

In vivo and in vitro healing potential of aqueous extract ointment *Ageratum conyzoides* Linn. applied to excision and incision wounds induced in Wistar rat and human cells

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Abstract

The healing effect of the aqueous extract and ointment at 4 and 8 g/kg doses of *Ageratum conyzoides* Linn. on tracing and excision-induced wounds in the Wistar rat was the subject of our study. We used the classical tests described by some authors to carry out pharmacological work and organoleptic and toxicological analyses of the extract and formulation of this plant. The results, derived from this methodology, show that this ointment is homogeneous, weakly aromatic and acidic (pH : 5,6), rich in dissolved and mineral substances, no lethality was observed up to the dose of 3200 mg/kg in acute. Also, the aqueous extract of this plant has an abundance of Flavonoids. In addition, the healing effect of *Ageratum conyzoides* L. ointment was evaluated on deep wounds in Wistar rats. The planimetry results and measurements made over twenty-six (26) days proved that the ointment at 4 and 8 g/rat weight; Madecassol[®] at 400 mg/kg (reference product) significantly reduced the mean area of the deep wounds in Wistar rats compared to the negative controls. In addition, the aqueous extract of *Ageratum conyzoides* L. at the dose of 5,5 ml/kg administered in vitro on cell model wounds created by scraping the cell web, significantly reduces the wound in 24 hours. In addition, at the dose of 11 ml/kg this scarring is complete, there is no visible wound trace. This reflects the presence of a cell thrust at the level of the scratch compared to the control lot, whose wound remained almost gaping. Thus, are confirmed the data of literature and traditional medicine on spa summarized efficacy.

Keywords: Healing, Wound, healing, Aqueous extract, Ointment, *Ageratum conyzoides* Linnée.

Introduction

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The human body is frequently attacked. These are either of internal or external origin and are not without consequences on human life. They manifest themselves in lesions and pathologies of infectious, ulcerative, dermatological types and even cause wounds.

Indeed, the wounds are desquamations of the skin and these different tissues, namely the epidermis, the dermis through trauma [1]. As well as bad, open wounds eventually close after degradation, this is the phenomenon of healing. Because scarring is a natural phenomenon through which animal tissues repair localized lesions [2].

Nowadays, we are witnessing an escape of cases of wounds due to certain chronic pathologies or their complications and various accidents, which lead to localized lesions, whose repair and regeneration processes can be brief: This is the case with

superficial and deep wounds. However, there are wounds that do not follow this logic.

In Congo Brazzaville, a study of penetrating wounds carried out on 110 cases of victims of the 1997 civil war had revealed 11.08% etiology by stabbing weapons on 88.18% by firearms with a mortality rate of 3,6% [3].

However, 80% of pressure sores develop in the first two weeks of hospitalization and 96% in the first three weeks.

The cost of treating pressure wounds is a real burden on the health care system :

27,632 % for pressure wounds, three months at home;

24,50 %for pressure ulcers three months in extended care [4].

In view of all these challenges and with the concern to help the population in the therapeutic management of wounds, up to the totality of wound healing and aesthetic care. We are oriented towards the pharmacopoeia and traditional medicine of our country, using a plant known for its medicinal virtues by the population, called *Ageratum conyzoides* Linnée [5-9].

But even less used in wound therapy, of which it is presumed to be healing and effective on infectious pathologies. For this reason, we approach this scientific aspect, with the aim of once again bringing a remedy to this public health problem, by assessing the healing power of *Ageratum conyzoides*, on wounds induced in Wistar rat and *in vitro* cell tracing wounds.

Materials and Methods

Plant material

Fresh leaves of *Ageratum conyzoides* Lin. Identified at the herbarium of the Laboratory of Botany of the IRSEN under the No. 648 of 18/01/1963 NERE, by Dr. Jean Marie Moutsamboté., were used. The harvest was made in the Talangai district, in the northern part of Brazzaville. This, during the rainy season, which covers the months of October to December.

Drying and grinding of the plant

Fresh leaves of the plant of *Ageratum conyzoides* Linn. were air-dried in the laboratory at 25°C for 7 days. Then, once dried, they were ground with a grinder. The collected powder allowed us to prepare the aqueous extract according to the routine method [10].

Preparation of the aqueous extract

100 g of plant leaf powder (1:1) is mixed with 1000 ml of distilled water and heated for 30 minutes at 55°C, then filtered with Wattman N°1 paper. The collected solution is evaporated under vacuum using a rotating evaporator R114 at 55°C. The dry residue obtained is soluble in NaCl at 9.

Preparation of ointment based on aqueous extract of *Ageratum conyzoides* Linnée.

2, 4 and 8g/kg of the dry residue of the aqueous extract were diluted in 10 ml of distilled water and then solubilized in 10 mL of Tween 80. The collected solution was mixed with Vaseline and homogenized with a porcelain mortar. The resulting product was distributed in clean, pre-washed and sterilized jars. The dough is then solidified in the refrigerator at 4°C, for 30 minutes before use [11].

