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Wound Healing Properties of *Landolphia calabarica* (Apocynaceae)

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Abstract

Leaves of *Landolphia calabarica* are used traditionally in Benin to treat wounds. This survey aims to value the healing properties of *Landolphia calabarica* at Wistars rats. Analysis phytochimique, the content in total phenols and the cytotoxicity has been done on the aqueous extract of *Landolphia calabarica* leaves. The healing activity of *Landolphia calabarica* leaves is valued by the local application of the aqueous extract and the total powder on wounds misled in dorso-lumbar part of rats *Wistars*. The aqueous extract of *Landolphia calabarica* is not toxic and contains a strong rate of phenols. Screening phytochimique revealed some chemical compounds to healing and vascularisantes properties. The value of the LC50 is 1,65 mL/mg and total phenols compounds are $286,33 \pm 5,22$ cent organ/mL. After eight (08) days of treatment, the wound percentage is 100% with the aqueous extract, compare to the reference (Betadine) and the total powder (respectively 85% and 89%). The healing is very meaningful with the aqueous extract of *Landolphia calabarica* compared to Betadine. These results justify the traditional use of this plant in the treatment of wounds.

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Keywords

Landolphia calabarica, Aqueous extract, Wound, Treatment, Bénin

Introduction

Wounds are traumatic pathologies that cause significant mortality. They constitute a public health problem, in particular because of their frequency, their potential severity, the consequences that they can generate, and the means to be deployed for their treatment and prevention (Abdeldjelil, 2016). The research undertaken for their therapeutic management has greatly improved their prognosis. However, the use of many conventional products is often limited because of their changeable efficiency, possible adverse effects, unavailability and

high prices, particularly in developing countries (Abdeldjelil, 2016). The solution to these many problems is to have recourse to plants. These traditional medicine products have long proven their efficiency in the treatment of wounds, and have very interesting advantages in terms of their availability, safety and low cost (Javakumar, 2015). Statistical data from the World Health Organization (WHO) show that 80% of Africans only treat with the plants around them for economic, social and cultural reasons (Who, 2017). Moreover, the African flora, renowned for its richness, includes thousands of plant species, some of which have been

scientifically studied and have led to medications that can be used in primary healthcare according to WHO recommendations. This is the case, for example, of *Athemisia annua* and *Salix alba*, respectively used in the manufacture of atheremeter and aspirin which have antimalarial activities (Onodje, 2014). This assumes that traditional African medicine could have solutions to pathologies in general and wound healing in particular. Among the medicinal plants with healing properties, for example, is leaves of *Tectona grandis* extract which, applied locally in a cream (5% and 10%) or administered orally (250 and 500mg / kg) promotes tensile strength, wound contraction and reepithelialization in different experimental wound models (Majumdar et al, 2007). The extract of the leaves of *Zanthoxylum zanthoxyloides*, rich in polyphenol and antioxidant composition, is also said to have healing and anti-inflammatory properties (Onodje, 2014). These authors also suggested that the antioxidant property of *Tectona grandis* leaves and their tannin content may be responsible for the healing action of this extract. In Benin, many plants are used in traditional medicine to heal wounds, but few have been scientifically studied. These are roots of *Strophantus hispidus*, calcined leaves of *Desmodium velutinum*, leaves of *Wralteriaindica* and *Uvaria chama* roots famous in wound healing (Sambou, 1998). Many traditional healers base their diagnosis and therapy on empirical grounds. This is the case of *Landolphia calabarica* leaves used in the treatment of acute and chronic wounds. They are charred, powdered and sprinkled on chronic wounds. For acute wounds, a decoction is used to clean the wounds and the powder from the dried leaf is applied to the wound. The same decocté is also used as a face bath to also prevent skin conditions. The present study aims to assess the healing properties of *Landolphia calabarica* in Wistar rats.

