

## Anticoagulant and anti-inflammatory activities of *Griffonia simplicifolia* and *Parquetina nigrescens* traditionally used against hemorrhoidal disease

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**ABSTRACT:** Hemorrhoidal disease is manifested by formation of thrombosis accompanied by intense pain. This pathology is proving to be a worldwide public health problem. This study aims to evaluate the anticoagulant and anti-inflammatory activities of aqueous root bark extracts of *Griffonia simplicifolia* and *Parquetina nigrescens*. Anticoagulant activity was assessed by prothrombin time (PT) and activated partial thromboplastin time (aPTT) tests. The anti-inflammatory one was evaluated in rats pretreated with plant extracts using carrageenan-inducing paw edema method. The results show that both plant extracts significantly increased aPTT, with values of 106 s and 367.15 s respectively for *P. nigrescens* (80 mg/mL) and *G. simplicifolia* (20 mg/mL). However, only *G. simplicifolia* extract showed a significant effect on PT, with a value of 51.92 s at 100 mg/mL, comparable to that of heparin (52.15 s) at 50 IU/mL. The results of anti-inflammatory activity showed that plants extracts and diclofenac reached their maximum edema-inhibiting activity at the 2nd hour with inhibition of 76.44 %, 56.54 % and 38.03 % respectively for *P. nigrescens* (200 mg/kg), *G. simplicifolia* (200 mg/kg) and diclofenac (10 mg/kg). The elongation of clotting time and inhibition of rat paw edema by these plant extracts thus demonstrates their anticoagulant and anti-inflammatory properties.

**KEYWORDS:** Hemorrhoidal disease, anticoagulant activity, anti-inflammatory activity, *Griffonia simplicifolia*, *Parquetina nigrescens*.

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### I. INTRODUCTION

Hemorrhoids are complex vascular formations normally present in all individuals, whose function is to contribute to anal continence<sup>1</sup>. Their pathological appearance is due to swelling and inflammation of the veins of the rectum and anus, transforming this normal anatomical state into a pathological one. The main symptoms are prolapse, bleeding and thrombosis<sup>2</sup>.

Hemorrhoidal disease is the most common pathology of the terminal intestine, affecting 13-36% of the general population<sup>3-5</sup>. It most often appears after the age of 30, and its incidence is estimated at around 50% of the population aged 50 years and over<sup>6,3,7</sup>. This pathology significantly influences the quality of life of patients and can cause substantial discomfort and disability<sup>8</sup>. It also has a social impact and represents an economic burden for healthcare systems in terms of direct costs and lost working days<sup>9,10</sup>.

Therapeutic management of hemorrhoidal disease is based on the adoption of hygienic-dietary rules, the use of topicals, venotonics, analgesics and non-steroidal anti-inflammatory drugs<sup>11</sup>. Other methods, such as endoscopy, do not fully treat hemorrhoidal disease, with a diminishing effect over time<sup>11,12</sup>. Similarly, the use of surgery on hemorrhoidal disease provides good results, but is too costly, with inconvenient side effects<sup>13,14</sup>.

The search for natural substances, particularly medicinal plants, to treat this vascular and painful pathology is proving necessary. Plants are rich sources of bioactive molecules that can be used to treat a wide range of diseases. It is in this context that the present study, focusing on *Griffonia simplicifolia* (Fabaceae) and *Parquetina nigrescens* (Aponynaceae), two plants traditionally used to treat hemorrhoidal disease in Côte d'Ivoire<sup>15</sup>, was carried out. As hemorrhoidal disease manifests itself in clot formation (thrombosis), often

causing severe pain, this study aims to evaluate the anticoagulant and anti-inflammatory effects of *Griffonia simplicifolia* and *Parquetina nigrescens*.

## II. MATERIALS AND METHOD

### 2.1. Plant material

Root barks of *Griffonia simplicifolia* and *Parquetina nigrescens* constituted the plant material. Roots of these plants were collected in the region of N'douci, Southern Côte d'Ivoire, and samples of each were sent to the National Floristic Center, Felix Houphouët-Boigny University of Abidjan for botanical identification. These samples were authenticated by comparison with specimens deposited at the National Floristic under numbers UCJ009412 for *Griffonia simplicifolia* and UCJ014295 for *Parquetina nigrescens*.

### 2.2. Blood samples

Blood samples were taken from 10 adult healthy volunteers of both sexes aged 18 to 30 years old. Sick people, people with hemorrhagic diseases, including those who had been transfused in the previous two months or who had taken anticoagulant drugs did not participate in this study. The blood was collected separately over sodium citrate in blue capped tubes.

