflammatory products through physiotherapy. Future scientific investigation should characterize which flavonoid molecules are concern with the anti-inflammatory effect.

Conclusion

In conclusion, the leaves of *Parquetina nigrescens* showed efficacy on acute carrageenan inducted paw edema, with better activity at the dose of 4800 mg/kg by oral administration. These organs of plant have anti-inflammatory properties. The results are interesting and comparable to the activity of diclofenac 25mg used by modern medicine against inflammation. These results could justify the empirical use of Parquetina *nigrescens* leaves in the treatment of inflammation in Côte d'Ivoire. The present study could also be considered as a scientific basis for the research of other solutions to solve inflammatory problems with lower risk.

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