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Phytochemical screening, acute oral toxicity, Analgesic effects and wound-healing activity of ethanolic and aqueous stem bark extracts of *Pavetta owariensis* P. Beauv.

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Abstract
In a word, *Pavetta owariensis* (Rubiaceae) is one of the important medicinal plants using in the African pharmacopeia. The present study deals with the phytochemical analysis stem bark of *Pavetta owariensis*; alkaloids, anthraquinone, anthocyanins, catechic and gallic tannins, free quinone, saponins, sterols and polyterpenes, flavonoids, polyphenols and terpenoids are present in the extracts (AEPO and EEPO). Moreover, many researchers claim that its secondary metabolites obtained from phytochemical analysis are responsible for the pharmaceutical activity of plants. There is not much to be added apart from the fact no detailed scientific data are available regarding acute toxicity, analgesic effect and wound-healing activity of *Pavetta owariensis*. As no mortality and no adverse effects have been observed in animals, it can be concluded that the LD50 of AEPO and EEPO is greater than 2000 mg/kg body weight. Therefore, the plant studied can be consumed safely. Experiments have shown that AEPO and EEPO have analgesic properties in the same way as Acetaminophen. Ethanolic and aqueous stem bark extracts of *Pavetta owariensis* based on shea butter (AEPO_10% and EEPO_10%) were found to have significant healing activity which was evidenced by increase in the rate of wound contraction and skin-breaking strength.

Keywords: *Pavetta owariensis*, phytochemical, analgesic, excision, incision, wound-healing

1. Introduction
One of the reasons herbal remedies are so popular is that herbal remedies are safer and cause fewer side effects. However, herbal remedies are not always safe and like drugs of all kinds, they need to be used with caution [1]. *Pavetta owariensis*. P. Beauv is a plant of the Rubiaceae family. It is used in traditional African medicine for the management of malaria, anthelmintics and other health problems such as wound healing [2]. This present study was carried out with the aim of evaluating the phytochemical composition, acute oral toxicity, analgesic effects and wound healing activity of ethanolic and aqueous stem bark extracts of *Pavetta owariensis*.

2. Materials
2.1 Vegetal material
The stem bark of *Pavetta owariensis* P. Beauv was collected in a forest in the village of Abatta (Abidjan, Côte d'Ivoire) in July 2019. It was identified and authenticated by the botanists of the Laboratory in Ivory Coast. First, the fresh stem bark of the plant was cut and broken into small pieces and oven dried. Then it was crushed to obtain a red powder and finally kept in a jar.

2.2 Experimental animals
Animals including albino mice and albino rats were donated by the animal facility of the University Jean Lorougnon Guede in Ivory Coast for experimental research. The animals were acclimatized and fed for a week before the experiments.

3. Methods
3.1 Extraction
The preparation of the extracts was done by the standard cold maceration extraction method because the heat destroys the active constituents of the medicinal plant, cold maceration is more suitable than a decoction.

Two solvents of different polarity are used, namely distilled water and ethanol. The maceration was then filtered, and the filtrate was dried in a hot air oven set at 80 °C for 24 h and weighed again [3].
AEPO = Aqueous stem bark Extract of *Pavetta owariensis.*
EEPO = Ethanolic stem bark Extract of *Pavetta owariensis.*

3.2 Phytochemical study
The aqueous and ethanolic extracts of the stem bark of *Pavetta owariensis* were subjected to a qualitative phytochemical study to detect the different chemical families, Table 1 [4].

Table 1: Usual methods of phytochemical screening

Secondary metabolite	Reagent of identification	Indicator (positive reaction)
Sterols and polyterpenes	Acetic anhydride acid and H_2SO_4	Color from purple to blue or green
Polyphenols	$FeCl_3$ (2%)	Dark blue or greenish color
Flavonoids	Hydrochloric alcohol, Magnesium shavings and Isoamyl 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
Sennosides	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
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 Acute toxicity study
The acute toxicity study was performed for aqueous and ethanolic stem bark extracts of *Pavetta owariensis* (AEPO and EEPO) according to OECD guidelines.
To begin with a total of 30 mice (6 mice per group), were randomly selected. The mice were divided into 5 groups of 6:
Group 1: Control (normal saline),
Group 2: Treated with 5 mg/kg bw.
Group 3: Treated with 50 mg/kg bw.
Group 4: Treated with 300 mg/kg bw.
Group 5: Treated with 2000 mg/kg bw.

Lastly, the animals were observed almost constantly for behavioral changes, mortality and appearance during different hours and then every day for a period of two weeks [5].