### 2.3. Animals

Acute toxicity and anti-inflammatory tests were carried out using Wistar albino rats aged 12 to 13 weeks and weighing between 160 and 180 g. Rats were bred at the animal house of the Normal Superior School of Abidjan. They were fed daily with pellets and had free access to water. All animals were housed in plastic cages and kept at room temperature with 12 hours of light during the day and 12 hours of darkness at night. All experimental procedures were approved by the Health Sciences Ethics Committee of the Felix Houphouët-Boigny University of Abidjan.

### 2.4. Chemicals

Reagent kits for prothrombin time test (Dade® Innovin®) and activated partial thromboplastin time test (Actin® FS) were purchased from Siemens Healthcare Diagnostics (France). Heparin® was obtained from Ceplapharm (France). Carrageenan was procured from Sigma Chemical Co. (St Louis, MO, USA). Diclofenac sodium tablets (25 mg, AdvaCare Pharma, USA) was used as a reference anti-inflammatory.

### 2.5. Extract preparation

The collected plant material (root barks of *G. simplicifolia* and *P. nigrescens*) was shade at room temperature for 3 weeks and were later pulverized using a grinder. One hundred (100) grams of each plant powder was dissolved separately in 1 L of distilled water. The mixture was then homogenized for 5 min using an electronic mixer, and the resulting homogenate was successively filtered twice on cotton and once on Whatman filter paper (3 mm)<sup>16</sup>. The process was repeated three times for each plant, and the filtrates obtained were concentrated to dryness under reduced pressure at 30°C using a rotary evaporator (BÜCHI). The resulting extracts represent the aqueous root bark extracts of *G. simplicifolia* and *P. nigrescens*, which were stored at 4°C for subsequent analysis.

### 2.6. Determination of anticoagulant activity

Prothrombin time (PT) and activated partial thromboplastin time (aPTT) assays were used to determine the anticoagulant activity of aqueous root bark extracts of *G. simplicifolia* and *P. nigrescens*. Blood samples placed in sodium citrate tubes were centrifuged at 3200 rpm for 5 min. The plasma resulting from this operation was then collected in hemolysis tubes and used for clotting test.

#### 2.6.1. Prothrombin time test

Prothrombin time (PT) test was carried out using commercial reagent kits (Dade® Innovin®). Calcium thromboplastin reagent was reconstituted with distilled water based on manufacturer's instruction and pre-warmed at 37 °C for 10 min in a water bath. Plasma (90 µL) was mixed with 10 µL of plant extracts (20-100 mg/mL) and incubated at 37°C for 5 min. Then, 200 µL of PT assay reagent (calcium thromboplastin) was added to extract concentration range and the clotting time was recorded by a coagulometer (SYSMEX CA-104). Plasma with only NaCl 0.9% was used as negative control (absence of anticoagulant activity). Heparin® (50 IU/mL) was used as positive control<sup>17</sup>.

## 2.6.2. Activated partial thromboplastin time (aPTT) test

The test was carried out using commercial reagent kits (Actin® FS). The partial thromboplastin reagent (Kaolin Platelet substitute mixture), a mixture of Kaolin and Phospholipid, was reconstituted according to the manufacturer's instruction. The resulting suspension and calcium chloride (0.025 M) were separately pre-warmed at 37 °C for 10 min in a water bath. Plasma (90 µL) was mixed with 10 µL of plant extracts (10-100 mg/mL) and incubated at 37°C for 5 min. Then, 100 µL of partial thromboplastin reagent was added to extract concentration range and the contents rapidly mixed. After addition of 100 µL of pre-warmed calcium chloride, the mixture was further incubated for 2 min and the clotting time was recorded by a coagulometer (SYSMEX CA-104). Plasma with only NaCl 0.9% was used as negative control (absence of anticoagulant activity). Heparin® (50 IU/mL) was used as positive control<sup>17</sup>.

## 2.7. Acute toxicity

Acute toxicity of aqueous root bark extracts of *G. simplicifolia* and *P. nigrescens* was performed according to the Organization for Economic Cooperation and Development (OECD) Guideline No. 423<sup>18</sup>. Two groups of three rats, fasted overnight, were each orally administered a single dose of 2000 mg/kg bw of aqueous root bark extract of *G. simplicifolia* or *P. nigrescens*. Another group of three rats received distilled water. After the treatment, food was withheld for further 3-4 hours. Animals were observed individually at least once during the first 30 min after treatment, regularly during the first 24 hours and daily for 14 days in order to record clinical signs of toxicity.

