Research Article

Study of the Antioxidant and Febrifuming potential of aqueous and hydroethanolic extracts of *Pavetta corymbosa* (DC) F.M. Williams (Rubiaceae) leaves

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Abstract

Objective: *Pavetta corymbosa* is a plant widely used in sub-Saharan Africa to treat conditions such as malaria, pain, and fever. There is no scientific literature on its antioxidant and antipyretic potential, hence the interest of this study, which is to evaluate the antioxidant and antipyretic potential of *Pavetta corymbosa* leaves, which would justify its use in traditional medicine. **Materials and methods:** The in vitro antioxidant effect of the aqueous and hydroethanolic extracts of *Pavetta corymbosa* leaves was evaluated using DPPH assay. The antipyretic potency of the aqueous and hydroethanolic extracts at doses of 200 mg/kg body weight (bw) against paracetamol (reference antipyretic) at a dose of 150 mg/kg bw was evaluated using Brewer's yeast induced pyrexia method. This method consisted of administering these extracts to rats following the induction of fever by subcutaneous injection of 20% of an aqueous suspension of brewer's yeast (*S. cerevisiae)* at a rate of 10 mL/kg. The temperature of the feverish rats was then measured 2 hours 30 minutes after this administration; then at regular 2-hour intervals. **Results**: Results of present study was showed that the hydroethanolic extract had significant antioxidant activity and febrifuge action compared to the aqueous extract ($p < 0.05$). However, the febrifuge effect of the hydroethanolic extract was comparable to that of paracetamol ($p>0.05$). **Conclusion:** Hence the interest in its use in traditional medicine against certain pathologies.

***Keywords*:** *Pavetta corymbosa*, febrifuge, hydroethanolic extract and antioxidant, paracetamol

Introduction

In recent years, the global population has become aware that fever management is necessary to improve patient comfort and therefore accelerate recovery (Kasonia et al., 1991) Moreover, traditional recipes have proven highly effective in treating certain pathologies such as malaria, pain, diabetes, hemorrhages, bacterial infections, and fever with plants (Dohou, 2015). It is in this context that *Pavetta corymbosa* has caught our attention. *Pavetta corymbosa* is an African shrubby plant. It can reach between two and four meters in height. The leaves of this plant are used in traditional Ivorian and Beninese medicine. The decoction and infusion of the leaves are used for the treatment of diabetes, malaria, fever, pain, inflammation, skin infections, and arthritis (Adjanohoun et Aké-Assi, 1979). Indeed, phytochemical exploration has highlighted the presence of certain chemical compounds in the aqueous and hydroethanolic extracts of *Pavetta corymbosa* leaves which are endowed with pharmacological activities (Miezan et al., 2024). The predominant active compounds are flavonoids, polyphenols, polyterpenes and sterols. However, in addition to the compounds mentioned above, the hydroethanolic extract contains saponosides and quinones (Miezan et al., 2024). Furthermore, the globally harmonized classification system considers the aqueous and hydroethanolic extracts of *Pavetta corymbosa* leaves as non-toxic substances (Hodge and Sterner, 1943). Thus, the aim of this study is to evaluate the antioxidant and febrifuge potential of the leaves of this plant which is commonly used in African folk medicine.

Materials and methods

Plant Material

For the evaluation of the antioxidant and febrifuge activity of Pavetta corymbosa, the plant material consisted of leaves harvested in the Aboisso region (Côte d'Ivoire).

Animal detail

Albino rats (Rattus norvegicus) of the Wistar variety,

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weighing between 117 and 121 g, were used during the in vivo study of febrifuge activity. These animals were obtained from the animal facility of the Department of Biological and Pharmaceutical Sciences at the Félix Houphouët Boigny University (Côte d'Ivoire). The rats were maintained under favorable breeding conditions in accordance with standards and good practices for laboratory animals. They were fed a standard complete feed in pellet form. They received continuous tap water from baby bottles.

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 plant material due to its accessibility and the abundance of *Pavetta corymbosa* there. 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).

