

## STUDY OF THE HEPATOPROTECTIVE POTENTIAL OF AQUEOUS AND HYDROETHANOLIC EXTRACTS OF *PAVETTA CORYMBOSA* (DC) F.M. WILLIAMS (RUBIACEAE) LEAVES

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### ABSTRACT

As part of the promotion of traditional medicine, a pharmacological study was conducted on aqueous and hydroethanolic extracts of *Pavetta corymbosa* leaves, a plant used in traditional medicine. The study focused on evaluating the hepatoprotective potential of these two types of extracts. As part of this evaluation, rats whose livers had been poisoned by carbon tetrachloride (5 ml/kg bw) were treated with aqueous and hydroethanolic extracts of *Pavetta corymbosa* leaves at a dose of 200 mg/kg bw and silymarin, a hepatoprotective drug, at a dose of 100 mg/kg bw. This study shows that the hydroethanolic extract and silymarin significantly ( $p < 0.05$ ) reduce hepatotoxicity in rats compared to the aqueous extract. This effectiveness of the hydroethanolic extract could be explained by the presence of saponosides and quinones present in the said extract compared to the aqueous extract. The effectiveness of the hydroethanolic extract of *Pavetta corymbosa*

leaves similar to reference drugs for the treatment of hepatotoxicity could be a hope for traditional medicine.

**KEYWORDS:** Aqueous extract, hydroethanolic extract, *Pavetta corymbosa*, hepatoprotective.

### INTRODUCTION

Traditional medicine, which generally involves the use of medicinal plants to cure or prevent certain pathologies, is an ancient practice widely used throughout most of the globe. Plants are a major source of medicines because they contain numerous bioactive molecules, most of

which likely play a role in defense or protection of the body **Usha (2014)**. Demand for services in this area is growing **OMS (2013)**. This traditional medicine is less expensive and accessible. It is with this in mind that the plant species *Pavetta corymbosa*, from the Rubiaceae family, was used in this study. *Pavetta corymbosa* leaves are used in decoctions and infusions for the treatment of diabetes, malaria, fever, pain, inflammation, skin infections, and arthritis **Adjanohoun and Aké-Assi L (1979)**. Indeed, triphytochemistry has revealed the presence of chemical compounds in the aqueous and hydroethanolic extracts of *Pavetta corymbosa* leaves that are endowed with pharmacological potential **Miezan *et al.*, (2024)**. The predominant active compounds are flavonoids, polyphenols, polyterpenes, and sterols. However, in addition to the compounds listed above, the hydroethanolic extract contains saponins and quinones **Miezan *et al.*, (2024)**. Furthermore, the Globally Harmonized Classification System considers the aqueous and hydroethanolic extracts of *Pavetta corymbosa* leaves to be non-toxic substances **(5)**. However, despite the widespread use of *Pavetta corymbosa* leaves, no scientific studies of their effectiveness on hepatotoxicity have been conducted. Therefore, the objective of this study is to evaluate the hepatoprotective potential of the leaves of this plant, which is commonly used in sub-Saharan folk medicine.

## I. MATERIALS AND METHODS

### I.1. MATERIALS

#### I.1.1. Plant Material

To evaluate the hepatoprotective potential of *Pavetta corymbosa*, the plant material consisted of leaves harvested in the Aboisso region (Côte d'Ivoire).

#### I.1.2. Animal Material

Albino rats (*Rattus norvegicus*) of the Wistar variety, weighing between 118 and 120 grams, were used in this study. These animals were obtained from the animal facility of the Department of Biological and Pharmaceutical Sciences at Félix Houphouët Boigny University (Côte d'Ivoire). The rats were kept under favorable breeding conditions in accordance with standards and good practices for laboratory animals. They were fed a standard complete feed in pellet form. The animals received continuous tap water from feeding bottles.

## I.2. METHODS

### I.2.1. Sampling

In this study, the plant material consisted of 5 kg of *Pavetta corymbosa* leaves collected from the same site (Aboisso). This site was chosen for harvesting due to its accessibility and the abundance of *Pavetta corymbosa* in this location. The leaves were packed in biodegradable bags and transported in a van immediately after harvest. The harvested leaves were dried in the laboratory, protected from light, for three weeks before being ground using a mechanical grinder (IKAMAG, Japan).

### I.2. 2. Preparation of the aqueous extract of *Pavetta corymbosa*

The aqueous extract was prepared from 100 grams of *Pavetta corymbosa* leaf powder in 1 L of boiling distilled water for ten minutes. The resulting solution was filtered through cotton and then vacuum-filtered using Whatman filter paper. The filtrate was oven-dried at 40°C, which constituted the total crude aqueous extract of *Pavetta corymbosa*.