The yield of the extract

After extraction of the product, the quantity of the extract obtained is calculated according to the mass of the initial product, giving a final yield.

Animals:

Mice (25 1 weight) and randomized Albinos Wistar rats with a weight equal to 200 ± 50 g at least 6 months of age were used. These animals were supplied to us by the pet shop of the Superior Normalis School. Where they are elevated under standard temperature conditions varying between 25°C and 27°C, and are subjected to a complete power supply with access to tap water ad libitum. These animals also benefit from an

acclimatization period of 07 days under a light-diurnal rhythm and are fasted 16 hours before the experiment.

Cell lines:

Human cells, including the cell line of Hacat keratinocytes (Reference CLS 300493) and Hepg2 human hepatocytes (Reference ATCC HB-8065), are derived from the Cell Line Service GmbH laboratory (CLS, Eppelheim, Germany).

Investigation of organoleptic and toxicological parameters of aqueous extract and *Ageratum conyzoides* Linnée ointment.

Organoleptic parameters of *Ageratum conyzoides* ointment:

It consists in appreciating the taste, smell, and texture of the *Ageratum conyzoides* ointment [12].

Safe dose and acute toxicity (LD50):

Three (03) groups of five (05) mice each were used and the animals were given extract oral way (p.o) at doses of 800, 1600 and 3200 mg/kg body weight (bw) respectively. A control group was given normal saline (0.9% w/v NaCl) at 10 ml/kg bw. Mice were observed over 72 hours and clinical signs and mortality (LD50) were recorded [13].

In vitro toxicity of the aqueous extract of *Ageratum conyzoides* on human cells.

We tested the aqueous extract of *Ageratum conyzoides* on liver cells and skin cells, including keratinocytes according to the classical method, which results in: thawing, counting of cells using a KOVA blade, culture and cytotoxic assay itself.

Primary skin irritation test:

0,4 g of ointment in doses of: 4 and 8 g/kg were applied to the healthy and scarified skin of two batches of wistar rats. These dressings are held by compress and tape for 24 hours to 72 hours later. The degree of irritation is assessed visually, 30 min after removal of the dressing [14].

Eye irritation test:

0,5 mg ointment at doses of: 4 and 8 g/kg were applied to the right eye of each wistar rat in both batches used. These rats are held in a restraint cage before 18 hours before the test. Next, we make the observations with the naked eye, to:

- The conjunctiva to check the colour of the eye (enanthem), which indicates possible swelling (Table 1);
- Eyelids (chemosis) and watch for watery eyes soaked in ointment; for 1 h, 24 h and 48 h after [14].

Physico-chemical analysis of the ointment:

The hydrogen potential or pH was measured using a HYDROCHECK brand pH meter; the dissolved substances (TDS) and conductivity were determined.

Evaluation of *in vivo* healing activity of Aqueous extract of *Ageratum conyzoides* L. ointment from deep wounds.

In vivo and in vitro healing potential of aqueous extract ointment Ageratum conyzoides Linn. applied to excision and incision wounds induced in Wistar rat and human cells

Induction of deep wounds:

The technique used is that described by Koulongous, [15]. It consists of inducing wounds on previously anesthetized rats, then treating them with the different products divided into six (6) lots of five (5) rats each, such as : an untreated control lot, the wounds are cleaned with disinfectant (daklin); a batch receiving local application of the Vaseline (0, 4 g); two batches

thigh of rats, following the method described by Bensegueni et al. [16]. Animals previously anaesthetized with ethyl ether are placed in prone and their back and lower limbs shaved long and wide. The wounds of about 15000 mm² are created with a knife blade and cleaned with cotton before application of the products. The depth of a wound includes the three skin layers and the muscle.

Table 1. Effect of aqueous extract ointment of A.conyzoides on ocular irritation index of conjunctiva.

Lots/dose	Duration	Assessment of achievement at conjunctiva level		
		Watery	Chemosis	Enantheses
Ointment A.conyzoides 4 g/kg	1	3	1	1
	24	0	0	0
	48	0	0	0
Ointment A.conyzoides 8 g/kg	1	3	1	1
	24	0	0	0
	48	0	0	0

0 = Absence ; 1 = Light or very weak; Pom A.c = ointment of Ageratum conyzoides

of 4 and 8 g/kg plant ointment and one batch of 4g/kg reference product (Madecassol®) treatments. As a result, 1,5 cm long, 1 cm wide and 1 cm deep surgical wounds were caused in the

The planimetry :

Wound dressing is done every other day with Dakin due to once a day. A quantity of 0.4 g of the ointment and all the products was applied to the various cleaned wounds. The lengths and widths of the excision wounds of the different batches are measured using a graduated rule every two days until the wounds are completely healed. A planimetric study was carried out by the direct calculation of the mean wound surfaces in order to assess the healing effect and the healing time, according to the following formula :

$$\% = \frac{(\sum S_{J_0} - \sum S_{J_n})}{\sum S_{J_0}} \times 100$$

In vitro evaluation of wound healing by application of the aqueous extract of Ageratum conyzoides L.

