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Phytochemical composition, Antinociceptive and anti-inflammatory activities of ethanolic and aqueous stem bark extracts of *Pavetta owariensis* P. Beauv

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Abstract
In Africa, *Pavetta owariensis* P. Beauv. (Rubiaceae) is found in Ivory Coast, Guinea, Sierra Leone, Nigeria, Cameroon and Ghana. The roots, leaves, stem bark and twigs are used by traditional healers to treat various diseases. For example, the stem bark is used in children's baths to protect them from skin and scalp infections. First, it was a question of determining chemical constituents present in stem bark of the medicinal plant. The phytochemical study revealed the presence of sterols and polyterpenes, polyphenols, flavonoids, catechic tannins, gallic tannins, alkaloids, free quinones, saponins, anthraquinones terpenoids and anthocyanins in aqueous and ethanolic stem bark extracts of *Pavetta owariensis*. On the one hand, studies carried out on the stem bark of *Pavetta owariensis* have shown that the aqueous and ethanolic extracts are analgesics which suppress sensitivity to pain in the same way as paracetamol (Doliprane®). On the other hand, the extracts have also shown anti-inflammatory potential like meloxicam (Mobic®) but in high doses.

Keywords: *Pavetta owariensis*, phytochemical, anti-inflammatory, analgesics, extract, stem bark

1. Introduction
Pavetta owariensis is a shrub or forest tree up to 7 m tall bearing young pubescent twigs. The roots, leaves, stem bark and twigs are used to treat yellow fever, malaria, skin diseases, syphilis, arthritis jaundice, guinea worm, toothache and venereal disease [1, 2]. One part of a medicinal plant is safe while all other parts are poisonous, however with *Pavetta Owariensis* all parts of the plant are used in traditional medicine. Studies carried out on *Pavetta owariensis* have concerned the chemical composition, analgesic effects and anti-inflammatory activity.

2. Materials
2.1 Vegetal material
Dried stem bark of *Pavetta owariensis* was purchased from a specialist in medicinal plants (July 2019, Abidjan, Ivory Coast). The Botanists of the laboratory carried out the identification and authentication dried stem bark of the plant.

2.2 Animals
The albino rats were delivered by the University pet store. All laboratory instructions have been respected (acclimatization, food, temperature, weight ...)

3. Methods
3.1 Extraction
It is technique of maceration which is used for the extracts. It consists in leaving red powder of stem bark of *Pavetta owariensis* in distilled water or ethanol to extract the soluble parts [3].
AEPO = Aqueous stem bark Extract of *Pavetta owariensis* and EEPO = Ethanolic stem bark Extract of *Pavetta owariensis*

3.2 Phytochemical analysis
Qualitative phytochemical screening was carried out on stem bark extracts of *Pavetta owariensis* using standard procedures which allow detection of different chemical families by staining reactions, precipitation and UV observations, Table 1 [4].

Table 1: Phytochemical characterization

Phytochemical constituents	Revelation reagent	Indicator (positive reaction)
Polyphenols	$FeCl_3$ (2%)	Dark blue or greenish color
Flavonoids	Hydrochloric alcohol, Magnesium shavings and Iso-amyl alcohol	Pink-orange or purplish color
Catechic tannins	Formalin and HCl	Gelatinous precipitate
Gallic tannins	Sodium acetate and $FeCl_3$	Blue-black color
Free quinones	NH_4OH	Red to purple color
Saponosides	Foam index	Persistent foam
Alkaloids	$HgCl_2$ and KI (Mayer)	Reddish-brown precipitate
	Picric acid (Hager) I_2 and KI (Wagner)	Creamy-white precipitate
Coumarins	KOH and HCl	Trouble or precipitate
Sterols and polyterpenes	Acetic anhydride acid and H_2SO_4	Color from purple to blue or green
Anthraquinones	NH_4OH	Yellow color
Terpenoids	$CHCl_3$, H_2SO_4	Brown color
Mucilage	Absolute ethanol	Flocculent precipitate
Anthocyanin	H_2SO_4 and NH_4OH	Black color
Volatile oils	NaOH and HCl	Black color
Cardiac glycosides	$CHCl_3$, H_2SO_4	Brown color