Materials and Methods

Plant material

The plant material used for this investigation are fresh leaves from *Landolphia calabarica*, collected at Glo located in the south of Benin (Atlantic department). It was identified and authenticated by the National Herbarium of Abomey Calavi University where voucher specimen was filed under N° AA 6724/HNB. The leaves and stem of the plant were shade-dried and ground into a fine powder. The powder was then extracted with water.

Extraction

For the aqueous extraction, decoction was done on a heating plate during 30 minutes by addition of 1000 ml

distilled water to 100 g of powder. The mixture was filtered with Whitman paper and evaporated under rota vapor at 65-70°C.

Experimental animals

Artemia salina shrimp larvae were used to evaluate larval toxicity.

Male and female albinos Wistar rats weighing 200 to 210 g at the age of eight to ten weeks were used for the study. Animals were housed in polypropylene cages and maintained under standard conditions with an alternated cycle of twelve hours light and twelve hours dark. They had free access to food and water. Room temperature was maintained at 25°C with a relative humidity of 35-60%.

Method

Ethics statement

The Wistar rats used in this study were handled according to the Institutional animal safety guidelines (Animal facility, Faculty of Health Science, University of Abomey Calavi, Benin). The experiments were performed according to the Institutional Animal Ethics No. 084 MS/DC/SG/ DFRS/CNPERS/SA (University of Abomey Calavi, Benin).

Toxicity assessment

It consists of studying in vitro, the cytotoxicity and healing power of *Landolphia calabarica* extracts on Wistar rats.

Phytochemical analysis

Phytochemical screening which is a qualitative chemical analysis based on color and precipitation reactions of major families of chemical compounds in plants was carried out to find out the phytocomponents present in the aqueous extract (Houghton et Amla, 1998)

Surgical excision

A rectangle of 2 cm in length and 1 cm in width is bounded by four points on the skin of the dorsolombary region of rats which are then excised (Onodje, 2014). The excision wounds are immediately treated after surgery. Animals are placed in individual cage with clean bedding.

Wounds treatment

The rats are treated once every two days with a local application of aqueous extract of *Landolphia calabarica*, the powder of *Landolphia calabarica* and Betadin as a reference healing agent. The dimensions (length and width) of the wounds are measured and photographed every two days during 28 days. The percentage of wounds concentration evolution is calculated according to Lodhi et al. (Lodhi et al, 2006):

Percentage of concentration (%) = [Healing wound surface/Initial surface] x 100

Statistical analysis

The results are expressed as Mean values } Standard Error of Mean (SEM). The statistical treatments are achieved with the software STATISTICA 5.5 version, and the software Microsoft Excel 2013 of Windows 2013. The middle value comparisons have been done by means of the parametric tests: T test for independent samples. The results are considered statistically at probability level of $P < 0.05$.

Results and Discussions

Phytochemical screening

The search for chemical groups in aqueous extract of *Landolphia calabarica* gave the results shown in Table 1.

Analysis of this table shows that twelve groups of chemical compounds are present. These are alkaloids, tannins (catechic tannins), Flavonones, leuco anthocyanins, Saponosides, triterpenoids, Steroids, mucilages, reducing compounds, coumarins, free Anthracene compounds and C-heterosides.

Polyphenols content

The results (figure 1) show that the aqueous extract has high content of total phenols (286.33 ± 5.22 centime organ / ml).

Larval toxicity

The results of the toxicity test with aqueous extracts of *Landolphia calabarica* larvae of *Artemia salina* are illustrated in figure. 2. The LC_{50} of the aqueous extract is 1.65 mg / ml.

Wound treatment

All wounds showed a gradual reduction in size following treatment (Table 2).

On the sixth day of treatment, the percentage of concentration is significant with betadin, the aqueous extract and the powder ($p = 0.03$). From the eighth day healing is very significant with the aqueous extract ($p = 0.001$)

Effects of *Landolphia calabarica* on hyperthermia

On the third day after wound induction, the temperature of the rats increased very significantly in the different group (table 2). During the different types of treatment, the aqueous extract caused a very significant decrease in temperature compared to the total powder ($p = 0.01$) and betadine ($p = 0.04$) on the fifth day.