Implementation of the 2-Dimensional (2D) wound cell model in 6-well plates (In vitro scratch assay) :

The 2 D cell scraping wound model (Fibrinogen and cells) used in this study is a model of choice for assessing the healing activity of natural and synthetic substances [17]. The purpose of this test is to look for possible scarring at the cellular level.

As a result, the plate has 6 wells each containing 1 mL of a 2,5 mg/mL fibrinogen solution is incubated at 37°C for 1 hour. Then the fibrinogen solution is sucked and the plate is rinsed twice

with DPBS. Then seeded with the cells at a density of 40,000 cells/well and incubated in a humid atmosphere at 37°C and 5% CO₂ until they become 100% confluent to begin testing.

Cell model of scratch wound proper:

Once the cell confluence is at 100%, a scratch of about 700 µm, crossing the well from end to end, is made with a pipette tip in the cell layer as shown in the figure. The culture medium is then sucked to remove the detached cells and replace with fresh medium. The healing tests can then begin (Figure 1).

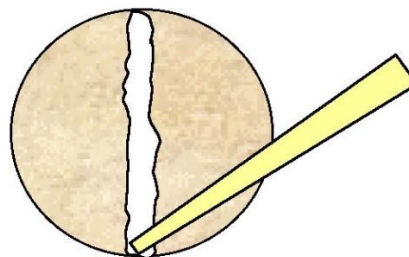


Figure 1. Cell scraping wound model.

Concentrations tested :

According to the cell toxicity studies performed on the extract, the concentrations selected are those that gave at least 80% cell viability in cytotoxic tests. These are 5.5 and 11 mg/kg. Thus the concentrations tested are prepared with DMSO and diluted with the culture medium.

Performing proper scarring tests :

After simulating the wound in the cell layer, the different concentrations are added to each well at 3 wells per concentration. The plates are then incubated in a humid atmosphere at 37°C and 5% CO₂ for 24 h and 48 h. At each time interval, the distance between the 2 cell populations on either side of the stripe is measured using an optical microscope with a micrometric lens. The percentage of cell proliferation is then determined based on incubation time. The photos are also taken to materialize the healing effect or not of the extract. The percentage of cell proliferation is given by the following formula :

$$\text{Cells Proliferative Pourcentage} = \frac{D_{\text{initiale}} - D_t}{D_t} \times 100$$

D_{initiale} (µm): distance between the 2 cell populations on either side of the stripe at T₀ (before application of the extract);

D_t (µm): the distance between the 2 cell populations on either side of the T_t stripe (after application of the extract).

Mechanism of action of *Ageratum conyzoides* L. on the scar process Proinflammatory cytokine.

Quantification of Tnfa (Tumor Necrosis Factor alpha):

The technique used in our study is that of ELISA in Sandwich. It is based on the principle of specific fixation of an antibody to an antigen, that is, the antigen is caught in a vice (trapped) between two (2) antibodies, one of which is fixed in the manufacturer's well and the other one is conjugated (antibody coupled to an enzyme) [18].

Blood sampling in rats :

For the exploration of this parameter, the sampling was carried out at the retro-orbital sinus of the eye (richly vascularized cartilage region) using a capillary tube. These samples were taken from fasting rats. The collected blood was placed in the numbered EDTA tubes and then immediately centrifuged for ten (10) minutes at 3000 rpm. The resulting serum was used for the determination of Tnfa. The samples for all batches were taken 1 day after the induction of the wounds (J₀) for all normal and diabetic batches, during the treatment of the wounds (on the 12th day) and at the end of the experiment (J₁₈).

Experimental protocol

The technique used is described by the supplier BOSTER antibody and ELISA expert. The procedure is used as follows:

- Prepare reagents+Standards+Controls at 37°C;
- Remove the number of ELISA strips needed for the test;
- Add 100 µL of Standard, Sample and Control into wells;
- Add 100 µL of sample diluent to a well corresponding to White;
- Cover plate with film paper and incubate for 90 minutes at 37°C;
- Gently vacuum the wells with their liquid (contents) using a washer;
- Add 100 µL of Biotinylated TNF anti-Rat antibody to each well;

- Cover and incubate at 37°C for 60 minutes;
- Wash the plate three (3) times with wash pad;
- Add 100 µL of AvidineBiotine-Peroxydase complex to each well, cover and incubate for 30 minutes at 37°C;
- Wash the plate five (5) times by adding 300 µL of buffer between each wash;
- Add 90 µL of Color Developer per well, cover plate and incubate for 25 minutes at 37°C in darkness;
- Add 100 µL of stop solution in each well, blue colors for 4 standards and clear 3 other standards turn yellow automatically;
- Read the Optical Densities at 450 nm at the Spectrophotometer.

Quantification of two growth factors (Tgfa and Tgfb) involved in the healing process.