3.3 Analgesic (antinociceptive) activity

The study of the analgesic effects of aqueous and ethanolic stem bark extracts of *Pavetta owariensis* (AEPO and EEPO) was evaluated using the method acetic acid-induced pain. A total of 30 rats (3 rats per batch) were randomly selected and marked for identification. The rats were divided into 10 groups of 3: Gp 1: negative control (0.25 mL/100 g, Distilled water), Gp 2: positive control (80 mg/kg bw, Paracetamol), Gp 3: treated with 15 mg/kg bw of AEPO, Gp 4: treated with 45 mg/kg bw of AEPO, Gp 5: treated with 80 mg/kg bw of AEPO, Group 6: treated with 115 mg/kg bw of AEPO, Gp 3': treated with 15 mg/kg bw of EEPO, Gp 4': treated with 45 mg/kg bw of EEPO, Gp 5': treated with 80 mg/kg bw of EEPO, Gp 6': treated with 115 mg/kg bw of EEPO. The mean number of writhes and the percentage inhibition of writhes were calculated as an indicator of analgesic activity according to equation [5].

$$\%\ \text{inhibition of writhing} = \frac{\text{writhes ctrol} - \text{writhes exp}}{\text{writhes ctrol}} \times 100$$

with writhes ctrol is the mean number of writhes in the negative control
and writhes exp is the mean number of writhes in the experimental

3.4 Anti-inflammatory activity (Carrageenan-induced paw edema model)

Anti-inflammatory activity of aqueous and ethanolic stem bark extract of *Pavetta owariensis* (AEPO and EEPO) was evaluated using the carrageenan induced paw edema. The rats, 30 in number, are divided into 10 groups of 3 each. Gp 1: negative control (0.75 mL/100 g, Distilled water), Gp 2: positive control (7.5 mg/kg bw, Meloxicam), Gp 3: treated with 7.5 mg/kg bw of AEPO, Gp 4: treated with 37.5 mg/kg bw of AEPO, Gp 5: treated with 67.5 mg/kg bw of AEPO, Group 6: treated with 82.5 mg/kg bw of AEPO, Gp 3': treated with 7.5 mg/kg bw of EEPO, Gp 4': treated with 37.5 mg/kg bw of EEPO, Gp 5': treated with 67.5 mg/kg bw of EEPO, Gp 6': treated with 82.5 mg/kg bw of EEPO. The percent inhibition (PI) at each time interval was calculated [6, 7]:

$$\text{PI} = \frac{(\text{Vx} - \text{Vh})\text{ctrol} - (\text{Vx} - \text{Vh})\text{trt}}{(\text{Vx} - \text{Vh})\text{ctrol}} \times 100$$

Vx = Volume of the paw edema at particular time interval (x hour)
Vh = Volume of the paw before induction of inflammation (0 hour)
(Vx-Vh)ctrol = Volume of edema of the control group
(Vt-Vo)trt = Volume of edema in the group of treated

3.5 Statistical analysis

The experimental result was expressed as standard error of the mean. The analysis of variance was used to compare the averages between more than two groups. Values with $p < 0.05$ were considered statistically significant. Graphs were obtained using the Microsoft Excel 2016 spreadsheet. Statistical analyzes were performed in GraphPad Prism for Windows.

4. Results

4.1 Yield of extracts

The percentage yield of ethanolic and aqueous extracts of stem bark of *Pavetta owariensis* is presented in Table 3.

Table 3: Yield (%) of ethanolic and aqueous stem bark extracts of *Pavetta owariensis*

Extract	Mass	Yield (%)
EEPO	4.85	09.70
EAPO	5.05	10.10

4.2. Phytochemical composition

The qualitative determination of phytochemicals composition presents in the ethanolic and aqueous stem bark extracts of *Pavetta owariensis* (AEPO and EEPO) is presented in Table 4.

Table 4: Phytochemical analysis

Secondary metabolite	EEPO	AEPO
Anthocyanins	+	+
Anthraquinones	+	+
Polyphenols	+	+
Flavonoids	+	+
Cardiac glycosides	-	-
Gallic tannins	+	+
Free quinones	+	+
Saponins	+	+
Alkaloids	+	+
Coumarins	-	-
Catechic tannins	+	+
Terpenoids	+	+
Mucilages	-	-
Sterols and polyterpenes	+	+
Volatile oils	-	-
(+) = Present, (-) = Absent		

4.3 Antinociceptive effects

The results of antinociceptive effects are presented in table 5 and figure 1, AEPO and EEPO showed significant analgesic activity.

