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Validation Of Herbal Ointment Wound Healing Efficacy Formulated From Gaertnera Phanerophlebia Baker

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Abstract:
Wounds are a common clinical entity in everyday life. They are defined as breaks in the continuity of living tissue caused by injury, which can lead to discomfort, infections, and other complications. Although a wide range of therapies have been suggested to promote wound healing, they all cause side effects, including allergic reactions. Therefore, there is a need for new drugs that are safe and effective. Herbal medicine still holds a unique place in providing an effective treatment without side effects. This study aimed to formulate a new herbal wound-healing ointment from Gaertnera phanerophlebia Baker named Tsitsiromade 5 % and establish the product's quality, wound-healing efficacy, and toxicity profile. The ointment was developed by fusion method. Standard tests for quality control and microbiological stability were performed. Investigations of the plant extract have shown its wound-healing efficacy and high antioxidant properties. Additionally, the formulated ointment significantly improved high rate of wound-healing process, including faster epithelial growth and prevention of infection and dermal irritation, compared to standard treatments. All quality parameters controlled on the ointment appear to comply with the European Pharmacopoeia guidelines. This new herbal ointment is safe and effectiveness in wound healing. Our research supports the efficacy of the product for wound healing and treating minor burns, cuts, and abrasions. This herbal ointment is a promising alternative medicine for wound management.

Key Word: *Gaertnera phanerophlebia, endemic, ointment, wound, healing, formulation, Tsitsiromade 5 %*

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I. Introduction

Wounds and burns can can lead to significant physical disability. Managing skin wound healing problems is a public health issue in which traditional herbal medicines could play a determining role. It has been estimated that 14 million people suffer from wounds and burns annually whith over 80 % of these live in low and middle income county [1]. The annual wound care product's market is expected to reach 15 to 22 billion dollar by 2024 [2,3]. These dermatological lesions have a significant impact on public health and have psychological consequences. This publication, brings the solutions by using an endemic plant of Madagascar which is used in traditional medicine for treating wounds healing.

Madagascar is widely recognized for its exceptional biodiversity. Nearly 80 % of the fauna and flora present in the island are endemic to the country. This makes it a biodiversity hotspot of global significance [3, 4]. Despite the increasing of research on Madagascar's plant biodiversity, a significant number of species remain unstudied, even as they face imminent threats of extinction [5]. The RUBIACEAE family, also known as the coffee family, is indeed a promising area for discovering new phytomedicines due to its large number of understudied species and their potential for containing bioactive compounds [6]. Many study have shown that RUBIACEAE plants are containing a variety of bioactive compounds, including alkaloids, terpenoids, and anthraquinones, which are relevant to human health [7, 8].

Gaertnera phanerophlebia Baker, or "Tsitsirontafika", belonging to the RUBIACEAE family is endemic to the northeastern part of Madagascar. Its twings leaves have been used in traditional medicine to treat wounds [9].

This healing potential has not yet been scientifically explored. The objectives of this present study are therefore to justify the healing power and the potential antioxidant of the plant, then to validate a wound healing ointment made from its aqueous extract. Reactive oxygen species (ROS) play a crucial role in the preparation of the normal wound healing response. Therefore, a correct balance between low or high levels of ROS is essential. Antioxidant dressings that regulate this balance are a target for new therapies.

To the best of our knowledge, this is the first research in validation of the wound healing use of the plant in folk medicine.

II. Material And Methods

Plant material

The fresh twings leaves of *Gaertnera phanerophlebia* were collected in December, 2019 from Alaotra Mangoro region's of Madagascar, Commune d'Ambohibary, Fokontany Ampitambe Ambatomainty.

The plant was identified and authenticated by the Departement of Botany of the National Center for Pharmaceutical Research Applications (CNARP). Then a voucher specimen (Voucher ST380RF1) was deposited with herbarium.