## 2.8. Anti-inflammatory activity

Anti-inflammatory activity of aqueous root bark extracts of *G. simplicifolia* and *P. nigrescens* was determined in rats using carrageenan-inducing paw edema method<sup>19</sup>. Thirty (30) rats were divided into six groups of five each and fasted for 18 h prior to experimentation. The thickness of each animal's right hind paw was first determined at time T<sub>0</sub> using a digital caliper, then the rats were treated as follows:

- Group 1 (edematous control) received distilled water orally;
- Group 2 received sodium diclofenac at 10 mg/kg bw orally;
- Groups 3 and 4 were orally administered with aqueous extract of *G. simplicifolia* at doses of 100 and 200 mg/kg bw;
- Groups 5 and 6 were orally treated with aqueous extract of *P. nigrescens* at doses of 100 and 200 mg/kg bw.

One hour after treatment, 1% carrageenan (0.1 mL) was administered subcutaneously to the plantar fascia of the right hind paw of each rat. The thickness of the paw that received carrageenan was measured after 1 h, 2 h, 3 h, 4 h, 5 h and 6 h using a digital caliper. Increase in thickness of the right hind paw was taken as an indicator of paw edema. Percentage increase in edema (% IE) was determined as the difference between thickness of the right hind paw at time zero (T<sub>0</sub>) and thickness of this paw at time t (T<sub>t</sub>):

$$\% \text{ IE} = \frac{T_t - T_0}{T_0} \times 100 \quad (1)$$

Percentage inhibition of the inflammatory reaction produced by carrageenan was calculated following formula:

$$\% \text{ Inhibition} = \frac{\% \text{ IEc} - \% \text{ IEt}}{\% \text{ IEc}} \quad (2)$$

Where IEc and IEt represent the mean percentage increase in paw thickness in control and treated groups, respectively.

## 2.9. Statistical analysis

Statistical analysis of the data was carried out using Graph Pad Prism 9.0 (Microsoft USA). Results were expressed as mean with standard errors of the mean (mean ± SEM). Differences between means were determined using one-way analysis of variance (ANOVA ONE WAY) followed by Turkey's multiple comparison test. The significance level was set at 5%.

### III. RESULTS

#### 3.1. Effect of *Griffonia simplicifolia* and *Parquetina nigrescens* extracts on prothrombin time

The results indicate that aqueous root bark extracts of *P. nigrescens* showed no significant effect ( $p > 0.05$ ) on clotting time relative to the prothrombin time (PT) test. *P. nigrescens* extract tested at different concentrations (20, 40, 80 and 100 mg/mL) on plasma generated PT values ranging from  $10.98 \text{ s} \pm 0.99$  to  $11.5 \text{ s} \pm 0.4$ . These values are statistically equal to those of the negative control (NaCl) with a PT of  $9.65 \text{ s} \pm 0.31$ . However, the reference product, Heparin, with a TP of  $52.15 \text{ s} \pm 3.88$ , showed significant anticoagulant activity ( $p < 0.001$ ) compared with *P. nigrescens* extract (Figure 1). For aqueous extract of *G. simplicifolia*, concentrations of 20 and 40 mg/mL showed no significant difference ( $p > 0.05$ ) of prothrombin time compared with the negative control. However, with concentrations of 80 and 100 mg/mL, the mean clotting time, which was  $37.33 \text{ s} \pm 6.04$  and  $51.92 \text{ s} \pm 1.01$  respectively, increased significantly ( $p < 0.001$ ) compared with that of the negative control ( $9.65 \text{ s} \pm 0.31$ ). Furthermore, the clotting time obtained with this extract at 100 mg/mL ( $51.92 \text{ s} \pm 1.01$ ) was statistically identical to that of Heparin ( $51.15 \text{ s} \pm 3.88$ ) (Figure 1).

