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Effect of *Cassia occidentalis* on Second-Degree Burn Wounds in Wistar Rats

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ABSTRACT

Cassia occidentalis, also known as false kinkeliba, is a medicinal plant known for its therapeutic properties. The aim of the present study was to demonstrate the healing properties of the aqueous extract of C. occidentalis leaves in a model of experimental second-degree burns in wistar rats, supported by phytochemical screening. These burn wounds were treated with C. occidentalis leaf extract at doses of 125, 250 and 500 mg/kg, 40 and 80 mg/ml respectively, and flukocin , a reference molecule for wound healing. The results of these studies showed that C. occidentalis leaf extract at 500 mg/kg and 80 mg/ml promotes rapid healing by reconstituting the skin layer. This healing process could be explained by the presence in the leaves of this plant of flavonoids, polyphenols, sterols and poly terpenes, alkaloids and saponosides revealed by phytochemical screening.

Keywords: C. occidentalis, wistar rats, flukocin, wound healing.

Introduction

Medicinal plants are an ideal alternative to chemical or specialty drugs. Throughout Africa, the observation is clear: populations faced with the problem of costs and/or accessibility of specialties in rural areas are increasingly turning to traditional pharmacies and traditional practitioners as an alternative to conventional treatment [1]. In Ivory Coast, the flora contains plants with healing properties. Numerous studies have shown the healing activity of some of these medicinal plants such as Tetrapleura tetraptera [2] and Kalanchoe crenata [3]. Also, plants have been used successfully as healing agents in the management of wounds [4]. Cassia occidentalis, also known as false Kinkeliba, is a plant whose leaves are used in the preparation of several remedies commonly used to relieve a wide variety of ailments, including wound healing [5]. However, despite its widespread traditional use, the biological mechanisms underlying this healing activity remain little explored scientifically. This study therefore aims to highlight the healing properties of Cassia occidentalis through an experimental evaluation, in order to validate its traditional use and to open the way to a pharmacological valorization of this endogenous plant.

Materials and Methods

Plant Material

The plant material used consisted of Cassia occidentalis leaves (**Figure 1**). They were harvested in the Daloa region in west-central Côte d'Ivoire.

Animal

Rats weighing 180 to 220 grams, Rattus novegicus, Wistsar strain, from the laboratory at the Félix Houphouët Boigny University in Cocody, were used for the experiments. They were placed in individual plastic cages with a mesh lid and provided with feeding bottles. A layer of wood shavings was placed at the bottom of the cages to constitute the bedding. They were fed daily with pellets from the IVOGRAIN company and tap water in the bottles.

Methods

1 Preparation of the Total Aqueous Extract

Harvested Cassia occidentalis leaves were washed and then dried away from light at room temperature (25-30°C) for three weeks in the Biochemistry Laboratory of Jean Lorougnon Guédé University. They were then reduced to powder using an electric grinder (Retsch sk 100). The powder obtained was macerated according to the method of 6: One hundred grams (150g) of Cassia occidentalis powder were put in one and a half liters of distilled water. The mixture was homogenized 10 times at a rate of 2 minutes per revolution using a Binatone brand mixer. The homogenate obtained was wrung out in a square of white cotton cloth and then filtered three times through absorbent cotton and once through Whatman paper (3 mm). The filtrate was evaporated at 50°C using a venticelle® type oven.

2 Secondary Metabolite Detection

This study was conducted on the total aqueous extract of Cassia occidentalis using the precipitation or staining technique according to the methods described in [7]. (**Table 1**)

3 Rat Treatment

3 1 Rat Weighing

Twenty-five (25) individually marked rats were weighed on D_0, D_7, D_{14}, D_{21}, and D28, then divided into five (05) groups.