In this study two growth factors (Tgfa and Tgfb) were quantified by E.L.I.S.A. tests [19,20]. 100 µl of cell supernatant are collected from each well as a function of time in aliquots in Eppendorf tubes, then stored at -80°C until dosing.

Statistical Analysis

The results obtained are expressed as an average more or less standard error (ESM). The batch comparison is performed by the test "t student -Fischer coupled to the ANOVA test". The significance threshold is set at p<0,05.

Results

Macroscopic analysis of the dry residue: Following the extraction of aqueous extract of *Ageratum conyzoides*, a dry résidus of 19,42 g, yielded 5,55%.

Organoleptic and galenic control of the ointment: Macroscopic examination (with the naked eye) revealed that the ointment based on aqueous extract of *Ageratum conyzoides* is dark brown, homogeneous, creamy to the touch, stable and has a pasty consistency. Also, it is not very aromatic, slightly bitter and soluble in Tween 20%.

In vivo and in vitro toxicity of aqueous extract of *A. conyzoides* L.

In vivo Acute toxicity :

We noted that the aqueous extract of *Ageratum conyzoides*, administered intragastric in mice at doses ranging from 800 to 3200 mg/kg body weight, showed no mortality or behavioural change in the general condition of the animals. This means that the DL50 would be between 5000 mg/kg and more.

In vitro toxicity on humans cells :

In vitro toxicity of aqueous extract of *Ageratum conyzoides* on human hepatocytes (hepg2). Figure 2 shows the effect of aqueous extract of *Ageratum conyzoides* on hepatocytes. This indicates that at concentrations of 22 and 50 mg/mL, this extract

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significantly reduces the viability of hepatocyte cells ($p < 0,05$ and $p < 0,01$) (Table 2).

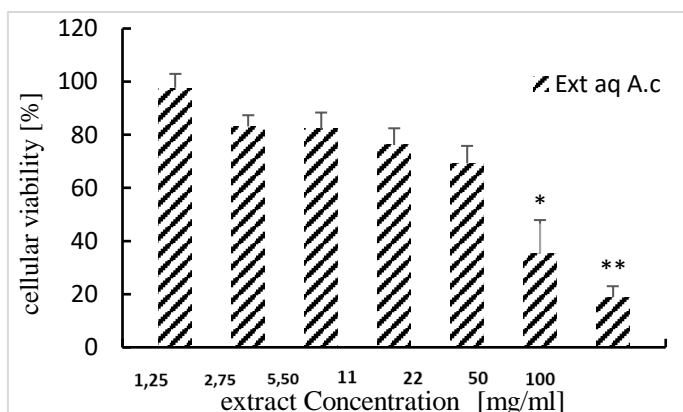


Figure 2. Effect of aqueous extract of *Ageratum conyzoides* (Ext aqA.c) on human hepatocytes. $n=3$, * $p < 0,05$ and ** $p < 0,01$: significance threshold.

In vitro toxicity of aqueous extract of *Ageratum conyzoides* to keratinocytes (hepg2):

The results of this test are presented in Figure 3, which shows that at concentrations of 50 and 100 mg/mL, the aqueous extract of *A. conyzoides* L. decreases significantly ($p < 0,05$ and $p < 0,01$) the viability rate of human keratinocytes (Figure 3). This means that at high concentrations, this extract would be cytotoxic on cells of the human line such as hepatocytes and keratinocytes.

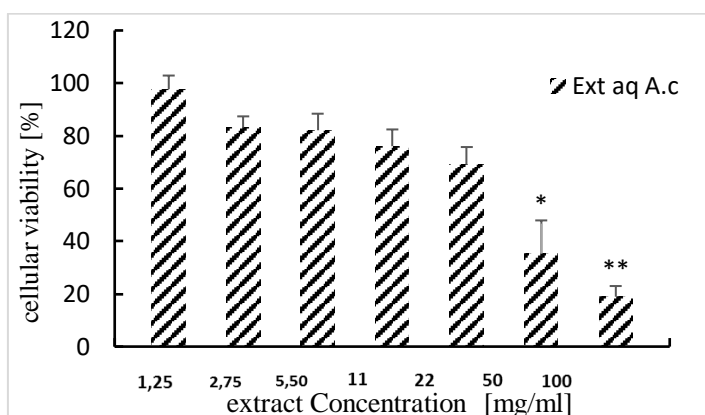


Figure 3. Effect of aqueous extract of *Ageratum conyzoides* (Ext aqA.c) on human keratinocytes. $n=3$, * $p < 0,05$ and ** $p < 0,01$: significance threshold.

Skin and eye irritation test :

The results from these experiments are expressed in Table 2. It is noted that, the aqueous extract of this plant at a given dose and depending on the time would cause temporary irritation in the skin and eye scarified area in the one (1) and 24 hours following administration. The irritability index varies by dose and time.

Table 2. Effect of *A. conyzoides* aqueous extract ointment on primary skin irritation.