#### 3.2. Effect of *Griffonia simplicifolia* and *Parquetina nigrescens* extracts on activated partial thromboplastin time

The results show that the activated partial thromboplastin time (aPTT) obtained with aqueous extract of *P. nigrescens* are significantly higher ( $p < 0.05$ ) than with NaCl ( $28.83 \text{ s} \pm 3.04$ ), used as a negative control. The highest aPTT was recorded with the 80 mg/mL concentration ( $106.0 \text{ s} \pm 5.95$ ). However, this value is well below that of Heparin ( $420 \text{ s} \pm 0$ ). The aPTTs obtained with the other concentrations of aqueous extract of *P. nigrescens* were  $62.78 \text{ s} \pm 2.52$  for 20 mg/mL;  $80.55 \text{ s} \pm 6.78$  for 40 mg/mL and  $74.97 \text{ s} \pm 10.65$  for 100 mg/mL (Figure 2). For the aqueous extract of *G. simplicifolia*, the results revealed a highly significant difference ( $p < 0.0001$ ) between the aPTT obtained with the different extract concentrations and that of the negative control (NaCl). At a concentration of 10 mg/mL, extract of *G. simplicifolia* already showed a significant coagulant effect, with an aPTT of  $194.0 \text{ s} \pm 14.13$ , significantly higher than that of the negative control ( $28.83 \text{ s} \pm 3.04$ ). For concentrations above 20 mg/mL, the clotting time (aPTT) values recorded were very high ( $> 400 \text{ s}$ ), comparable to those obtained in the presence of Heparin (Figure 2).

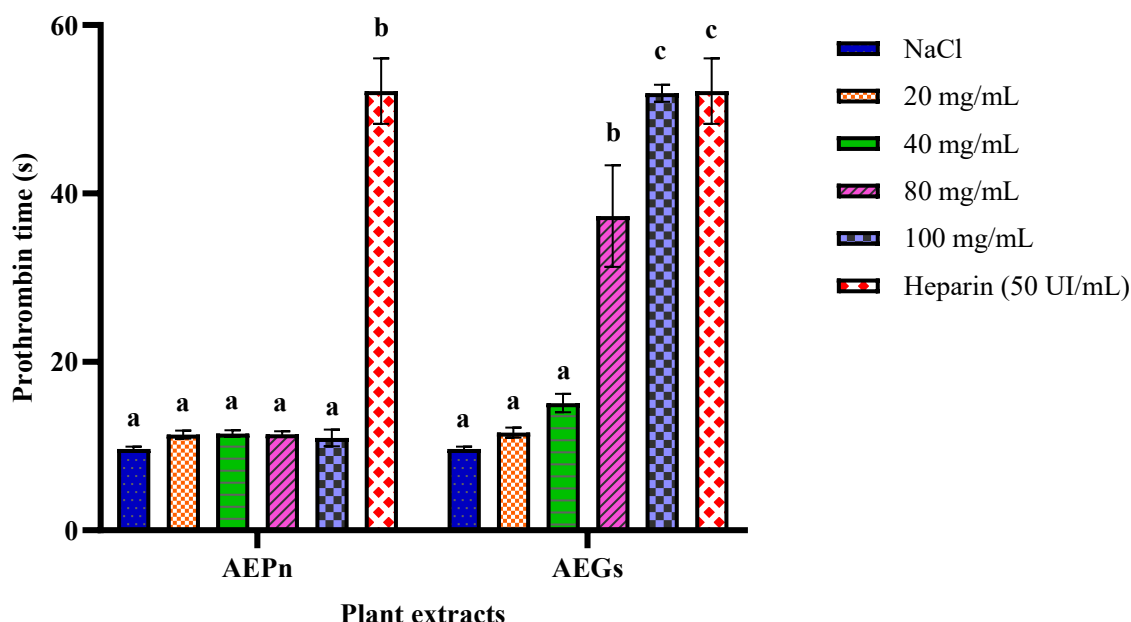
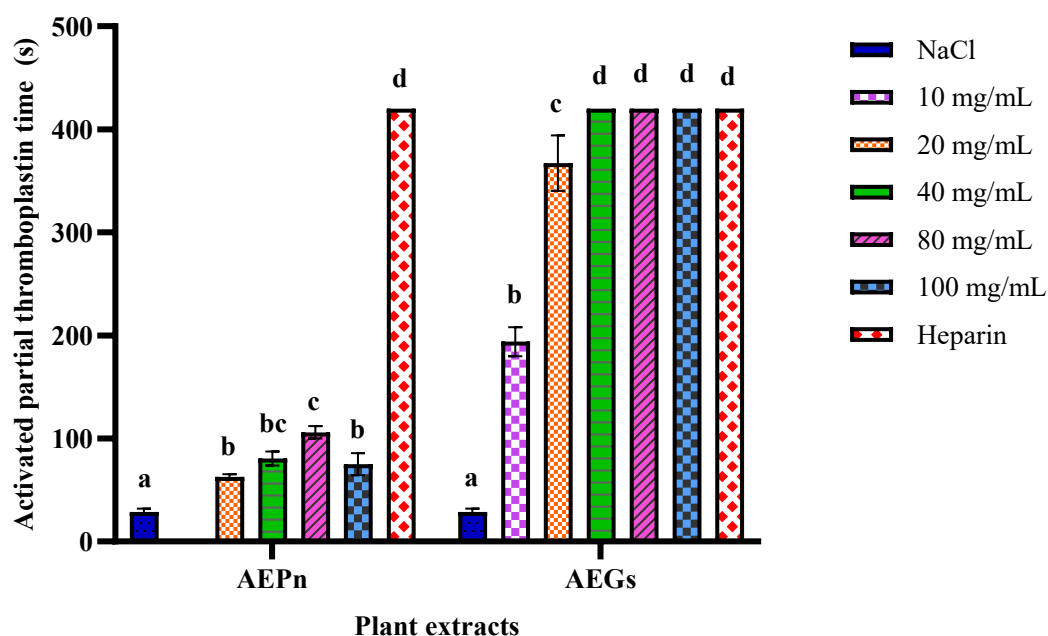