### I.2. 3. Preparation of the hydroethanolic extract of *Pavetta corymbosa*

For the 70% hydroethanolic extract, the Guédé-Guina method **Guédé-Guina *et al.*, (1993)** was used. 100 g of *Pavetta corymbosa* leaf powder was used for this purpose. The resulting mixture was homogenized using a magnetic stirrer for one (1) day. The solution was filtered through cotton and then under vacuum under the same conditions as before. The filtrate obtained was concentrated using a rotary evaporator and then dried in an oven at 40°C. The powder obtained constituted the hydroethanolic extract of *Pavetta corymbosa*.

## I.3. Hepatoprotective Activity

The experiment was conducted on four groups of six rats. The hepatoprotective potential of the two types of extracts was determined using the method described by **Raj and Gothandam (2014)**. This method demonstrates the preventive properties of aqueous and hydroethanolic extracts of *Pavetta corymbosa* leaves against carbon tetrachloride (cc14) poisoning. The aqueous and hydroethanolic extracts were compared to those of silymarin (Sil), the reference hepatoprotective drug **Raj and Gothandam (2014)**. Thus, the animals received the following treatments:

- Group 1 received the CCL<sub>4</sub> poisoning solution at 5 ml/kg body weight intraperitoneally for three successive days, followed by 10 ml of distilled water for four successive days.

- Group 2 received 5 ml/kg bw of the CCl<sub>4</sub> intoxication solution intraperitoneally for three consecutive days, followed by 10 ml of silymarin (Sil) at 100 mg/kg bw for four consecutive days.
- Group 3 received 5 ml/kg bw of the CCl<sub>4</sub> intoxication solution intraperitoneally for three consecutive days, followed by 10 ml (200 mg/kg bw) of aqueous extract for four consecutive days.
- Group 4 received 5 ml/kg bw of the CCl<sub>4</sub> intoxication solution intraperitoneally for three consecutive days, followed by 10 ml (200 mg/kg) of hydroethanolic extract for four consecutive days.
- Group 5 (control) received 10 ml of distilled water for four successive days.

The different treatments were carried out every day at the same time. The animals were deprived of food for 12 hours and water for only one hour before the manipulations. They were fed one hour after these manipulations, 24 hours after the last treatment. Their blood was collected by tail puncture on day 8 of the experiment into dry tubes, and the serum was used to assess serum transaminase levels (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)) using a Cobas C311 analyzer.

### I.3.1. Assay of Enzymatic Activities

#### I.3.1.1. Measurement of Aspartate Aminotransferase Activity

Aspartate Aminotransferase (ASAT) was assayed using the **Tietz** method (2015) and the recommendations of the International Federation of Clinical Chemistry (IFCC). ASAT transfers its amino group to the carbon atom of  $\alpha$ -ketoglutarate, forming glutamate and oxaloacetate. Oxaloacetate is then reduced to malate by malate dehydrogenase in the presence of reduced NADH, which is oxidized to NAD<sup>+</sup>. The amount is proportional to the amount of oxaloacetate present in the serum and the amount of ASAT activity. This catalytic activity is obtained by determining the disappearance of NADH at 340 nm. In practice, in a heparinized EDTA tube containing 500  $\mu$ L of reagents (total volume) pre-incubated at 37°C for 2 to 3 min, 50  $\mu$ L of the sample to be assayed was added. After shaking for 1 min, the optical densities were read with a spectrophotometer at 350 nm for the determination of the enzymatic activity of ASAT. This activity is calculated from  $\lambda$  at 340 nm and the SGOT factor which is equal to 1745 according to the formula below.

$$\text{Enzymatic activity of SGOT (U/I)} = \frac{\Delta DO}{\lambda} \times 1745 \quad (1)$$

OD = Optical Density Variation.

$\lambda$  = Wavelength (nm).

### I.3.1.2. Measurement of Alanine Aminotransferase Activity

Alanine aminotransferase (ALAT) was assayed using the **Bergmeyer and Horder** method (1980) following the International Federation of Clinical Chemistry (IFCC). Alanine aminotransferase (ALAT) catalyzes the transfer of the amine group from alanine to  $\beta$ -cetoglutarate to form pyruvate and L-glutamate. Pyruvate is converted to lactate by lactate dehydrogenase. The amount of NADH reduction is proportional to the amount of pyruvate formed in the reaction medium and the enzyme's activity. This activity is determined by measuring the absorbance at 350 nm against a control. In a heparinized EDTA tube containing 500  $\mu$ L of reagents (total volume) pre-incubated at 37°C for 2 to 3 min, 50  $\mu$ L of the sample to be assayed was added. After shaking for 1 min, the optical densities were read with a spectrophotometer at 350 nm for the determination of the enzymatic activity of ALAT. This activity is calculated from  $\lambda$  at 340 nm and the SGOT factor which is equal to 1745 according to the formula below.