Plants are healing agents and widely preferred due to their wide availability. They constitute a potential source of new bioactive molecules. The aqueous extract and total powder of *Landolphia calabarica* are used in this study to treat the wound. This allows to get closer to the usual form of preparation and use of this plant in traditional medicine.

The phytochemical screening of leaves of *Landolphia calabarica* reveals the presence of Alkaloids, Catechic tannins, Flavones, Leuco-Anthocyanins, Steroids, Saponosids, Triterpenoids, Mucilages, Coumarins, Free Anthracene Derivatives and C-heteroside. But, we note the absence of gallic tannins, anthrocynes, quinone derivatives, cyanogenic derivatives and O-heteroside.

The polyphenols identified (mainly tannins, flavonoids, and leuco-anthocyanins) are very high in the aqueous extract. These compound is known for their antioxidant activities (Curtay et Robin, 2000 ; Han, 1998 ; Latha et Rejesh, 2007). Tannins, waterproof the most external layers of the skin and mucous membranes, protecting the underlying layers and promoting tissue regeneration in the event of superficial injury or burns (Bruneton, 2005). As regards flavonoids, they are able to modulate the enzymes activity and alter several cellular systems behavior, giving them a multitude of biological activities, including antioxidant, anti-inflammatory and anti-ulcerous activities (Zannou, 2014). Coumarin is antiviral, immunostimulating, tranquilizing and very beneficial in skin conditions (Bruneton, 2005). Mucilages have an analgesic, antiseptic effect and treat dermatological

complaint. All these groups of compounds have been found in the extract of *Landolphia calabarica* (L.c.) and could be responsible for the healing activity revealed by aqueous extract and the powder of L.c. Our results are

consistent with the work of several Algerian authors who have shown that plants with all these groups of compounds have healing power (Abdeldjelil, 2016).

Table.1 Results of tests to identify chemical families in aqueous extract of *Landolphia calabarica*

	Families sought		Observations
Alkaloides			+
Polyphenolic compounds	Tanins catechic		+
	Tanins gallic		-
	Flavonoids		+
	Anthocyanins		-
	Leucoanthocyanes		+
Quinone Derivatives			-
Saponin			+
Triterpenids			+
Steroids			+
Mucilages			+
Coumarins			+
Compounds reducing			+
Anthracene compounds	free		+
	receiver	O - Heterosides	-
		C - Heterosides	+

Presence of compounds : (+, ++), absence of compounds : (-), trace of compound : (±)

Table.2 Wound concentration following treatment

Day	Percentage (%) of wound concentration			
	Betadin	Withness rats	Aqueous extract	Powder
J0	0	0	0	0
J2	18	-5	18	14
J4	33	5	43	27
J6	62 *	38	77 **	67 *
J8	85 **	46	100 ***	89 **
J10	100 ***	62	100 ***	100 ***
J12	100	79	100	100
J14	100	90	100	100
J16	100	100	100	100