Animals

White albino rat « Swiss » (230-300g) and mice « Wistar » (20-30g) were obtained from the animal house of the Department of IMVAVET (Institut Malgache des Vaccins Vétérinaires). They were allowed to acclimatize in the research laboratory for 1 week before the beginning of the study and were fed with standard livestock pellets. The animals were allowed unrestricted access to clean drinking water. Animal testing was conducted in alignment with the OECD guidelines and the 3Rs principle. (Replacement, Reduction, and Refinement) for minimizing animal use in research and improving animal welfare [10, 11].

Method of extraction

Twings leaves of *Gaertnera phanerophlebia* Baker were shide dried and ground into a fine powder. Thereafter, it was placed in a reflux flask with distilled water (1/10: M/V) and the whole was heated to 100° C for 10 to 20 min. After cooling, the decoction was filtered and then evaporated to dryness. The obtained extract ($ST380RF_{Dec}$) was stored at 4° C in the refrigerator for the ointment preparation.

DPPH free radical scavenging assay

The antioxidant activity of the aqueous extract was determined by the method of DPPH assay, based on the degradation of DPPH radical (2, 2 Diphenyl-1Picrylhydrazyl) with some modifications. The addition of antioxidant reduces the DPPH radical and causes the mixture to discolor [12, 13].

Qualitative evaluation

The qualitative assay was performed according to the method of Takao *et al*. (1994) [14] and Ruiz-Terán *et al*. (2008) [15]. Two milligrams of the dried extracts were diluted with 1 mL of the methanol. Then, 20 (µL) aliquots of each dilution were carefully loaded individually onto the baseline of the TLC plates (20 cm x 10 cm) and the sample was allowed to dry. DCM-Methanol (95 :5) was used as the mobile phase. Once the plate was dry, it was sprayed with a solution of DPPH radicals in methanol (25 %). Compounds with radical-scavenging activity showed a yellow-on-purple spot due to the discoloration of DPPH.

Quantitative evaluation

The antiradical activity of the plant extract samples was quantified using a spectrophotometer, via Popovici *et al*. (2009) and Razafintsalama *et al*. (2016) methods with slight modifications [16, 17]. Fist, a solution of DPPH in methanol (4.5 %) was prepared. Then, 3.800 mL of this solution was blended with 0.200 mL of the plant extract samples in methanol, which included 3.125-50 µg/mL of the sample. The reaction mixtures were placed in the dark at room temperature for 30 min. The absorbance of the mixtures was recorded spectrophotometrically at 517 nm [18,19,20]. In this study, α-tocopherol (3.125 µg – 50 µg) was used as positive control replacing the extracts [21].

The DPPH scavenging activity of the extract was expressed by the concentration providing 50 % of inhibition (IC_{50}). This was calculated using linear regression equation between the percentage of inhibition and the concentration of the sample [22]. As cited by Abas *et al*. (2006) and Ahmad *et al*. (2010) extract with an IC_{50} value of less than 30 µg/ml, between 30 - 100 µg/ml and more than 100 µg/ml is considered to possess strong, moderate, and weak free radical scavenging activity, respectively [23, 24]. Each sample was measured in triplicate and the mean value of absorbance was obtained.

Formulation of the ointment

This study aims to formulate environmentally friendly ointments using a carefully selected and combined set of natural ingredients. This approach emphasizes sustainable practices by prioritizing natural components over synthetic ones.

The herbal wound healing ointment (named Tsitsiromade) was prepared using the conventional melting method by fusion (Fig.1). This method involved melting all of the ointment contituents of the base together at 70° C, then cooling the mixture while continuously stirring until it congeals. Plant extract was added towards the end of the process to prevent degradation [25]. The choice of excipients and their respective proportions are based on theoretical and availability considerations.

In this process, the formulated ointment was prepared by incorporating 5 % of the aqueous extract, the active ingredient, in the base. First, the powder extract was grounded with a pestle and mortar. Then, a sufficient quantity (Qs) of the excipient mixture was added to reach a final mass of 30 g. The mixtures were then divided into 10 g aluminum tubes, which are tightly closed for storage.