Lots/dose	Duration (hour)	Erythema and escarre		Edema	
		Escarified zone	No escarified zone	Escarified zone	No escarified zone
Ointment <i>A. conyzoides</i> 4g/kg	1/2	0	0	0	0
	24	0	0	1	0
	72	1	0	0	0
Ointment <i>A. conyzoides</i> 8g/kg	1/2	0	0	0	0
	24	1	0	0	0
	72	0	0	0	0

0 = Absence; 1 = Light or very weak; PomA.c = *Ageratum conyzoides* ointment; $n=4$.

Physicochemical parameters of the ointment:

The pH of this ointment is 5.6; it corresponds to that of a weak acid. Its conductivity is 4.10 mg/l and the TDS is 7.47 $\mu\text{S/cm}$.

Healing effect of aqueous extract and ointment of *Ageratum conyzoides* Linn.

In vivo scarring of deep excision wounds by ointment of aqueous *A. conyzoides* extract in wistar rats:

Figures 4 and 5 shows the effects of *Ageratum conyzoides* aqueous extract ointment on induced deep excision wounds in rats. This resulted in a significant reduction ($p < 0,001$) in wound size from day 8 to day 14 in rats treated with *Ageratum conyzoides* ointment at doses of 4 and 8 g and Madécassol® 1% compared to control lots. However, the healing is only complete on the 18th day. It is concluded that the extract-based ointment of *A. conyzoid* L. is effective on the contraction of the banks of the deep wound and promotes a réépithelialisation of the damaged tissue.

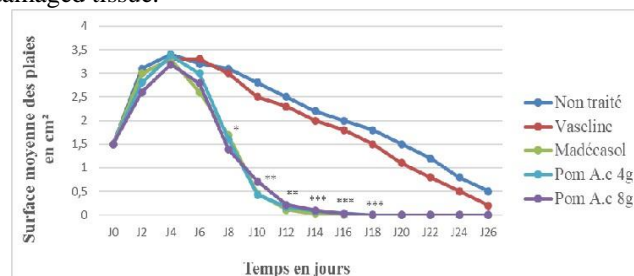


Figure 4. Effect of the *Ageratum conyzoides* ointment on the evolution of the average areas (cm^2) of the deep wounds $n=5$, with * $p < 0,05$; ** $p < 0,01$; *** $p < 0,001$.

In vitro wound healing by the aqueous extract of *A. conyzoides* L. applied to human cells:

Figures 6 and 7, represent the effect of the aqueous extract of *Ageratum conyzoides* on cell model wounds created by scraping of the cell web. The results were observed in two phases : 24 and 48 hours after the application of the extract. 24 hours later, there was a small reduction in wound diameter at the dose of 5.5 ml/kg, as opposed to the 11 ml/kg dose applied to keratinocytes compared to the control. In addition, 48 hours after incubation, there was a significant decrease ($p < 0,01$). from the size of the wound to 5,5 ml/kg and a near-complete wound-bank scarring, which results in a cell surge at the level of the scratch. While,

the wound from the witness sample remains gaping. It is noted that, the scarring kinetics of the aqueous extract on a keratinocyte lesion is concentration and time dependent.

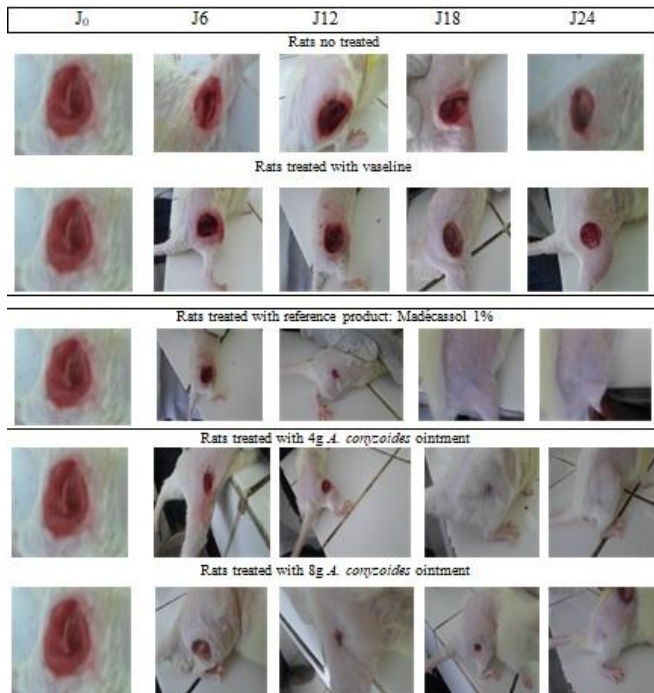


Figure 5. Illustrations of the dimensions of the wounds, their average surfaces throughout the experimental period [from J₀ to J₂₄].

After 24 hours of incubation.