Preparation of *Pavetta corymbosa* aqueous extract: EAPC

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

Preparation of the hydroethanolic extract of *Pavetta corymbosa:* EHPC

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 vacuum-filtered 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 resulting powder constituted the hydroethanolic extract of *Pavetta corymbosa.*

Evaluation of the Antioxidant Activity of *Pavetta corymbosa* Extracts

Assay of Antioxidant Compounds in *Pavetta corymbosa* Extracts

Quantification of Polyphenols in *Pavetta corymbosa* Extracts

To measure total polyphenols, the Wood et al., (2022) method was used. A volume of 2.5 mL of Folin-Ciocalteu reagent, previously diluted (1/10), was added to 30 µL of extract. The mixture was kept in the dark for 2 minutes at room temperature. Subsequently, 2 mL of calcium carbonate solution (75 $g.L^{-1}$) was added. The mixture was then placed in a water bath at 50°C for 15 minutes, then rapidly cooled. Absorbance was then measured at 760 nm, using distilled water as a reference. A calibration line was created using different gallic acid concentrations. The analyses were performed in triplicate and the polyphenol concentration was expressed in grams of gallic acid equivalent per gram of dry extract matter (g Eq FA.g^{-1}).

Quantification of flavonoids in *Pavetta corymbosa* extracts

For the determination of total flavonoids, the Marinova et al. (2005) method was used. In a 25 mL flask, 0.75 mL of 5% (w/v) sodium nitrite (NaNO2) was added to 2.5 mL of extract. The mixture was then incubated for 6 minutes in the dark. After incubation, 5 mL of sodium hydroxide (1N NaOH) was added and the volume was made up to 25 mL. The mixture was shaken vigorously before being measured using a UV-visible spectrophotometer. The reading was taken at 510 nm. The tests were carried out in triplicate. The flavonoid content was expressed in grams per liter of quercetin equivalent extract.

Free Radical Scavenging Activity of *Pavetta corymbosa* Leaf extracts with 2, 2'-Diphenyl-1-picrylhydrazyl (DPPH)

The free radical scavenging activity of *Pavetta corymbosa* leaf extracts was measured using the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay, according to the Parejo et al. (2002) method with some modifications. A range of concentrations (0-200 µg/mL) of aqueous and hydroethanolic extracts of *Pavetta corymbosa* leaves or vitamin C (reference antioxidant) was prepared in an ethanol/water (70/30) (v/v) solution. A volume of 100 µL of this solution was mixed with 3.9 mL of DPPH (70 µM) prepared in methanol. After homogenization, the mixture was incubated at room temperature (25°C) away from light. After 15 minutes of incubation, the absorbance was measured at 517 nm against a blank containing only methanol. The percentage inhibition of the DPPH radical was calculated using the following equation:

$$\text{DPPH inhibition (\%)} = \frac{(A_0 - A_{extract})}{A_0} x\ 100$$

Where: A0 = absorbance obtained with the control,

$A_{extract}$ = absorbance obtained with the test (extract).

Evaluation of the febrifuge power of *Pavetta corymbosa* extracts

The animals were fasted, with free access to drinking water for 24 hours. Their initial temperature was recorded before

fever induction. The rectal temperature of each rat was recorded using a digital thermometer just before fever induction by subcutaneous injection of twenty percent (20%) of an aqueous suspension of brewer's yeast at a rate of 10 mL/kg by Bhowmick et al. (2014). Eighteen (18) hours (T0) after pyrexia induction, the temperature was recorded. Pyrexia was confirmed by a temperature rise ranging between 0.3 °C and 0.5 °C. Rats with a temperature rise of less than 0.3 °C were excluded from the study (Diallo and Diouf, 2000; Muhamma et al., 2012). The rats were then randomly divided into several groups as follows:

- Group 1 (control): The animals in this group received distilled water orally at a rate of 10 mL/kg body weight (BW).

- Group 2: The animals in this group received orally a dose of 200 mg/kg/BW of the aqueous extract of *Pavetta corymbosa* leaves.

- Group 3: The animals in this group received orally a dose of 200 mg/kg/BW of the hydroethanolic extract of *Pavetta corymbosa* leaves.

- Group 4 (reference): The animals in this group received orally the reference antipyretic drug, paracetamol, at a dose of 150 mg/kg body weight used in therapeutics.

All these treatments were administered as soon as the temperature was recorded after pyrexia induction. Then, temperature measurements of the feverish rats were taken 2 h 30 min after this administration; then at regular intervals of 2 h. The observations were thus successively made at the following times T, corresponding to the times after pyrexia induction: 2 h 30 min (T1); 4 h 30 min (T_2); 6 h 30 min (T_3); 8 h 30 min (T_4) and 10 h 30 min (T_5) At each analysis time, the percentage reduction in pyrexia (R in %) was calculated according to the following formula:

$$\boldsymbol{R}\ (\%) = \frac{(T_p - T_n)\ x\ 100}{(T_p - T_i)}$$

With: Tp: temperature after induction of the fire; Tn: temperature after x time; Ti: initial temperature

Statistical Analysis

Results were expressed as mean plus or minus standard deviation (SD) of the mean (mean ± SD). Data were plotted using Graph Pad Prism 5.0 software (Microsoft USA).