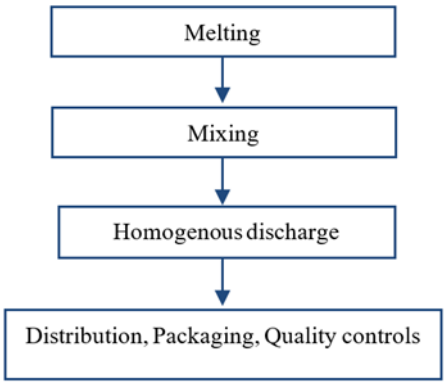

Fig.1 : Process for preparing the ointment

Quality and stability control

The following qualitative tests were carried out on the prepared ointment as described by Le Hir *et al*. (2011), in order to evaluate the quality and the stability of the prepared ointment [26].

Appearance: The general appearance of the formulated ointment was observed and recorded. Qualities included color, odor, granular or lumpy surface.

Consistency : A small amount of ointment was slowly rubbed between the thumb and fore finger to gauge consistency of the ointment.

Spreadability : A small amount of ointment was rubbed on the back of the hand and the ease with which it is spread over the skin was noted.

Washability : A small amount of ointment was rubbed on the back of the hand, after which it was washed off with warm water.

Homogeneity : It was determined by spreading a thin layer of approximately 0.2 g on a flat surface using a spatula. The regularity or unevenness of the mixture's distribution and the presence or absence of lumps were checked by eye and noted.

pH value : The pH of each preparation was determined using potentiometry, according to the guidelines of the European Pharmacopoeia, and measured with a pH meter at room temperature. The experimental procedure involved melting 10 g of each sample at 40° C followed by a direct cold reading on the preparations, with each reported value being the average of three measurements.

Centrifugation effect : Ointment instability can indeed be visually identified by the separation of the ointment into distinct layers or phases, indicating a loss of homogeneity. Often, this separation involves a liquid component, like oil, separating from the rest of the ointment. This test refers to a centrifugal sedimentation assay, which uses centrifugal force to separate components of a mixture based on their density and size. The presence or absence of a sedimentation phase (a distinct layer formed at the bottom of the container) after centrifugation indicates the mixture's stability and the characteristics of its components [27].

A quantity of each preparation was introduced into test tubes. The entire setup was then subjected to three successive centrifugations. Each centrifugation step was performed for a duration of 10 minutes. The first centrifugation was conducted at a speed of 1 600 rpm. This was followed by a second centrifugation at 3 000 rpm. Finally, a third centrifugation was performed at 4 000 rpm [28].

Physical and chemical stability: The developed product in its packaging was observed every three days for physical changes that were visible to the naked eye. The ointments were subjected to the following conditions : open and exposed to air, and closed at room temperature, both in light and in darkness.

Accelerated degradation test : In the pharmaceutical industry, temperature control is crucial for maintaining the efficacy and stability of drugs because some drugs can degrade or lose their potency at higher temperatures. The accelerated degradation test aimed to determine the impact of temperature on the preparations which were subjected to different storage temperatures [29, 30]. The experience was conducted as follow: five batches, each containig three jars were subjected to different temperatures conditions ranging from –80° C, –20° C, 4° C, 20° C, 40° C, and 60° C. They were examined at predetermined intervals throughout the storage period. Inspections were carried out after 3 days, 1 week, 2 weeks, 3 weeks, 1 month, 2 months, and 3 months of storage. The different batches of jars were categorized into three distinct groups based on their handling during the experiment: permanently open batch, permanently closed batch and opened and tested batch at each inspection [31].

Sterility test

This is a mandatory step for products intended for use on open wounds or severely damaged skin [32]. The objective is to confirm that the purity of the finished product meets the microbiological requirements specified in the monographs of the European Pharmacopoeia. This test also allows estimation of the formulated product's shelf life. The method consists of searching and counting bacterial and fungal germs that may be present in the preparation. Preparations for external use must be free of pathogenic microorganisms such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*, and *Aspergillus niger* because they can cause infections and other health issues.