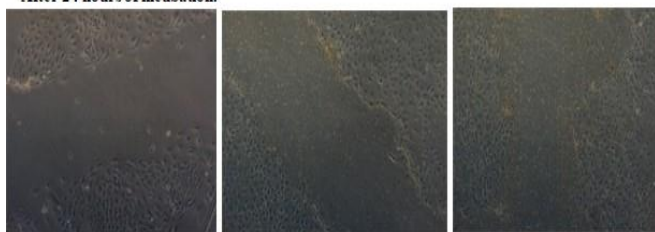


Figure 6. Effect of aqueous extract of *Ageratum conyzoides* at doses of 5.5 and 11 mg/ml on the wound induced by scraping of the keratinocyte cell layer 24 h after incubation.

After 48 hours of incubation.

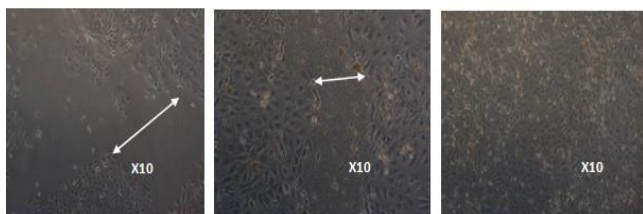


Figure 7. Effect of aqueous extract of *Ageratum conyzoides* at doses of 5.5 and 11 mg/ml on the wound induced by scraping of the keratinocyte cell layer 48 h after incubation.

Mechanism of action of *Ageratum conyzoides* in the healing process.

Effect of aqueous extract of *Ageratum conyzoides* Linn. on the rate of $Tnf\alpha$:

Figure 8 shows the effect of aqueous extract and *A. conyzoides* L. ointment on the variation in $Tnf\alpha$ levels in normal and diabetic rats. It follows from this test that the ointment of the

aqueous extract of *A. conyzoides* L. administered at the studied doses (4 g/kg and 8 g/kg) significantly inhibits ($p < 0,01$ and $p < 0,001$) over time (J₁₂ to J₂₀), the release of inflammatory substances $Tnf\alpha$ at the level of damaged tissue in normal non-diabetic rats, with a variation of around : 9,730 pg/mL; 6,391 and 0 pg/ml. Similarly, this ointment and Cicatryl® loosely block the release of $Tnf\alpha$ in diabetic rats, compared to the control lots. It is assumed that the aqueous extract of *A. conyzoides* L. easily heals the single excision wounds, as those of diabetics.

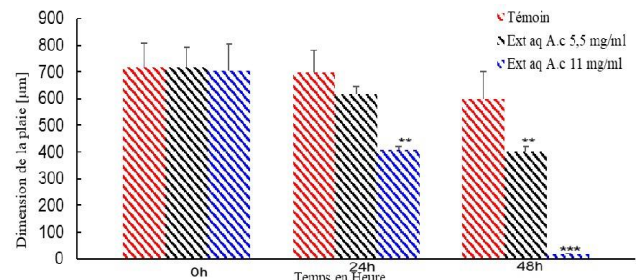


Figure 8. Effect of aqueous extract of *Ageratum conyzoides* on the distances (width) of the induced wounds (μm) on the keratinocyte cell layer as a function of time. $n=3$; * $p < 0,01$; ** $p < 0,001$: differences significant relative to the witness.

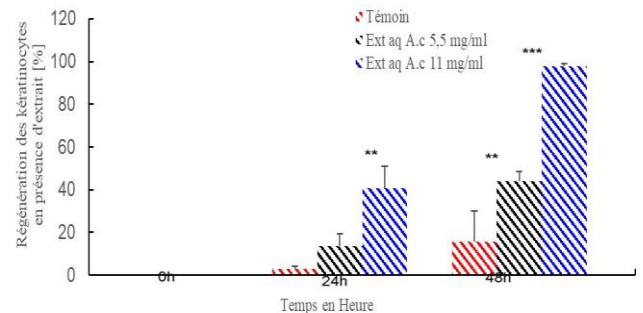


Figure 9. Changes in the rate of $Tnf\alpha$ (pg/ml) in normal non-diabetic rats (NDA.c:4 and 8 g) and diabetic rats (DT) compared to the different products applied to the wound as a function of time $n=4$; *** $p < 0,01$; **** $p < 0,001$: significant differences compared to nondiabetic rats.

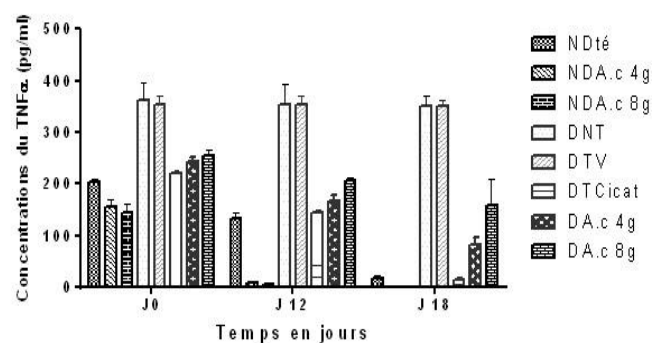


Figure 10. Changes in the rate of $Tnf\alpha$ (pg/ml) in normal non-diabetic rats (NDA.c:4 and 8 g) and diabetic rats (DT) compared to the different products applied to the wound as a function of time $n=4$; *** $p < 0,01$; **** $p < 0,001$: significant differences compared to nondiabetic rats.