3.4 Analgesic activity
The study of the analgesic effects of aqueous and ethanolic stem bark extracts of *Pavetta owariensis* were evaluated using the method of writhing induced by acetic acid on mice. First, a total of 60 mice (6 mice per batch) were randomly selected and marked for identification. The mice were divided into 10 groups of 6:
Group 1: Negative control (1 mL/100 g, distilled water).
Group 2: Positive control (100 mg/kg bw, Acetaminophen).
Group 3: Treated with 50 mg/kg bw of AEPO.
Group 4: Treated with 75 mg/kg bw of AEPO.
Group 5: Treated with 100 mg/kg bw of AEPO.
Group 6: Treated with 500 mg/kg bw of AEPO.
Group 3': Treated with 50 mg/kg bw of EEPO.
Group 4': Treated with 75 mg/kg bw of EEPO.
Group 5': Treated with 100 mg/kg bw of EEPO.
Group 6': Treated with 500 mg/kg bw of EECG.

The mean number of writhes and the percentage inhibition of writhes were calculated as an indicator of analgesic activity according to equation [6].

$$\text{Percentage inhibition of writhing} = \frac{\text{writhes control} - \text{writhes experimental}}{\text{writhes control}} \times 100$$

With writhes control = the mean number of writhes in the control and writhes experimental = the mean number of writhes in the experimental.

3.5 Healing activity
To assess healing activity of aqueous and ethanolic stem bark extracts of *Pavetta owariensis* (AEPO and EEPO), standard models of excisional and incisional wounds were used.

3.6.1 Excision wound model
A full-thickness circular excisional wound measuring approximately 500 mm^2 and 1.7 mm in depth was performed on shaved dorsal thoracic region of experimental rats while respecting all laboratory recommendations. The rats were divided into 3 groups of 6: Group 1: placebo control (Shea butter), Group 2: treated with 10% ointment of the aqueous stem bark extract of *Pavetta owariensis* (AEPO_10%), Group 3: treated with 10% ointment of the ethanolic stem bark extract of *Pavetta owariensis* (EEPO_10%). The wound closure rate was assessed by measuring wound on days 0, 2, 4, 8, 12, 16, 18, and 20. The percentage of wound contraction at each time interval was calculated [7]:

$$\text{Percentage of wound contraction} = \frac{\text{wound 0} - \text{wound t}}{\text{wound 0}} \times 100$$

With:
Wound 0 = wound area at 0 hour,
Wound t = wound area at particular time (t)

Ointment's formulation
A single ointment (shea butter) and two mixed ointments were prepared according to formula described in Ivorian Pharmacopoeia (Table 2).
Shea butter, stem bark extract aqueous and ethanolic of *Pavetta owariensis* (AEPO and EEPO) and potassium benzoate (preservative = E 212) were triturated in a mortar with a pestle to obtain a homogeneous paste. Two ointments, AEPO_10% and EEPO_10% were packaged in jars and stored at room temperature [8].

Table 2: Formula used for different ointments

Composition	Placebo Shea butter	Ointment AEPO_10%	Ointment EEPO_10%
AEPO (g)	0	4	0
EEPO (g)	0	0	4
Shea butter (g)	39.6	35.6	35.6
Potassium benzoate (g) (E 212)	0.4	0.4	0.4
Total (g)	40	40	40

3.6.2 Incision wound model

Under the same conditions as for the previous model, a longitudinal paravertebral incision of 5 cm in length was made and sutured by 1 cm this time [9]. After 24 h of wound creation, on the first day, the rats were divided into 3 groups of 6.

Group 1: Placebo control (Shea butter + E 212).
Group 2: Treated with the 10% ointment of the aqueous stem bark extract of *Pavetta owariensis* (AEPO_10%).

Group 3: Treated with the 10% ointment of the ethanolic stem bark extract of *Pavetta owariensis* (EEPO_10%).

The sutures were removed on day 8 post-incision and the treatment was continued. Then, the tensile strength was measured on the 10th day and calculated using weight technique [10].

$$\text{Percentage of tensile strength} = \frac{\text{tensile strength experimental} - \text{tensile strength control}}{\text{tensile strength control}} \times 100$$

with tensile strength control = The mean tensile strength of placebo control and tensile strength experimental = The mean the tensile strength of group treated.

3.7 Data 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 Graph Pad Prism for Windows.

4. Results

4.1 Yield of extracts

The percentage yield of ethanolic and aqueous stem bark extracts of *Pavetta owariensis* (AEPO and EEPO) was presented in Table 3.

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

Extract	Mass (g)	Yield (%)
EEPO	4.25	8.50
EAPO	5.15	10.30

4.2 Phytochemical screening

Phytochemical screening of the ethanolic and aqueous stem bark extracts of *Pavetta owariensis* (AEPO and EEPO) was done to qualitatively identify presence or absence of secondary metabolites and results were presented in Table 4.