Microbial contamination control was carried out using the germ count method [33]. The tests were conducted at at specific time intervals: 3 days, 1 week, 2 weeks, 3 weeks, 1 month, 2 months, and 3 months after the preparation was stored. Mueller Hinton Agar (MHA) and Sabouraud Dextrose Agar were used as culture media. In this case, a small amount (0.1 ml) of ointment is spread across the surface of the agar in Petri dish. Specifically, Sabouraud medium, was incubated at 24° C to encourage fungal growth, while MHA (Mueller Hinton Agar), which is often used for bacterial growth, was incubated at 37° C. Microorganism growth was observed after 24 and 48 hours.

Finally, the number of germs found in a sample is determined and expressed as Colony-Forming Units (CFU) per milliliter (ml) or gram (g). This number is then compared to the limits outlined in the European Pharmacopoeia's microbiological criteria for herbal medicinal products. This process, known as microbial limit testing, helps assess the product's microbiological quality, and ensure that it meets safety standards [34, 35].

The herbal ointment preparation is deemed acceptable if its microbial contamination level falls within the limits specified by either general recommendations or the monographs of the European Pharmacopoeia [36]. The table below gives the acceptance criteria for sterile pharmaceutical forms.

Table no 1: Acceptance criteria for the microbiological quality of non-sterile pharmaceutical forms

Administration route	TAPC (CFU/g ou CFU/ml)	TYMC (CFU/g ou CFU/ml)	Specified microorganisms
Cutaneous route	10^2	10^1	Absence of *S. aureus* (1 g ou 1 ml) Absence of *P. aeruginosa* (1g ou 1 ml)

TAPC: Total Aerobic Plate Count; TYMC: Total Mold and Yest Count. [37].

Acute dermal toxicity test

The tests were carried out following the guidelines of the Organization for Economic Co-operation and Development or OECD (2008b).

Then, acute dermal toxicity test was performed according to the Draize Acute Primary Irritation procedure on male mice. The experiments were carried out *in vivo* on SWISS mice weighing between 20 and 30 grams. Three animals were assigned to each of four groups and acclimated in a cage at a temperature of ± 23° C for one week. The mice had free access to tap water and were fed a standard diet. The total study duration was 72 hours from the day of application. This allows the effects of the product coming into contact with the skin to be assessed and categorized [38].

For this investigation, the application site (the mid-dorsal region) was shaved and cleaned with distilled water. Then, 150 mg/kg of the prepared herbal ointment was applied to the site. The area was inspected within 24 hours for cutaneous changes, such as erythema (redness), edema (swelling), and vesicular eruptions [29, 39]. The skin reaction was assessed by determining scores according to the Draize scale based on the Primary Irritability Index (PII) value (Table. 2; Fig. 2).

Table no 2: Interpretation of the Primary Irritability Index (IIP) value according to Draize scale

IIP value	Categories	IIP value	Categories
IIP < 0.5	Non-irritating	2.1 < IIP < 5	Irritant (Moderate Reaction)
0.5 < IIP < 2	Slightly irritating	5.1 < IIP < 8	Very irritating (Severe reaction)

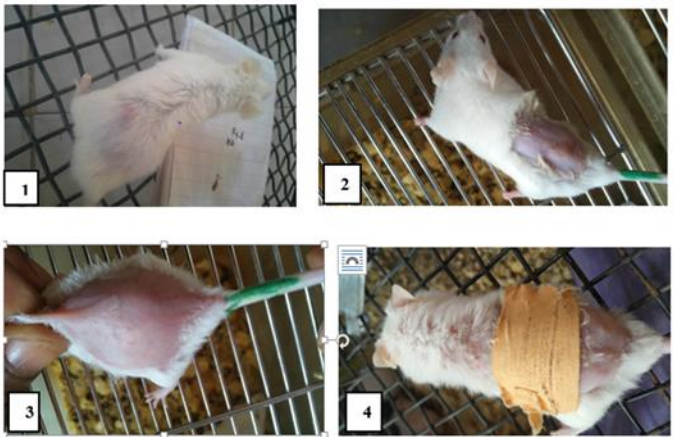

Fig. 2: Acute dermal toxicity test on mice

Wound healing test

This study involves two key stages of healing process: first, lesion induction, and second, observation and evaluation of healing kinetics.