Table 5: Effects of AEPO and EEPO on acetic acid-induced pain

	Writhing frequency	Inhibition of writhing (%)
Gp 1: Negative control with 0.45 mL/100 g bw of Distilled water	92.84 ±1.34	0
Gp 2: Positive control with 80 mg/kg bw of Paracetamol	11.78± 1.24	87.31
Gp 3: Group treated with 15 mg/kg bw of AEPO	70.16±1.89	24.43
Gp 4: Group treated with 45 mg/kg bw of AEPO	57.97±1.46	37.56
Gp 5: Group treated with 80 mg/kg bw of AEPO	22.91±1.29	75.32
Gp 6: Group treated with 115 mg/kg bw of AEPO	07.46±1.47	91.96
Gp 3': Group treated with 15 mg/kg bw of EEPO	74.87±1.91	19.35
Gp 4': Group treated with 45 mg/kg bw of EEPO	59.81±1.23	35.58
Gp 5': Group treated with 80 mg/kg bw of EEPO	24.27±1.19	73.86
Gp 6': Group treated with 115 mg/kg bw of EEPO	09.42±1.37	89.85

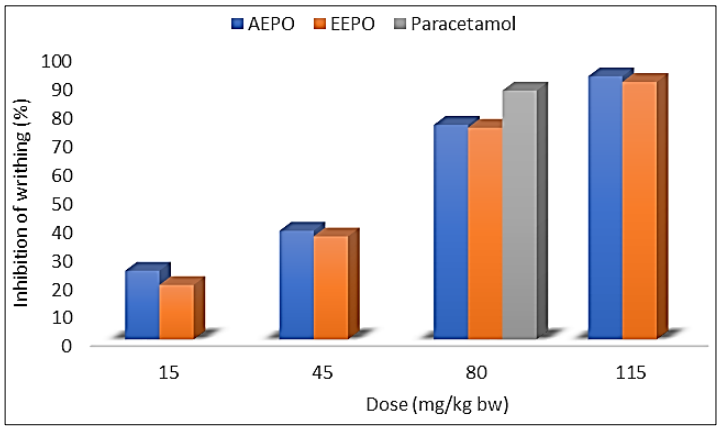

Fig 1: Comparison between the effects of Paracetamol, AEPO and EEPO on acetic acid-induced pain

4.4 Anti-inflammation study (Carrageenan-induced paw edema model)

Result of anti-inflammatory activity of AEPO and EEPO are shown in table 5 and figure 2. AEPO and EEPO inhibit inflammation induced by carrageenan. However, anti-inflammatory activity is greater with AEPO and EEPO at high doses than Meloxicam used as a reference.

Table 5: Effect of the AEPO and EEPO with carrageenan-induced paw edema in rats

Paw volume (mL) PI = Percentage inhibition (%)						
Groups	**Before**	**0 h**	**0.5 h**	**1.5 h**	**3.5 h**	**5.5 h**
Distilled water, 0.75 mL/100 g bw	0.85 ± 0.01	0.95 ± 0.02	1.96 ± 0.01	2.92 ± 0.01	3.05 ± 0.01	2.93 ± 0.01
Meloxicam, 07.50 mg/kg bw	0.86 ± 0.01	0.96 ± 0.01	1.76 ± 0.01* (20.79)	1.52 ± 0.02* (44.55)	1.43 ± 0.02* (77.61)	1.35 ± 0.01* (80.30)
AEPO, 07.50 mg/kg bw	0.85 ± 0.02	0.96 ± 0.02	1.81 ± 0.02* (15.84)	1.73 ± 0.01* (23.76)	1.66 ± 0.01* (66.66)	1.32 ± 0.02* (81.81)
AEPO, 37.50 mg/kg bw	0.86 ± 0.02	0.95 ± 0.02	1.80 ± 0.01* (15.84)	1.72 ± 0.01* (23.76)	1.57 ± 0.02* (70.48)	1.31 ± 0.02* (81.81)
AEPO, 67.50 mg/kg bw	0.87 ± 0.02	0.95 ± 0.01	1.79 ± 0.02* (16.83)	1.70±0.01* (25.74)	1.56 ± 0.01* (70.95)	1.29 ± 0.01* (82.82)
AEPO 82.50 mg/kg bw	0.87 ± 0.01	0.96 ± 0.02	1.78 ± 0.02* (18.81)	1.65 ± 0.02* (31.68)	1.55 ± 0.02* (71.90)	1.25 ± 0.01* (85.35)
EEPO 07.50 mg/kg bw	0.86 ± 0.01	0.95 ± 0.01	1.80 ± 0.01* (15.84)	1.72 ± 0.01* (23.76)	1.62 ± 0.01* (68.09)	1.29 ± 0.01* (82.82)