Analysis of the results was performed using analysis of variance (ONE-WAY ANOVA). Differences between means were determined using Dunnett's test, ($P < 0.01$) (highly significant difference) and ($P < 0.05$) (significant difference).

Results and discussion

Phenol and Flavonoid content

Contents Quantitative photochemical assay of *Pavetta corymbosa* extracts indicates that EHPC contains a relatively higher level of polyphenols than EAPC (0.94 ± 0.38 versus 0.93 ± 0.25 mg EAG/g; $P < 0.05$). Similarly, the flavonoid content in EHPC (0.52 ± 0.45 mg EQ/g) is high compared to that determined in EAPC (0.37 ± 0.15 mg EQ/g). This difference is statistically highly significant ($P < 0.01$, Table 1).

Antiradical activity of *Pavetta corymbosa* leaf extracts using the DPPH assay

The percentages of inhibition of the DPPH radical indicate that EHPC has a higher antiradical activity than EAPC (81.21 versus 66.25%; $P < 0.05$; Table 2). This antiradical activity of EHPC is comparable to that of vitamin C (82.33%), used as a reference antioxidant in this study ($P > 0.05$; Table 2).

Febrifugal Potential of *Pavetta Corymbosa* Leaf Extracts

EAPC and EHPC administered to febrile rats at a dose of 200 mg/kg bw significantly ($P < 0.001$) reduced pyrexia compared to control rats (a ± b and c ± d versus e ± f; Table 3) after 2 hours of observation. Paracetamol (150 mg/kg bw) also reduced pyrexia by approximately 65.00 ± 45% ($P < 0.05$) during this same period (Table 3). EHPC and

Table 1. Total polyphenol and total flavonoid content of the extracts

Extracts	Polyphenol (mg EAG/g of DS)	Flavonoid (mg EQ/g of DS)
EAPC	0,93 ± 0,01b	0,37 ± 0,12 b
EHPC	0,94 ± 0,01 a	0,52 ± 0,10a

Values represent the mean ± SD, with n=3. GAE: Gallic acid equivalent; EQ: Quercetin equivalent ; DM: Dry matter ; a and b are letters indicating the means for the comparison test.

Table 2. Antiradical activity of aqueous and hydroethanolic extracts of *Pavetta corymbosa* leaves

Extracts	Inhibition percentages (%)
EAPC	$66,25 \pm 2,20^{b}$
EHPC	$81,21 \pm 3,26^{a}$
Vitamin C	$82,33 \pm 4,35^{a}$

The values represent the percentages of inhibition of the different substances; a and b are letters subscripted to the means for the comparison test. They come from the statistical test applied to the analysis of the experimental data. In this way, in each column, the values followed by different letters present significant differences with ($P < 0.05$).

Table 3. Percentage of inhibition of pyrexia of aqueous and hydroethanolic extracts of *Pavetta corymbosa* leaves

Percentage of inhibition	T_1	T_2	T_3	T_4	T_5
Distilled water (%)	3,85 ± 0,52	2,24 ± 0,67	1,19 ± 0,37	1,85 ±0,08	0,92 ± 42
Paracetamol (%)	65,00 ± 0,45[a]	75,00 ± 0,33[a]	85,03 ± 0,56[a]	85,01 ± 0,89[a]	87,5± 0,25[a]
EAPC (%)	51,84 ± 0,33[b]	56,70 ± 0,37[b]	72,16 ± 0,30[b]	75,47 ± 0,14[b]	76,07 ±0,23[b]
EHPC (%)	63,45 ± 0,65[a]	74,31 ± 0,67[a]	83,60 ± 0,44[a]	83,31 ± 0,31[a]	85,60 ± 0,07[a]

T_1, T_2, T_3, T_4, and T_5 represent the observation periods at different times: 2H: 30, 4H: 30, 6H: 30, 8H: 30, and 10H: 30. Distilled water (%): Percentage of pyrexia inhibition in rats treated with distilled water following pyrexia induction by brewer's yeast. Paracetamol (%): Percentage of pyrexia inhibition in rats treated with distilled water following pyrexia induction by brewer's yeast Aqueous extract (%): Percentage of pyrexia inhibition in rats treated with distilled water following pyrexia induction by brewer's yeast Aqueous extract (%): Percentage of pyrexia inhibition in rats treated with distilled water following pyrexia induction by brewer's yeast Each value is expressed as mean ± standard deviation, with n = 6 rats. a and b are letters indicating the means for the comparison test. Thus, in each column, values followed by the same letter do not show significant differences at the threshold ($P < 0.05$).

paracetamol had similar febrifugal effects throughout the observation period. Thus, after 10 hours of observations, EHPC and paracetamol induced a decrease in pyrexia of approximately 85.60 ± 0.7 and 87.5 ± 0.25 respectively (Table 3). This variation is not statistically significant (P > 0.05) (Table3).