$$\text{Enzymatic activity of SGOT (U/I)} = \frac{\Delta DO}{\lambda} \times 1745 \quad (2)$$

OD = Optical Density

$\lambda$  = Variation Wavelength (nm)

## I.4. Statistical Analysis

Results were expressed as mean plus or minus standard deviation (SD) of the mean (mean  $\pm$  SD). Data representation and analysis were performed using Graph Pad Prism 5.0 software (Microsoft USA). Differences between means were determined using Dunnett's test,  $p < 0.01$  (highly significant difference) and  $p < 0.05$  (significant difference).

## II. RESULTS AND DISCUSSION

### II.1. RESULTS

#### II.1.1. Hepatoprotective Potential

##### II.1.1.1. Effect of Different Treatments on Serum Aspartate Aminotransferase (AST) Concentration

It should be noted that the initial (baseline) transaminase concentrations were  $40 \pm 1.37$  IU/L for aspartate aminotransferase (AST) and  $38 \pm 1.35$  IU/L for alanine aminotransferase (ALT) for each group of rats. The results of the aspartate aminotransferase assay show that before

the experiment (D0), there was no significant difference in serum AST concentration between the different groups of rats ( $p > 0.05$ ). Seven days after the experiment (D7),  $\text{CCl}_4$  significantly increased ( $p < 0.001$ ) the serum AST concentration in the untreated intoxicated group (distilled water +  $\text{ccl}_4$ ) ( $275 \pm 22.5$  IU/L). However, the serum concentration of ASAT in the intoxicated group treated with Silymarin (Sil) ( $247.5 \pm 9.6$  IU/L) in the aqueous and hydroethanolic extracts decreased. The serum concentrations of ASAT were respectively  $130.7 \pm 5.50$  IU/L for the aqueous extract (200 mg/kg bw) and  $112.3 \pm 10.2$  IU/L for the hydroethanolic extract (200 mg/kg bw). However, silymarin and the hydroethanolic extract significantly reduced intoxication ( $p < 0.05$ ) compared to that of the aqueous extract. There was no significant difference in intoxication between silymarin and the hydroethanolic extract ( $p > 0.05$ ). (**Table I**).

#### II.1.1.2- Effect of different treatments on serum alanine aminotransferase (ALAT) concentration

The results of the alanine aminotransferase assay show that before the experiment (D0), there was no significant difference in serum ALAT concentration between the different groups ( $p > 0.05$ ). Seven days after the experiment (D7),  $\text{CCl}_4$  significantly increased ( $p < 0.001$ ) the serum ALAT concentration in the untreated intoxicated group ( $143.09 \pm 22.5$  IU/L). However, the serum ALAT concentration of the intoxicated group treated with Silymarin (Sil) ( $42.67 \pm 19.2$  IU/L) in the aqueous and hydroethanolic extracts decreased. Serum ALT concentrations were  $54.33 \pm 5.8$  IU/L for the aqueous extract (200 mg/kg bw) and  $43.93 \pm 17.8$  IU/L for the hydroethanolic extract (200 mg/kg bw), respectively. However, silymarin and hydroethanolic extract significantly reduced the concentration ( $p < 0.05$ ) compared to that of the aqueous extract. There was no significant difference in concentration between silymarin and hydroethanolic extract ( $p > 0.05$ ). (**Table I**).

Each value was the mean  $\pm$  standard deviation,  $N = 6$  rats, a and b are letters subscripted for the means for the comparison test. Thus, in each column, values followed by the same letter do not show significant differences at the  $p < 0.05$  threshold.

**Table I: Effects of different aqueous and hydroethanolic extracts of *Pavetta corymbosa* leaves and silymarin on transaminase concentrations during hepatotoxicity.**

Treatment	D0		D7	
	ASAT (UI/L)	ALAT (UI/L)	ASAT (UI/L)	ALAT (UI/L)
Water + CCL <sub>4</sub>	253,3 ± 27,43	84,3 ± 5,68	275 ± 22,5	143,09 ± 22,5
Sil + CCL <sub>4</sub>	252 ± 38,69	83,67 ± 9,07	109 ± 3,6 <sup>a</sup>	42,67 ± 19,2 <sup>a</sup>
A.E + CCL <sub>4</sub>	255 ± 20,3	82,03 ± 49,96	130,7 ± 5,50 <sup>b</sup>	54,33 ± 5,8 <sup>b</sup>
H.A + CCL <sub>4</sub>	254,7 ± 17,67	85,75 ± 41,55	112,3 ± 10,2 <sup>a</sup>	43,93 ± 17,8 <sup>a</sup>
Control	40 ± 1,37UI/L	38 ± 1,35UI/L	40 ± 1,37UI/L	38 ± 1,35UI/L

D0: Transaminase concentrations in rats following induction of hepatotoxicity before treatment. D7: Transaminase concentrations after seven days of treatment following induction of hepatotoxicity by carbon tetrachloride (CCl<sub>4</sub>).