3 2 Wound Induction

Rats were anesthetized by inhalation in a bell jar containing cotton soaked in ethyl ether for 2 to 3 minutes. Their dorsal flanks were shaved and cleaned with 70% surgical alcohol 24 hours before burn induction. Experimental burns were induced using a 3 cm diameter metal cylinder connected to a rod with a handle. The cylinder heated to a temperature of 200 °C was applied for 20 seconds by lightly pressing on the surface of the shaved skin of the rats in order to cause extensive deep second-degree burns [8]. This burn is characterized by damage to the epidermis and dermis with the presence of blisters (liquid of vascular origin forming a bubble which develops at the epidermis-dermis interface) with a red background, whitish areas.

3 3 Force-feeding the rats

The twenty-five (25) rats, divided into five (05) groups, were treated as follows.

-The rats in group 1 were treated with distilled water (control rats).

-The rats in group 2 were treated with the plant extract at a dose of 125 mg/kg and a concentration of 40 mg/ml.

Rats in group 3 were treated with the plant extract at a dose of 250 mg/kg with a concentration of 40 mg/ml.

-Rats in group 4 were treated with the plant extract at a dose of 500 mg/kg with a concentration of 80 mg/ml.

-Rats in group 5 were treated with Flukocin 500 at a dose of 25 mg/kg with a concentration of 6.66 mg/ml. All rats were evaluated daily for twenty-eight days after wound induction.

4 Evaluation of healing activity

The healing activity of the extract was evaluated at the macroscopic level (observation of wound moisture or dryness and measurement of wound surface reduction diameter) and at the biochemical level.

4 1 Macroscopic Assessment

At the macroscopic level, wound healing was assessed by observing the wound to determine its moisture or dryness. Skin recovery was assessed by observing the presence or absence of granules in the wound on D_0, D_1, D_7, D_{14}, D_{21}, and D_{28}.

4 2 Planimetric Evaluation

The planimetric evaluation consisted of measuring the wound diameters using a caliper, and calculating the wound surface areas using the following formulas:

$$\text{Wound surface} = \frac{\text{Large diameter}}{2} \times \frac{\text{Small diameter}}{2} \times \pi$$

5 Statistical Analyses

Results are expressed as means plus or minus standard errors of the mean (M ± SEM). Means between treated and control groups were compared using the Student t-test. The significance threshold was set at $p < 0.05$.

Results

1. Secondary Metabolite Investigation

Qualitative phytochemical analysis of the extract revealed the following chemical compounds: alkaloids, phenols, flavonoids, saponins, and polyphenols and sterols, with an absence of tannins and quinones. (**Table 2**)

2 Effect of the different treatments on rat body mass

There was a slight loss in body mass in all rats on day 7, followed by a slight increase from day 14. However, a significant increase until the end of treatment was observed in the groups treated with fluckocin 500 g (reference molecule) and the extract at a dose of 500 mg/kg. (**Table 3**)

3 Macroscopic Observation

Large wounds that were moist from the first few days became dry wounds after two weeks of treatment, then reduced in size in groups 4 and 5 at the end of the experiment. (**Figure 2**)

4 Planimetric Evaluation

The average surface area gradually decreased for all lots, but at a faster rate in groups 4 and 5 (**Figure 3**).