Figure No 1: Effect of plant extracts and Heparin on prothrombin time.

AEPn: aqueous extract of *P. nigrescens*; AEGs: aqueous extract of *G. simplicifolia*. Results are mean  $\pm$  SEM values ( $n=10$ ). Letters represent statistical significance. Mean prothrombin times with different letters are significantly different ( $p < 0.05$ ).



**Figure No 2: Effect of plant extracts and Heparin on activated partial thromboplastin time.**

AEPn: aqueous extract of *P. nigrescens*; AEGs: aqueous extract of *G. simplicifolia*. Results are mean  $\pm$  SEM values ( $n=10$ ). Letters represent statistical significance. Mean activated partial thromboplastin times with different letters are significantly different ( $p < 0.05$ ).

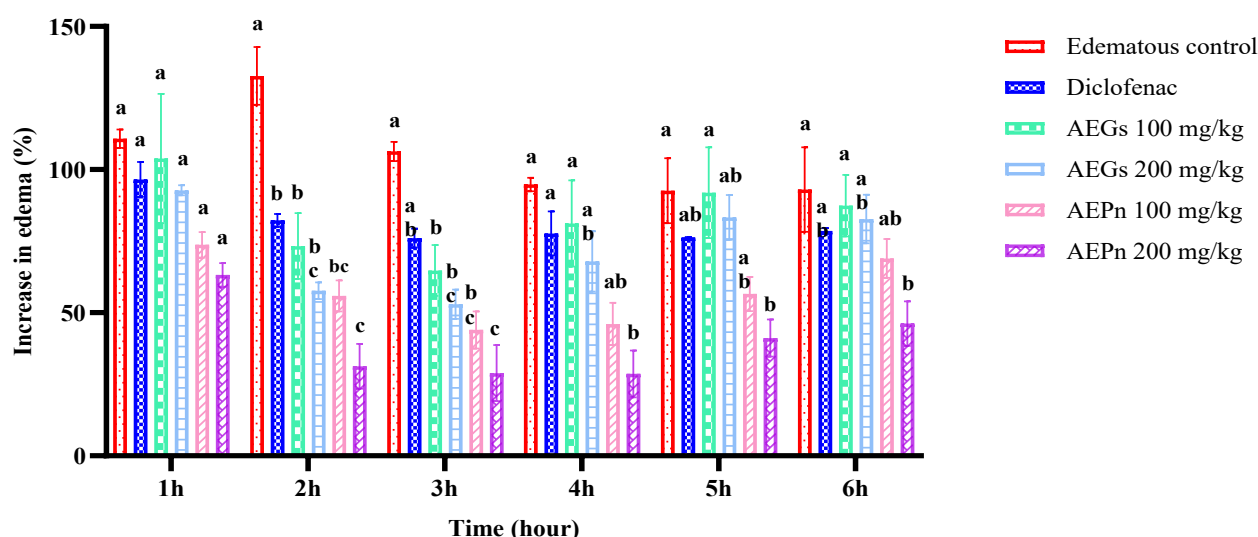
### 3.3. Acute toxicity

The acute oral toxicity of aqueous root bark extracts of *P. nigrescens* and *G. simplicifolia* at a single dose of 2000 mg/kg bw to rats revealed several reactions. In rats given aqueous extract of *P. nigrescens*, a reduction in mobility was observed 10 min after administration. At 30 min, in addition to reduced mobility, drowsiness and tremors were observed in these animals, compared with controls. After 1 h, the rats' behavior remained unchanged; they showed a weakening, but no mortality was observed. Two hours later, they returned to their usual behavior and fed properly during the two-week observation period. In rats given aqueous extract of *G. simplicifolia*, somnolence and reduced mobility were also observed 30 min after administration. However, after 1 h, they returned to their usual behavior and fed. After 24 h, no mortality was observed. The same was true after two weeks' observation. The lethal dose 50 ( $LD_{50}$ ) is therefore estimated to be greater than 2000 mg/kg bw for both plant extracts.