Effect of aqueous extract of *Ageratum conyzoides* Linn. The behaviour of growth factors ($Tgf\alpha$ and $Tgf\beta$) involved in the healing process:

Figures 9 and 10 representative the effects of aqueous extract of *conyzoid* *Ageratum* on keratinocyte growth factor concentrations ($Tgf\alpha$ and $Tgf\beta$) during the healing process of

In vivo and *in vitro* healing potential of aqueous extract ointment *Ageratum conyzoides* Linn. applied to excision and incision wounds induced in Wistar rat and human cells

damaged rat tissue. It is noted that the aqueous extract of *Ageratum conyzoides* Linn., at administered doses significantly increases the concentrations of Tgf α and Tgf β as a function of time, compared to the control lot. However, this increase in Tgf α is double that of Tgf β . So the aqueous extract of *Ageratum conyzoides* Linn., increases the concentration of growth factors of keratinocytes during wound healing (Figures 9-12).

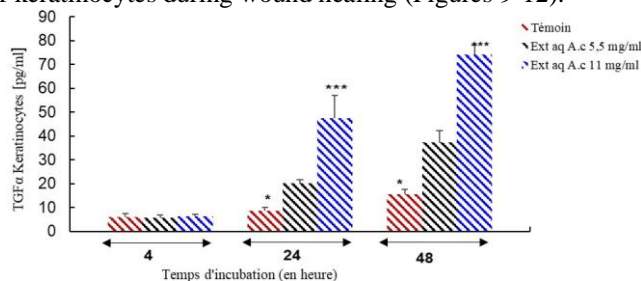


Figure 11. Effect of aqueous extract of *Ageratum conyzoides* (Ext aqA.c) on Tgf α concentration (pg/ml) of keratinocytes. n= 3; *p<0.05; ***p<0.001: significant differences from control.

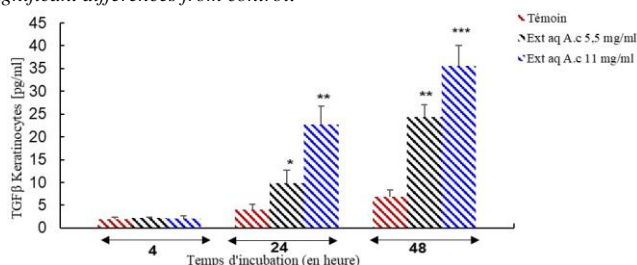


Figure 12. Effect of aqueous extract of *Ageratum conyzoides* (Ext aqA.c) on Tgf β concentration (pg/ml) of keratinocytes. n= 3; *p<0,05; **p<0.01 ***p<0,001: significant differences from control.

Discussion

Organoleptic, toxicological and physico-chemical analyses of the aqueous extract and ointment of *Ageratum conyzoides* Linn.

These analyses showed that the *Ageratum conyzoides* ointment is dark brown in colour, homogeneous, creamy, with a pasty consistency and a slightly bitter taste. It has a low acidic pH and contains dissolved substances. In terms of its toxic potential, it is noted that the aqueous extract of *Ageratum conyzoides* is not lethal up to 5000 mg/kg intragastric administration in mice. However, it is hepatotoxic at concentrations of 22 and 50 mg/mL and harmful to skin keratinocytes at concentrations of 50 and 100 mg/mL respectively. Nevertheless its ointment is not irritating on the skin in local application. But, may cause slight eye irritation stimulating tears. Indeed, *Ageratum conyzoides* possesses chemical substances or molecules, which would be hepatotoxic and cause necrosis of keratinocytes has a some concentration. The literature has shown that this plant is rich in alkaloids, tannins and flavonoids etc [18]. However, a small amount of pyrrolizidine (alkaloid) and traces of furan have been found in this plant, which are molecules capable of being carcinogenic, teratogenic and genotoxic [14,19,20].

This probably explains the necrosis and low viability observed on hepatocytes and keratinocytes. Because hepatocytes are sensitive and versatile cells because of their ability to synthesize, accumulate, and break down different substances [21,22]. Also,

they are self-regenerating and can proliferate actively to recover lost segments [23].

Healing effect of *Ageratum conyzoides* extract and ointment Linnaeus on deep wounds and cell wounds

The results obtained from this study show, on the one hand, that the aqueous extract of *Ageratum conyzoides* Linnée administered *in vivo* is effective on wounds, by reducing the diameter and contracting their banks. This leads to a complete healing in a given time. It could be said that, the aqueous extract of *Ageratum conyzoides* contains in its phytochemical composition one or more molecules having a power of reepithelialisation of skin cells such as keratinocytes. On the other hand, the ointment based on this extract applied locally at different concentrations (5.5 and 11 mg/mL) in the injured area causes a healing dependent on the contact time.