Water + CCL<sub>4</sub>: Group of rats treated with distilled water following induction of hepatotoxicity by carbon tetrachloride (CCl<sub>4</sub>).

Sil + CCL<sub>4</sub>: Batch of rats treated with silymarin (reference hepatoprotective drug) following induction of hepatotoxicity by carbon tetrachloride (CCl<sub>4</sub>).

A.E + CCL<sub>4</sub>: Batch of rats treated with the aqueous extract following induction of hepatotoxicity by carbon tetrachloride (CCl<sub>4</sub>).

H.E + CCL<sub>4</sub>: Batch of rats treated with the hydroethanolic extract following induction of hepatotoxicity by carbon tetrachloride (CCl<sub>4</sub>).

Control: Group of rats treated with distilled water.

## II.2. DISCUSSION

Regarding hepatoprotective activity, test results showed that treatment with aqueous and hydroethanolic extracts of *Pavetta corymbosa* leaves protects the liver and repairs damage caused by CCl<sub>4</sub>. The protective effects of the various extracts are believed to be due to an increase in the activity of antioxidant enzymes. Indeed, ccl4 is a dose-dependent hepatotoxicant, and its toxicity is primarily due to the formation of free radicals or toxic forms of oxygen that cause lipid peroxidation, leading to the destruction of liver cell membranes. CCl<sub>4</sub> is also a hepatotoxicant with an obligate and predictable indirect action. The increase in serum transaminase levels after CCl<sub>4</sub> injection is evidence of significant liver damage. CCl<sub>4</sub> induced liver injury is commonly used as a model for hepatic drug screening,



and the extent of damage is assessed by circulating cytoplasmic transaminase levels (ALAT and ASAT) **Hegde and Joshia (2009)**. The decrease in liver enzyme levels by extracts is an indicator of the regeneration of the liver tissue damage repair process due to CCl<sub>4</sub> (**Suresh *et al.*, 2000**); **Moselhy and Ali (2009)**. We can therefore state that repeated administration of the hydroethanolic extract of *Pavetta corymbosa* leaves would protect against CCl<sub>4</sub> induced hepatotoxicity with an efficacy close to that of silymarin. This work showed that both aqueous and hydroethanolic extracts of *Pavetta corymbosa* leaves protect the liver. This is consistent with the work of **Maxime (2012)**, who showed that at a dose of 500 mg/kg bw, the aqueous extract of *Gomphrena celosioides* leaves has a restorative effect on the liver. The same was true in **Bamba *et al.*, work (2016)**, which showed that at a dose of 200 mg/kg bw, the hydroethanolic extract of *Cola nitida* protects the liver. The aqueous and hydroethanolic extracts of *Pavetta corymbosa* leaves contain sterols, polyphenols, and flavonoids, most of which are recognized for their hepatoprotective activities **Wegner and Blast (2001)**. The hepatoprotective activity of the tested extracts could also be due to the presence of flavonoids and phenols in said extracts. However, the hepatoprotective effect of the hydroethanolic extract, much more pronounced than that of the aqueous extract and similar to silymarin, could be explained by the presence of saponins and quinones in said extract. This is in agreement with the work of **Miezan *et al.*, (2017)** who showed that the protective effect of aqueous and hydroethanolic extracts of *Erythrococca anomala* leaves at a dose of 200 mg/kg was linked to the presence of these same metabolisms.

## CONCLUSION

The results obtained during this study show that both aqueous and hydroethanolic extracts of *Pavetta corymbosa* leaves possess hepatoprotective properties. However, the hydroethanolic extract demonstrated more effective activity than the aqueous extract. These results provide a scientific basis for the traditional use of *Pavetta corymbosa* leaves in disease management.

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## ETHICS APPROVAL

The experimental procedures and protocols used in this study were approved by the ethics committee, Health Sciences Committee, Félix Houphouët-Boigny University. These guidelines were in accordance with those of the European Council Legislation 87/607/EEC



for the protection of experimental animals. Every effort has been made to minimize animal suffering and reduce the number of animals used.

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