### 3.4. Effect of *Griffonia simplicifolia* and *Parquetina nigrescens* extracts on carrageenan-induced edema

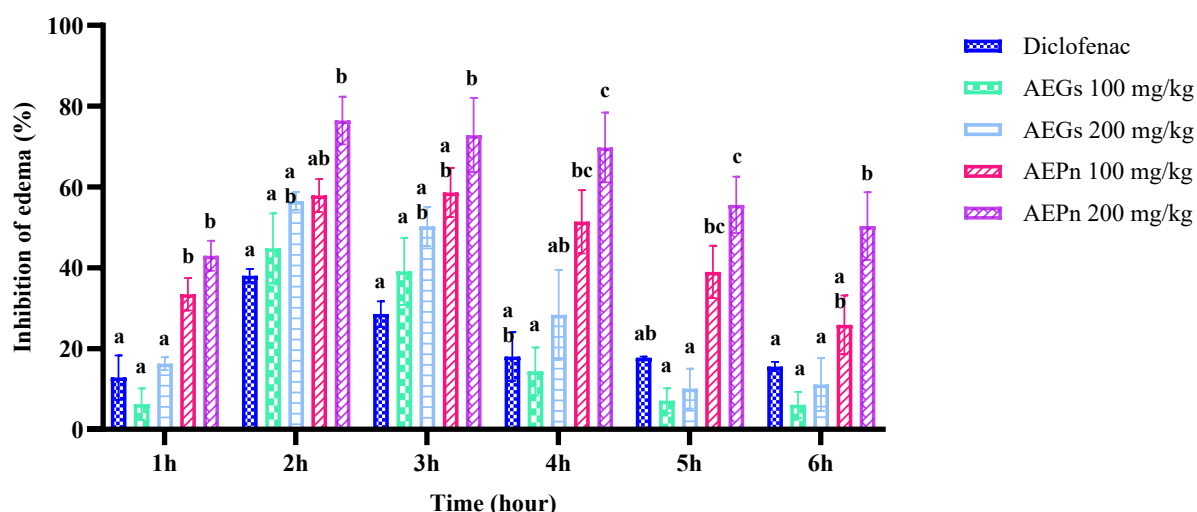
The results show that carrageenan induced an increase in paw thickness in all animals one hour after injection (Figure 3). This increase revealed no significant difference between the different groups of rats. In animals receiving distilled water (edematous control), the peak increase in edema ( $132.7\% \pm 10.09$ ) was recorded at the 2<sup>nd</sup> hour, before falling slowly from the 3<sup>rd</sup> hour ( $106.4\% \pm 3.36$ ) and to the 6<sup>th</sup> hour ( $93.04\% \pm 14.78$ ). In rats pretreated with aqueous extract of *G. simplicifolia*, the peak increase was reached one hour after carrageenan injection, with values of  $103.9\% \pm 22.58$  and  $92.75\% \pm 1.75$ , in groups receiving 100 and 200 mg/kg bw extract respectively. Significant decreases in edema ( $p < 0.05$ ) were observed from the 2<sup>nd</sup> to the 3<sup>rd</sup> hour after induction of inflammation, where the increase in edema fell to  $64.76\% \pm 8.85$  and  $52.94\% \pm 5.14$  for doses of 100 and 200 mg/kg bw extract respectively. A slight increase in rat paw thickness was subsequently observed from the 4<sup>th</sup> to the 6<sup>th</sup> hour after carrageenan injection. These changes in edema were statistically identical ( $p > 0.05$ ) to those observed in rats pretreated with diclofenac sodium. The peak increase ( $96.58\% \pm 6.08$ ) was reached in these animals one hour after carrageenan injection. This was followed by a reduction until the 3<sup>rd</sup> hour ( $76.04\% \pm 3.33$ ), and a slight increase from the 4<sup>th</sup> to the 6<sup>th</sup> hour when the increase in rat paw thickness was  $78.55\% \pm 1.07$ . Analysis of the percent inhibition of rat paw edema revealed no significant difference ( $p > 0.05$ ) between aqueous extract of *G. simplicifolia* (100 and 200 mg/kg) and diclofenac sodium (10 mg/kg) from the first hour to the 6<sup>th</sup> hour after carrageenan injection (Figure 4).