Table 4: Results of phytochemicals analysis

Secondary metabolites	EEPO	EAPO
Alkaloids	+	+
Anthraquinones	+	+
Anthocyanins	+	+
Catechic tannins	+	+
Gallic tannins	+	+
Free quinones	+	+
Saponins	+	+
Sterols and polyterpenes	+	+
Coumarins	-	-
Polyphenols	+	+
Terpenoids	+	+
Mucilages	-	-
Flavonoids	+	+
Volatile oils	-	-
Cardiac glycosides	-	-
(+) = Presence, (-) = Absence		

4.3 Acute toxicity study

The results of oral acute toxicity study revealed that all tested of aqueous and ethanolic stem barks extracts of *Pavetta owariensis* (AEPO and EEPO) were appeared safe to the dose of 2000 mg/kg as none of the mice was died and even did not show any sign of toxicity during the observation period of 14 days. Therefore, LD_{50} of aqueous and ethanolic stem bark extracts of *Pavetta owariensis* are greater than 2000 mg/kg bw.

4.4 *In vivo* analgesic effects

The result of the analgesic effects of aqueous and ethanolic stem bark extracts of *Pavetta owariensis* (AEPO and EEPO) is presented in Table 5. AEPO and EEPO showed significant analgesic activity in reducing number writhing induced by acetic acid (Figure 1).

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

	Writhing frequency	Inhibition of writhing (%)
Group 1: Negative control with 1mL/100 g bw of Distilled water.	97.54 ±1.93	0
Group 2: Positive control with 100 mg/kg bw of Acetaminophen.	14.81±1.74	84.82
Group 3: Group treated with 50 mg/kg bw of AEPO.	48.12±1.32	50.67
Group 4: Group treated with 75 mg/kg bw of AEPO.	31.56±1.29	67.64
Group 5: Group treated with 100 mg/kg bw of AEPO.	16.37±1.46	83.22
Group 6: Group treated with 500 mg/kg bw of AEPO.	08.59±1.89	91.19
Group 3': Group treated with 50 mg/kg bw of EEPO.	62.98±1.31	35.43
Group 4': Group treated with 75 mg/kg bw of EEPO.	47.69±1.83	51.10
Group 5': Group treated with 100 mg/kg bw of EEPO.	24.57±1.37	74.81
Group 6': Group treated with 500 mg/kg bw of EEPO.	13.42±1.47	86.24

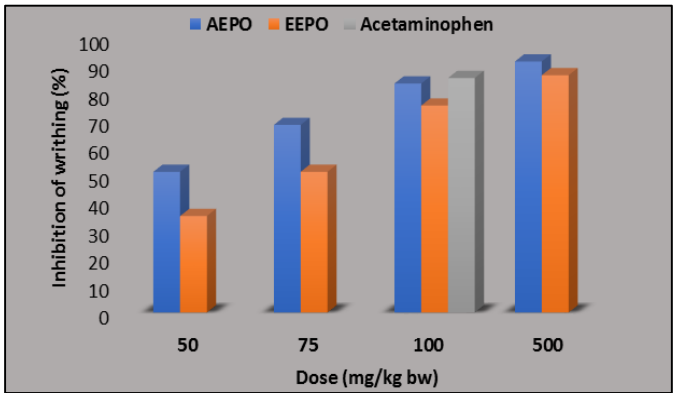

Fig 1: Comparison between the effects of AEPO, EEPO and Acetaminophen on acetic acid-induced writhing

4.5 Wound-healing activity

4.5.1 Excision wound model

As depicted in Table 6, AEPO_10% and EEPO_10% showed significant wound contraction against control placebo on days 2 to 20. The data from (Table 6) confirmed that considerably shorter healing time was recorded by EEPO_10% and AEPO_10% against control placebo (Figure 2).