EEPO 37.50 mg/kg bw	0.87 ± 0.01	0.96 ± 0.01	1.79 ± 0.02* (17.82)	1.65 ± 0.01* (31.68)	1.55 ± 0.02* (71.90)	1.28 ± 0.01* (83.83)
EEPO 67.50 mg/kg bw	0.86 ± 0.01	0.96 ± 0.02	1.78 ± 0.02* (18.81)	1.64 ± 0.02* (32.67)	1.54 ± 0.01* (72.38)	1.27 ± 0.01* (84.34)
EEPO 82.50 mg/kg bw	0.87 ± 0.01	0.95 ± 0.02	1.77 ± 0.01* (18.81)	1.62 ± 0.02* (33.66)	1.47 ± 0.02* (75.24)	1.23 ± 0.01* (85.85)

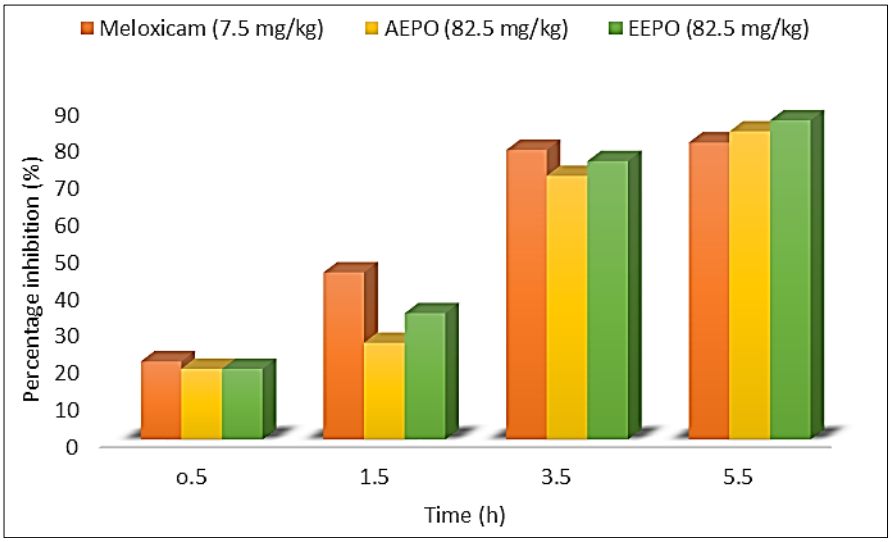

Fig 2: Effect of the AEPO and EEPO on inflammation induced by carrageenan

5. Discussion

Secondary metabolites can be broken down into three main groups: alkaloids, terpenes and phenolic compounds. They represent an important part of natural substances not only in number but also by the biological and pharmacological activities that they confer on the plants in which they are found [8, 9]. Polyphenols are known for their anti-inflammatory, antioxidant cytotoxic, anticancer, antitumor, antiparasitic, antimicrobial and cardioprotective activities. Alkaloids play a defensive role in the plant against herbivores and pathogens. Due to their potent biological activities, many known alkaloids have been exploited as pharmaceuticals, stimulants, narcotics and poisons. Terpenes are responsible for the colors varying from yellow to red in flowers and fruits. This coloring attracts pollinators (flowers) and serves as a source of food for herbivores, thus aiding in the dispersal of seeds [10, 11, 12].

Doliprane® is an analgesic (relieves pain) and an antipyretic (lowers fever). The active substance of this medicine is paracetamol. As soon as it is taken, paracetamol intervenes in the brain, spinal cord and peripheral nervous systems. For this, paracetamol blocks the synthesis of prostaglandins in the nervous system. it also limits the sensation of pain by directly attacking the peripheral system.

From our results, it is possible to affirm that AEPO and EEPO act in the same way as paracetamol at higher doses, therefore the same mechanism of action. Note that paracetamol is present in many drugs, alone or combined with other active substances. Used wisely, paracetamol is safe and effective [13, 14].

Mobic® contains an active substance called meloxicam. Meloxicam belongs to a group of medicines called nonsteroidal anti-inflammatory drugs (NSAIDs), which are used to reduce inflammation and pain in the joints and muscles. Meloxicam works by inhibiting the synthesis of prostaglandins, the substances responsible for inflammation. it more inhibits the COX-2 cyclooxygenase, while relatively sparing the constituent cyclooxygenase COX-1, but the selectivity of inhibition on COX-2 only appears at the minimum recommended dosages. Meloxicam decreases the hormones that cause pain, soreness, tenderness, stiffness, and inflammation. From the results of our research, it seems that AEPO and EEPO use the same mode of action as paracetamol. Stem bark of *Pavetta owariensis* is therefore vegetable paracetamol [15, 16, 17].

6. Conclusion

AEOP and EEPO intervene by inhibiting enzymes responsible for inflammation, which has the effect of preventing the formation of molecules involved in pain. They should also exert an action inducing heat dissipation. This explains its antipyretic effect (which lowers fever).

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