Discussion

In this study, the healing activity of phytoconstituents of the aqueous extract of C. occidentalis leaves in a second-degree experimental burn model was demonstrated in Wistar rats. Triphytochemical study revealed the presence of certain chemical compounds such as polyphenols, flavonoids, alkaloids, sterols and polyterpenes and saponosides in the leaves of C. occidentalis This result is consistent with the results of [9] who reported the presence of these same secondary metabolites in the aqueous extract of C. Occidentalis leaves. Since wound healing is a complex process characterized by homeostasis, re-epithelization, granulation tissue formation and extracellular matrix remodeling [10], the study of the effects of phyto-constituents of the aqueous extract of C. Occidentalis leaves focused, among other things, on the macroscopic evaluation of wounds. The humidity and increase in wound surface area observed from the first days of the burn could suggest that the effects of the extract were not yet effective. On the other hand, from the second week of treatment, these wounds dry up and their surfaces reduce. The effects of the wounds were effectively and progressively attenuated by the aqueous extract of the leaves of C. Occidentalis in all groups, particularly in the group at a dose of 500 mg/kg. These observations could suggest that the extract exerts both anti-inflammatory and antioxidant effects. Indeed, the polyphenols contained in the extract are known to be chemical molecules with antioxidant activity. Polyphenols on the one hand are known as chemical molecules that possess antioxidant activity and are believed to attenuate damage caused by free radicals at the macromolecular level [11]. On the other hand, saponins, for their part, would exert an anti-inflammatory activity [12]. These observed phenomena could be due to the action of flavonoids present in the aqueous extract of the leaves of C. occidentalis. Indeed, according to 13 these flavonoids would involve the stimulation of the production of fibroblasts and/or collagen fibers or a stimulation of angiogenesis to promote rapid healing.

Conclusion and Outlook

The results of this study showed that C. occidentalis leaf extract possesses healing properties commonly used in traditional medicine. This effect stems from chemical compounds such as polyphenols, flavonoids, sterols, polyterpenes, and alkaloids present in C. occidentalis leaves. However, further studies are needed to isolate and identify the active molecule(s) and subsequently elucidate their pharmacological mechanisms of action.

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Figures and tables

Figure 1: C. occidentalis leaves

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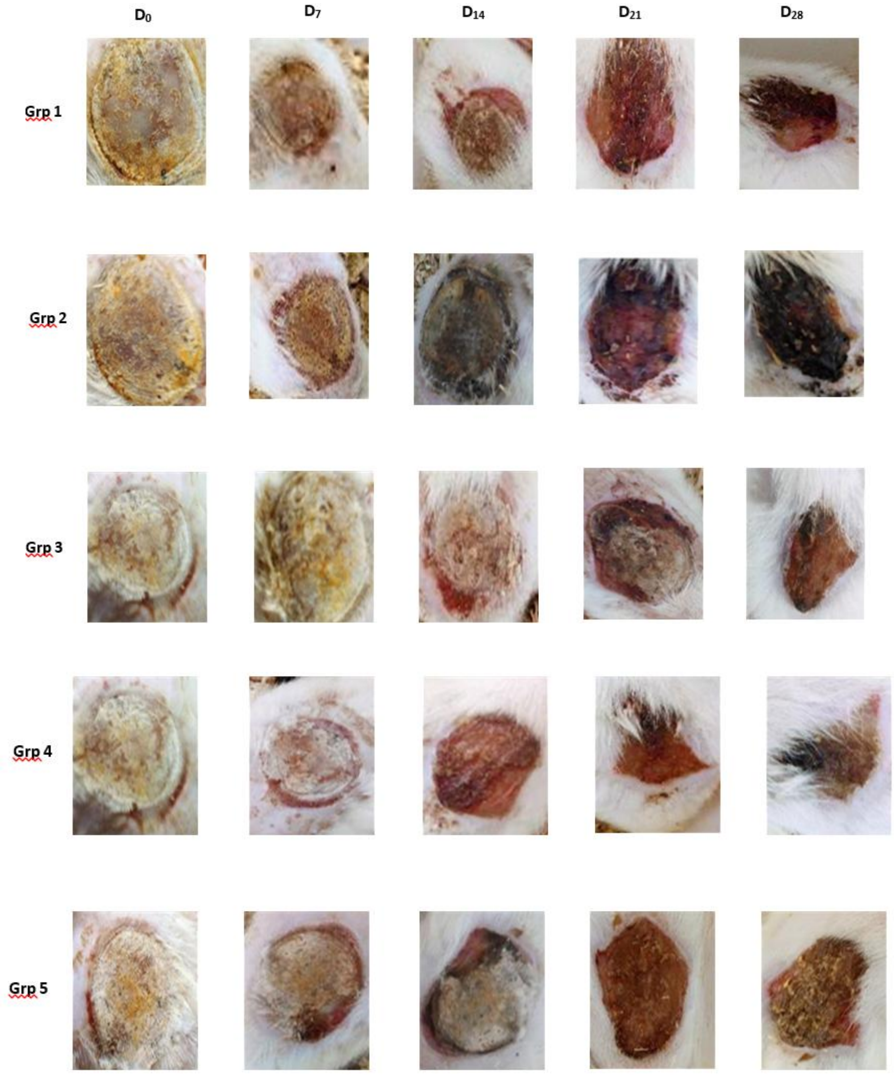