* : p = 0,03 ; ** : p = 0,01 ; *** : p = 0,001

Figure.1 Polyphenol calibration curve

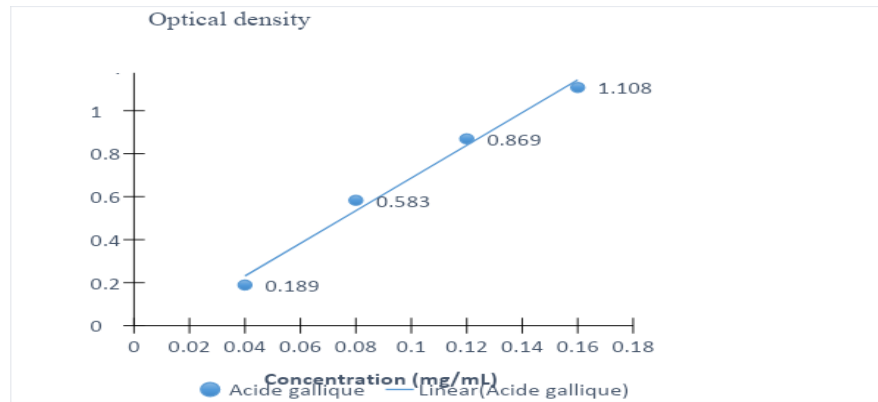


Figure.2 Variation in larval mortality as a function of the concentration of the aqueous extract of *Landolphia calabarica*

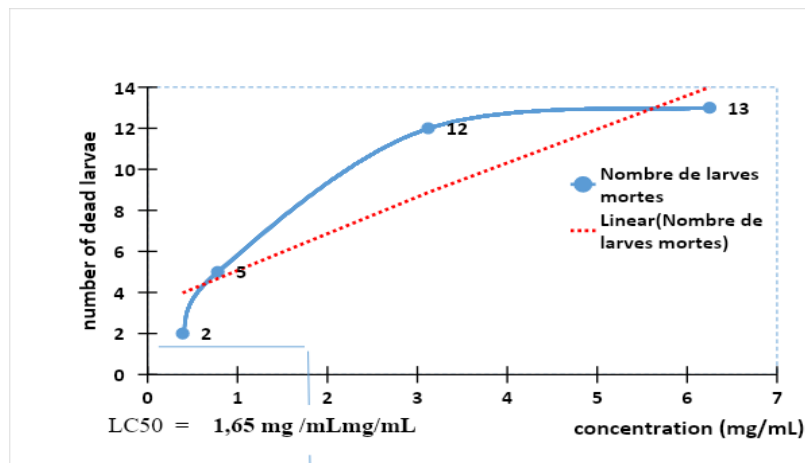
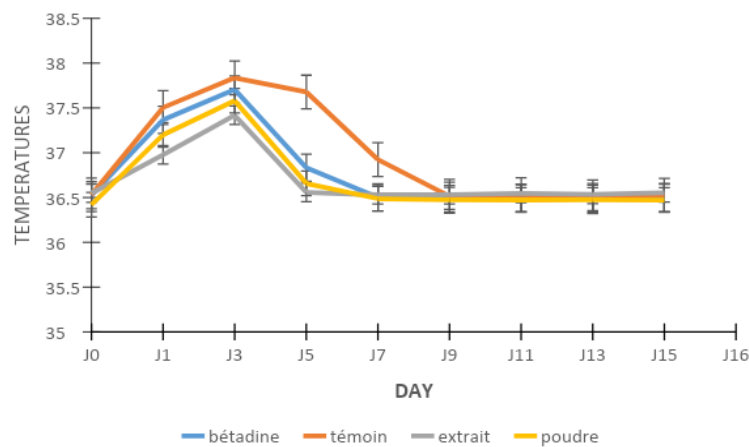


Figure.3 Curves of the evolution of rats' rectal temperatures after induction wound followed by treatments



N = 6 per group, values are mean \pm standard mean errors, $p < 0.05$ compared to the control group (ANOVA followed by the Student's test)

Larval toxicity results showed that the LC50 value is higher than 0.1 mg / ml. Any value above this, indicates that extract does not any toxicity (Mousseux, 1995). Following this result, we can therefore affirm that the aqueous extract of *Landolphia calabarica* have not any risk of intoxication, which justifies its use by the population. We found an average value of 286.33 ± 5.22 organ penny / milliliter of polyphenol. This value is much higher than that obtained by other authors in Togo [4]. Indeed, these authors have shown that *Zanthoxylum zanthoxyloides* has a high healing power with 83.39 ± 1.04 organ penny penny / milliliter of polyphenol.