Results also show that pretreatment of rats with aqueous extract of *P. nigrescens* at 100 and 200 mg/kg bw induced reductions in edema over the 6-hour experimental period compared with edematous control rats (Figure 3). The peak increase was reached one hour after carrageenan administration, with values of  $73.74\% \pm 4.47$  and  $63.18\% \pm 4.1$  respectively for doses of 100 and 200 mg/kg bw. Significant reductions in edema were observed from the 2<sup>nd</sup> to 3<sup>rd</sup> hour for the 100 mg/kg bw dose, where the increase in rat paw thickness fell to 43.98%, and up to the 4th hour for the 200 mg/kg bw dose, where the increase in rat paw thickness was 28.63%. A slight increase in edema was subsequently observed up to the 6<sup>th</sup> hour after carrageenan injection. The percentage edema inhibition of aqueous extract of *P. nigrescens* at 200 mg/kg bw was significantly higher ( $p < 0.05$ ) than that of 100 mg/kg bw, diclofenac sodium and aqueous extract of *G. simplicifolia* (Figure 4). The highest inhibition ( $76.44 \pm 5.92\%$ ) was recorded with the 200 mg/kg bw dose of aqueous extract of *P. nigrescens* at the 2<sup>nd</sup> hour, while the lowest ( $6.05\% \pm 3.25$ ) was obtained with aqueous extract of *G. simplicifolia* at 100 mg/kg bw at the 6<sup>th</sup> hour after carrageenan injection.



**Figure No 3: Time course of paw edema in rats treated with Diclofenac and plant extracts.**

AEPn: aqueous extract of *P. nigrescens*; AEGs: aqueous extract of *G. simplicifolia*. Values are expressed as means  $\pm$  SEM ( $n = 5$ ). Letters represent statistical significance. Mean percentages of edema increase with different letters are significantly different ( $p < 0.05$ ).



**Figure No 4: Time course inhibition of paw edema in rats treated with Diclofenac and plant extracts.**

AEPn: aqueous extract of *P. nigrescens*; AEGs: aqueous extract of *G. simplicifolia*. Values are expressed as means  $\pm$  SEM ( $n = 5$ ). Letters represent statistical significance. Mean percentages of edema inhibition with different letters are significantly different ( $p < 0.05$ ).

#### IV. DISCUSSION

The *in vitro* anticoagulant activity of aqueous root bark extracts of *Griffonia simplicifolia* and *Parquetina nigrescens* was assessed through two coagulation parameters, namely prothrombin time (PT) and activated partial thromboplastin time (aPTT). The coagulation system involves a complex set of reactions involving many different proteins<sup>20</sup>. These reactions convert fibrinogen into fibrin, which forms a thrombus with platelets. The initiation of coagulation cascades is divided into two pathways: the exogenous or extrinsic pathway and the endogenous or intrinsic pathway

Measuring PT provides an overall assessment of the activity of coagulation factors in the exogenous pathway (coagulation triggered by contact with thromboplastin released by the cells), while measuring aPTT assesses the activity of coagulation factors in the endogenous pathway. The results obtained show that aqueous extract of *G. simplicifolia*, at high concentrations, prolonged prothrombin time (PT). In fact, clotting time increased significantly ( $p < 0.001$ ) only with extract concentrations of 80 and 100 mg/mL. In the activated partial thromboplastin time (aPTT) test, *G. simplicifolia* extract significantly ( $p < 0.0001$ ) prolonged clotting time already at 10 mg/mL extract. This prolongation of clotting time increased with increasing extract concentration, and at 20 mg/mL, the aPTT recorded was statistically identical to that of Heparin (50 IU/mL). These results indicate that aqueous root bark extract of *G. simplicifolia* has an anticoagulant effect on both blood coagulation pathways, but this effect is better on the endogenous pathway. With aqueous extract of *P. nigrescens* at concentrations of 20 to 100 mg/mL, the results generated prothrombin times statistically identical to those of the negative control (NaCl). This extract therefore has no effect on the exogenous coagulation pathway. However, aPTT values indicate that *P. nigrescens* extract, at the different concentrations tested (20 to 100 mg/mL) significantly ( $p < 0.05$ ) lengthened clotting time compared with that of the negative control. The effect of aqueous extract of this plant was still significantly less than that of Heparin (50 IU/mL). These results indicate that aqueous root bark extract of *P. nigrescens* has an inhibitory effect on exogenous coagulation factors. The anticoagulant effect of both plants could be attributed to polyphenols and flavonoids, the presence of which has been revealed in extracts of *G. simplicifolia*<sup>21</sup> and *P. nigrescens*<sup>22</sup>. In fact, polyphenols and flavonoids are able to inhibit many enzymes, including several serine proteases involved in the coagulation cascade<sup>23,24</sup>. In addition, many epidemiological studies have shown a correlation between the anticoagulant/antithrombotic activity of natural extracts and their polyphenol and flavonoid content<sup>25</sup>.