Table 6: Wound-healing effect of AEPO_10% and EEPO_10% in excision wound model

Wound area (mm^2) and (% wound contraction)			
Post-wounding days	**Control placebo**	**AEPO_10%**	**EEPO_10%**
0	504.50±1.80	503.30±1.70	503.50±1.40
2	460.70±1.50 (8.68%)	457.50±1.20 (9.10%)	455.80±1.35 (9.47%)
4	415.80±1.40 (17.58%)	390.90±1.50 (22.33%)	384.50±1.90 (23.63%)
8	330.90±1.70 (34.41%)	255.50±1.80 (49.23%)	225.80±1.70 (55.15%)
12	210.50±1.30 (58.27%)	105.80±1.90 (78.98%)	97.76±1.10 (80.58%)
16	75.67±1.45 (85%)	20.16±1.30 (95.99%)	10.50±1.20 (97.91%)
18	20.35±1.80 (95.96%)	4.57±1.40 (99.10%)	00±00 (100%)
20	8.97±1.24 (98.22%)	00±00 (100%)	00±00 (100%)
Epithelialization (day)	21.3±1.50	18.35±1.40	16.15±1.50

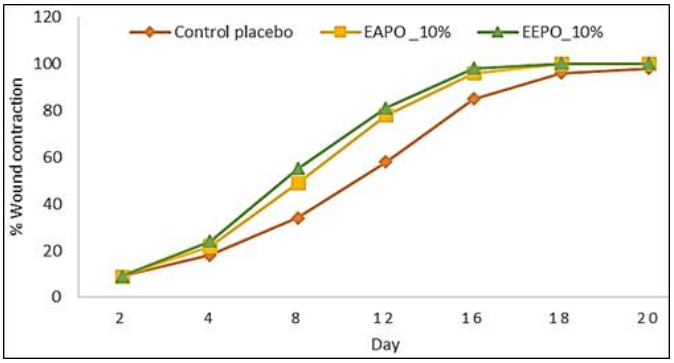

Fig 2: Wound-healing effect of AEPO_10%, EEPO_10% and Control placebo in excision wound model

4.5.2 Incision wound model

As shown in Table 7, AEPO_10% and EEPO_10% were effective in increasing breaking strength of healing wound. Comparing with Control placebo, AEPO_10% and EEPO_10% had a greater increasing effect on the tensile strength.

Table 7: Wound-healing effect of Control placebo, AEPO_10% and EEPO_10% in incision wound model

	Breaking strength (g)	**Tensile strength (%)**
Control placebo	295.90±1.79	0
AEPO_10%	489.34±1.88	65.37
EEPO_10%	570.70±1.35	92.87

5. Discussion

Phytochemical screening test of AEPO and EEPO revealed the presence of alkaloids, anthraquinones, anthocyanins, catechic and gallic tannins, free quinones, saponins, sterols and polyterpenes, flavonoids, polyphenols and terpenoids. By the way, these biologically active compounds are directly accountable for, antimicrobial, antifungal, antioxidant, anti-bacterial, anti-analgesic, anti-inflammatory and anticancer activities [11]. The terpenoids and tannins for example are also promote wound-healing process. Several arguments can be put forward, these compounds are responsible for fast wound contraction and shorter epithelialization period [12, 13].

Note that, the acetic acid-induced writhing test serves as a standard technique to assess analgesic efficacy of herbal drugs. It is good to know that acetic acid induces the release of various endogenous mediators involved in the pain mechanism. We can cite, histamine, it is precisely histamine that triggers the effects of allergy, this molecule is a neurotransmitter secreted by the body, in particular during allergic reactions. As for serotonin, it is involved in particular in the regulation of behavior, motivation, decision-making, mood, anxiety, this molecule is a neuromodulator produced by neurons to modulate communication between other neurons in our brain. Finally, bradykinin which is a peptide hormone which acts on smooth muscles, which dilates blood vessels and increases the permeability of capillaries, it plays an important role in the pain mechanism [14, 15]. In this study, EAPO and EEPO showed significant inhibition of writhing induced by acetic acid in a way that prevents the prostaglandin pathway in pain perception. Prostaglandins play a central role in communication and regulation between cells in the body. In this regard, they cause sensitivity to pain and worsen edema associated with inflammation; by their vasodilator action [16].

The healing process consists of three phases: firstly, the defense phase: phase during which body defends itself against all types of infections thanks to the immune cells; secondly, the repair phase: stage during which new tissues will form, it is at this time that a superficial layer forms on the skin to cover it; thirdly, the maturation phase: the longest stage because it can last from a few months to a few years, it is during this phase that the collagen and elastin fibers are strengthened to restructure the epidermis [17, 18].

The healing property of AEPO_10% and EEPO_10% appears to be due to the phytoconstituents present in the medicinal plant, and it also appears that the faster healing process may be a function of the individual or additive effects of the phytoconstituents [19].

6. Conclusion

There is not much to be added apart from the fact that, AEPO and EEPO have pain relieving effects. In addition, AEPO and EEPO have LD_{50} values greater than 2000 mg/kg bw and are therefore non-toxic. Therefore, they can be used as alternatives in pain management, as traditional medicine claims. AEPO_10% and EEPO_10% have properties which make them capable of promoting an accelerated wound healing activity compared to the placebo control.

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