Discussion

The results obtained during this study showed that the aqueous and hydroethanolic extracts of Pavetta corymbosa leaves possess antioxidant and antipyretic potential. Quantitative photochemical analysis of the extracts showed that EAPC contains fewer flavonoids and polyphenols compared to EHPC. The total flavonoid and polyphenol content of the hydroethanolic extract is consistent with that of the hydroethanolic extract of Zingiber officinalista rhizomes harvested in Cameroon (Fotsing et Ngogang, 2013). It appears that the ethanol and water mixture of Pavetta corymbosa leaf powder extracted more flavonoids and polyphenols than the aqueous extract. Regarding in vitro antioxidant activity, the DPPH test, which consists of trapping free radicals, was used. The results show that Pavetta corymbosa extracts neutralized free radicals using the DPPH test, with high potential for EHPC.

Any substance capable of capturing or neutralizing free radicals is called an antioxidant (Dufour et al., 2009). Antioxidant activity could be attributed to various mechanisms such as preventing the initiation of the peroxidation chain and the decomposition of peroxides (Gupta et al., 2008). The antiradical activity of both types of extracts could be explained by the presence of reducing and inhibiting compounds such as polyphenols and flavonoids present in Pavetta corymbosa leaves. These compounds act by donating a hydrogen atom to break the free radical chain. The high antioxidant potential of the hydroethanolic extract could be explained by its high polyphenol and flavonoid content, compared to the aqueous extract. This is in agreement with the work of (Kouakou et al., 2015) who showed that at a dose of 200 mg/kg bw, aqueous and hydroethanolic extracts of Trema guineensis leaves induce a significant antioxidant effect in rats. The induction of pyrexia in this study made it possible to evaluate the febrifuge potential of aqueous and hydroethanolic extracts of Pavetta corymbosa leaves on a model of inhibition of fever induced by brewer's yeast Saccaromyces cerevisae. Subcutaneous administration of brewer's yeast (Saccharomyces cerevisae) is a recommended protocol for evaluating antipyretic activities (Bhowmick et al., 2014). Thus, the rise in temperature caused by the injection of brewer's yeast (Saccharomyces cerevisae) is thought to be responsible for the production of various pro-inflammatory mediators. These stimulate prostaglandin synthesis by exerting their action at the hypothalamic center, thus causing an increase in this thermostat (Ouedraogo et al., 2012). The inhibition of prostaglandin synthesis could thus be a mechanism of the febrifuge action of EHPC similar to that exerted by paracetamol. This mechanism is responsible for blocking the enzymatic activity of cyclooxygenase by inhibiting prostaglandin synthesis. Both types of aqueous and hydroethanolic extracts of Pavetta corymbosa have antipyretic potential. However, that of the hydroethanolic extract is significantly superior to that of the aqueous extract, due to its high polyphenol and flavonoid content. This finding is consistent with the work of (Tidou et al., 2024), who showed that the hydroethanolic extract on Nephrolepsis biserrata had pyretic activity before the interaction at a dose of 200 mg/kg. The antipyretic effect of EHPC, similar to that of paracetamol, could be explained by the fact that this extract contains pharmacologically active molecules, such as flavonoids, saponins, polyphenols, polyterpenes, and sterols, which interfere with the synthesis

of prostaglandins, acting in a similar manner to non-steroidal anti-inflammatory drugs (NSAIDs) (Victor and Gaël, 2019). This result is corroborated by the work of Victor and Gaël (2019), who showed in their study that the methanoic extract of V. congolensis at 300 mg/kg of PC had excellent febrifuge power, similar to that of paracetamol, the reference molecule commonly used in cases of fever.

Conclusion

The results of this study showed that the aqueous and hydroethanolic extracts of Pavetta corymbosa leaves possess antiradical and antipyretic properties. However, the hydroethanolic extract (EHPC) demonstrated significant antioxidant activity and febrifuge action compared to the aqueous extract (EAPC). These results provide a scientific basis that could justify the traditional use of Pavetta corymbosa leaves in the treatment of pathologies.

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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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