The acute oral toxicity study of aqueous root bark extracts of *G. simplicifolia* and *P. nigrescens* revealed no mortality in rats during the two-week observation period. The lethal dose 50 (LD<sub>50</sub>) of these extracts is estimated to be greater than 2000 mg/kg b.w. Both plant extracts are therefore classified in category 5 of the globally harmonized system of classification of chemicals, which characterizes substances of low toxicity<sup>18</sup> (OECD, 2001).

The anti-inflammatory activity of aqueous root bark extracts of *P. nigrescens* and *G. simplicifolia* was determined after carrageenan-induced edema in the right hind paw of rats. The validity of the method was verified by testing diclofenac sodium, a non-steroidal anti-inflammatory drug, effective against carrageenan-induced edema<sup>26</sup>. The change in edema was assessed in rats 1h, 2h, 3h, 4h, 5h and 6h after carrageenan injection. Carrageenan is a mucopolysaccharide that induces maximum edema at 3 hours post-injection<sup>27</sup>. The increase in paw thickness in edematous control rats from the first to the 6<sup>th</sup> hour indicates that any reduction in edema during this period could be due to the effects of the administered products. The results revealed that aqueous root bark extracts of *P. nigrescens* and *G. simplicifolia*, like diclofenac sodium, inhibited edema with a maximum effect between the 2<sup>nd</sup> and 3<sup>rd</sup> hour after injection of carrageenan. This indicates the anti-inflammatory activity of that extracts. The effect exerted by *P. nigrescens* extract (200 mg/kg) is better than that of *G. simplicifolia* (100 and 200 mg/kg), which is nevertheless comparable to that of diclofenac sodium. The maximum anti-inflammatory activity of these extracts observed between the 2<sup>nd</sup> and 3<sup>rd</sup> hour could be explained by the presence within them of cyclooxygenase inhibitors, which would induce inhibition of prostaglandin synthesis at the time of their release into the inflammatory site. Previous studies have revealed the presence of several secondary metabolites including alkaloids, saponins, tannins, polyphenols, flavonoids, etc. in extracts of *G. simplicifolia*<sup>21</sup> and *P. nigrescens*<sup>22</sup>. Total flavonoid and phenol contents recorded in the hydroethanolic crude extract of *G. simplicifolia* were 224 mg QE/100 g of extract and 4.37 mg GAE/g of extract, respectively<sup>21</sup>. For *P. nigrescens*, the total phenolic and tannin contents in the ethanol leaf extract were estimated to be 9.19 mg GAE/g of extract and 11.51 mg GAE/g of extract, respectively<sup>22</sup>. It is also known that phenolic compounds, notably flavonoids and tannins, inhibit the enzymatic activities of arachidonic acid metabolism and reduce the production of inflammation mediators such as arachidonic acid, nitrogen monoxide, prostaglandins and leukotrienes<sup>28,29</sup>. The anti-inflammatory effect of aqueous root bark extracts of *G. simplicifolia* and *P. nigrescens* could therefore be due to the presence of phenolic compounds within them, as shown by Nyarko *et al.*<sup>21</sup> and Airaodion *et al.*<sup>22</sup>.

## V. CONCLUSION

The present study revealed that aqueous root bark extracts of *Griffonia simplicifolia* and *Parquetina nigrescens* possess anticoagulant and anti-inflammatory properties, reflected in their ability to prolong blood plasma clotting time and inhibit edema. These results justify the use of these two plants in traditional medicine for the treatment of hemorrhoidal disease, a pathology manifested by the formation of clots (thrombosis) accompanied by intense pain.

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## CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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