The aqueous extract of *Landolphia calabarica* is therefore endowed with a strong power in phenolic compound. This would be responsible for many biological activities including antioxidant activity; because supplementing wounds with antioxidants helps prevent cellular oxidative damage and accelerates healing (Soneja et al, 2005). Phenolic compounds are widely distributed in plant tissues, among which are found numerous anti-radical and anti-oxydant molecules (Chen et Ho, 1995). Furthermore, some authors (N'guessan et al, 2007) have shown the existence of a correlation between the total phenol contents and the anti-radical activity.

The antioxidant activity of *Landolphia calabarica* is therefore linked to its high content of Total Phenols. These results are also in agreement with the work of several authors who have proved that plants which have good antioxidant activity contain high contents of phenolic group (Adedakpo et al, 2008). This antioxidant activity contributes to various therapeutic activities such as wound healing (Onedje, 2014).

Plant extracts rich in phenolic compounds inhibit oxidative stress, reduce lipid peroxidation not only by preventing or delaying cell necrosis, but also by improving vascularization (Nayak et al, 2006; Sun et al, 2002). The healing activity of *Landolphia calabarica* is therefore at least partly linked to its oxidizing properties.

To confirm this, tests were carried out on Wistar rats. The wound induction caused the temperature in each batch of rats to increase until the third day ($36.53\text{ }^{\circ}\text{C}$ to $37.83\text{ }^{\circ}\text{C}$ for the controls, $36.54\text{ }^{\circ}\text{C}$ to $37.41\text{ }^{\circ}\text{C}$ for extracts, $36.42\text{ }^{\circ}\text{C}$ to $37.58\text{ }^{\circ}\text{C}$ for powders and $36.52\text{ }^{\circ}\text{C}$ to $37.70\text{ }^{\circ}\text{C}$ for betadine).

During the different types of treatment, the aqueous extract caused a very significant decrease in temperature

compared to the total powder ($p = 0.01$) and betadine ($p = 0.04$) on the fifth day.

In the control group, the decrease in temperature was very slow compared to the animals treated. In the treated group, the return to the start temperature is obtained on the seventh day and two days after in the controls.

The treatment with the aqueous extract of *Landolphia calabarica* therefore dropped the temperature significantly compared to the total powder and the reference product. The total powder and the aqueous extract of *Landolphia calabarica* therefore contain compounds with antipyretic property. These results corroborate those carried out on the *Zanthoxylum zanthoxyloides* in Togo (Onedje, 2014).

Regarding the healing activity, the animals were treated every other day in order to reduce the stress associated with handling. The risk of wound contamination was reduced as the rats were kept in individual and clean cages. Thus, the groups treated with the aqueous extract and the powder showed considerable signs of healing significantly ($p = 0.001$ for the aqueous extract and 0.01 for the powder) on the second and fourth day compared to those which treated with Betadine. These two groups recovered earlier compared to the control group (Table 3). Wound concentration is faster with treatment with aqueous extract followed by powder and Betadine compared to controls. These results confirm those of work on extracts of *Alafia multiflora* which have healing and antioxidant property (Tsala, 2009). From these observations, we deduce that the large chemical groups contained in the aqueous extract and the powder of *Landolphia calabarica* allow wound healing in rats. The bioactive substances contained in this extract would not disturb the normal sequence of events leading to good repair of the skin.

In conclusion, the phytochemical study showed the presence of large groups of chemical compounds such as alkaloids, catechic tannins, Flavonones, Leuco anthocyanins, Saponosides, Triterpenoids, Steroids, Cardenolides, Mucilages, reducing compounds, coumarins, free anthracene derivatives and C-heterosides. The toxicity test has also shown that this plant is not toxic. The aqueous extract of *Landolphia calabarica* has significant healing properties and good antioxidant activities by measuring total phenols. This is a function of the presence of active ingredients such as polyphenols. This antioxidant property of leaves of *Landolphia calabarica* contributes to various therapeutic

activities and is therefore responsible for the healing action.

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