For this purpose, there is an increase in the rate of keratinocytes depending on the concentration of the administered extract. This would mean that, the healing kinetics of the lesion, depends on the colonization of the damaged area by the keratinocytes, the concentration of the applied extract, but also the contact time. The higher the concentration of the extract, the faster the healing, therefore the faster the colonization of the lesion by keratinocytes, which will migrate to the center of the lesion.

This centripetal migration followed by the proliferation of keratinocytes at the level of the injured area will form a temporary membrane. These cells will migrate one after the other into a single cell layer and stop when the keratinocytes of one bank meet those of the other bank of the wound.

In addition, it is therefore possible that the healing effect of the aqueous extract and ointment used here is attributed to the presence of secondary metabolites, such as flavonoids in this plant, which have anti-inflammatory and antioxidant properties [24]. This elaborate study corroborates the work of: Adebayo et al., and Omotoso et Eze, [25,26]. Therefore, the results obtained with the extract and ointment of *Ageratum conyzoides* confirm the use of this plant in traditional medicine in the treatment of wounds, wounds and burns. Only it can be notified that the external use of the plant would be safe [27].

As regards the mechanism of action of *conyzoides Ageratum*, during the healing process of the skin wounds, we carried out the assay of Tnf α , followed by that of growth factors, in particular TNF α and TNF β .

Indeed, this study shows that, *Ageratum conyzoides* inhibits the massive release of Tnf α at the level of damaged tissue during the inflammatory phase and stimulates growth factors and cells, promoting healing. This could be explained by the fact that, the slowdown in the release of pro inflammatory cytokines, TNF- α promotes the transformation of these cytokines into anti-inflammatory cytokines. Knowing that, Tnf α participate in the recruitment of inflammatory cells following the adhesion of leukocytes with endothelium by chemotherapy and stimulate their degranulation. These increase the synthesis of prostacyclin

and FAP, initiates and maintains mechanisms involved in inflammatory disorders [28].

It is also noted that the aqueous extract of *Ageratum conyzoides* stimulates the production of growth factors including Tgfa and Tgfβ at the cellular lesion level. This means that the transforming growth factor (TGF), which is released at the liver level, promotes neovascularization, fibroblast migration and collagen production [29].

Probably, *Ageratum conyzoides* would activate the epidermal cells, which will migrate and proliferate to fill the wound bed. As a result, the liberated pro inflammatory cytokines are blocked and the wound is healed. If appropriate, proinflammatory cytokines may delay healing [30]. This delay is characterized by a wound re-epithelialization defect, angiogenesis inhibition and decreased cell proliferation.

This is the case with diabetic rat wounds, which is marked by a disturbed inflammatory process with an accumulation of proinflammatory cytokines, proteases and also a decrease of certain growth factors that interfere with the healing process [30]. Also, inflammatory macrophages (cytokine pro inflammatory) present at the onset of inflammation persist and delay the arrival of anti-inflammatory and restorative macrophages, [31,32].

The pro-inflammatory cytokines TNFα, IL-1β and IL-6, as well as others, have proved to be important cytokines modulated by natural products, such as : flavonoids and terpenes, which act as anti-inflammatory, analgesic, anti allergic substances, etc., are often cytokine modulators, and may therefore be interesting substances for the management of cytokines [33].

Our work confirms that carried out by : Faqueti et al. [34], demonstrated that flavonoids extracted from *Ageratum conyzoides* have a beneficial effect against pain and inflammation [35-38], which claimed that the methoxyflavone polymer inhibited the production of prostaglandin E2 (PGE2) and inflammatory cytokines, TNF-α and interleukin-1β (IL-1β), producers of oxide synthase (iNOS) and negative regulator of cyclooxygenase-2 (COX-2) [35-38].

Conclusion

This research work verified cytotoxicity and evaluated healing properties of the aqueous extract *Ageratum conyzoides* on human cells, including keratinocytes and hepatocytes. The aqueous extract of *Ageratum conyzoides* administered in acute shows no evidence of toxicity or lethal dose. Unlike in subacute, it has shown hepatotoxicity, because it reduces the viability of human hepatocytes at doses of 22 and 50 mg/kg and decreases the viability rate of keratinocytes at concentrations of 50 and 100 mg/mL. However, the aqueous extract and the ointment of *Ageratum conyzoides* have a healing dose and time dependent effect. So that applied *in vitro* to damaged skin cells, the ointment of *Ageratum conyzoides* causes regeneration and/or proliferation of keratinocytes during healing. In addition, the ointment of *Ageratum conyzoides* acts on the wound by

inhibiting the exit of cytokine pro inflammatory such as Tnfα, then stimulates the secretion of growth factors: Tgfa and Tgfb), triggering the re epithelialization and regeneration of necrotic skin tissue.

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