Figure 2 : Evolution of wounds over time

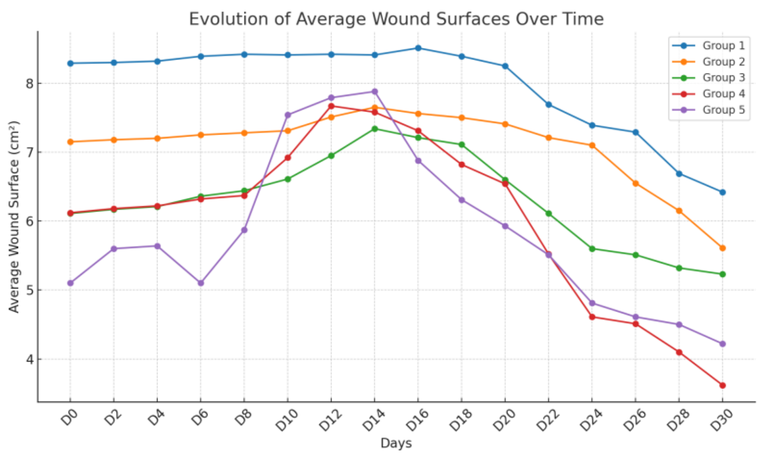

Figure 3: Evolution of average wound surfaces over time

Table 1 : Method for researching secondary metabolites contained in the leaves of C. occidentalis

Chemical families	Testing	Reagents	Comments
Alkaloids		Dragendorff and Vaser Mayer's Reagent	Precipitation or orange coloration
Polyphenols		ferric chloride ($FeCl_3$)	blue-blackish or green colored precipitate
Tannins		Stiasny	intense blue-black or dark green coloration
Flavonoids	WIL-STATER	magnesium shavings	red coloring
Saponoids	Emulsification test	Water	Very high foam production.
Polyterpenes and sterols	Libermann's reaction	Sulfuric acid in the presence of acetic anhydride	Purple or violet coloring, turning blue then green
Free or combined quinones		Borntraeger (ammonia diluted to half)	Red/pink/purple

Table 2 : Métabolites secondaires contenus dans les feuilles de C. occidentalis

Métabolites secondaires	Contents
Alkaloids	++
Tannins	-
Flavonoids	++
Polyphenols	++
Polyterpenes and sterols	++
Saponoids	++
quinones	-

Table 3: Periodic mean masses of rats

	Periodic **mean masses** (g)				
	D_0	D_7	D_{14}	D_{21}	D_{28}
group 1 (C_0: distilled water)	143 ± 8,84	133 ± 00	135 ± 01	140 ± 4,8	142 ± 4,2
group 2 (Ext dose 125)	115 ± 11	107 ± 01	116 ± 00	118 ± 5,1	118 ± 1,2
group 3 (Ext dose 250)	145 ± 2,91	135 ± 12,01	136 ± 10,02	137 ± 1,02	137 ± 2,3
group 4 (Ext dose 500)	155 ± 15,2	145 ± 5,5	141 ± 16,5	158 ± 1,2	175 ± 00
group 5 (Flukocin 500)	160 ± 0,5	155 ± 12,2	155 ± 4	164 ± 1,2	170 ± 1,5

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Conflict of Interest Statement: All authors have nothing else to disclose.

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