Study models

The wound healing effects were carried out on adult male Wistar rats over eight weeks old [40]. Prior to the experiment, the rats were randomized into groups of three. Then, they were placed in individual cages at a room temperature of 25° C ± -1° and given free access to water and food under normal diurnal and nocturnal conditions. The groups are shown in the table below (Table.3).

Table no 3: Groups of experimental animals

Model	Test	No.	Positive control	No.	Non tested control	No.
Fresh wound	Plant extract	3	Brulex®	3	Untested	3
	Herbal ointment	3				

Wound induction

An excision wound was inflicted under light ether anesthesia according to the methods described by Sagliyan *et al*. (2010) and Kodati *et al*. (2011) with some modifications [41, 42].

A 40 mm diameter round seal was applied to the sides of a depilated and sterilized central trunk with ethanol (70° C). Full skin thickness was excised from the marked area to create a 200 mm^2 wound. The wounds were cleaned with distilled water before any treatment was applied. Ointments were applied twice daily until complete healing occurred (Fig. 3).

For anaesthesia, it was carried out by using inhalation of diethyl ether in a glass bell jar [43].

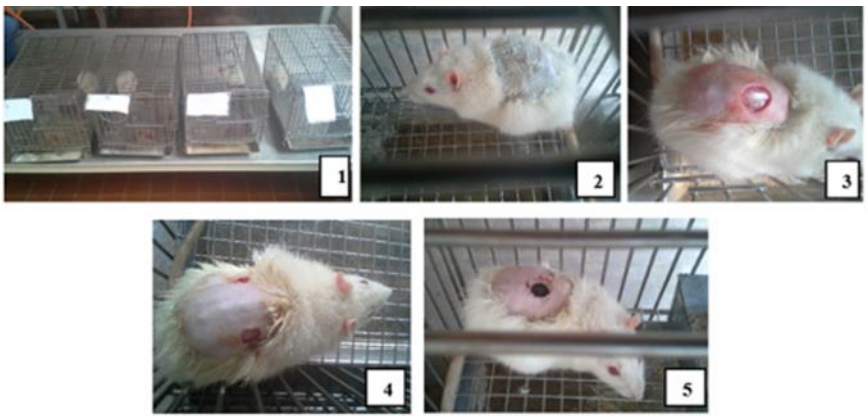

Fig. 3: Procedure for external excision healing in rats

1) Individual distribution of rats;

2) Shaving by applying depilatory cream;

3) Wound induction by excision;

4) Open circular experimental wounds on the right and left flanks of the animal; 5) Application of the ointment

Wound healing evaluation

Success in wound healing was evaluated by assessing the physical appearance of the wound, how long it took to heal, and changes in surface area over time across all treatment groups.

For 21 days, macroscopic observations of wounds were evaluated daily using a standardized scoring system developed by Qiu *et al.* (2003) and Azame *et al*. (2020) to detect signs of inflammation, such as swelling, redness, and exudates. This evaluation was performed before each new application in the study or treatment [44, 45]. This process was used to quantify the level of inflammation based on the presence of these visible characteristics.

A scoring system is used to evaluate wound healing progress. Appearance and color are assessed, and a score from 0 to 3 is given for size, swelling, exudate, and redness. These scores are then summed. A lower total score (0) indicates better wound healing, while a higher score (12) suggests worse healing. The statistical study compared the average scores (Table. 4).

Table no 4: Criteria for assessing the macroscopic appearance of wounds [44]

Evaluation criteria (1)	Score	Meaning	Evaluation criteria (2)	Score	Meaning
Swelling	0	Absent	Exsudate	0	Absent
	1	Low		1	Low
	2	Moderate		2	Moderate
	3	Significant		3	Significant
Totale area	0	Significant discount	Redness	0	Absent
	1	Moderate reduction		1	Low
	2	Low reduction		2	Moderate
	3	No reduction		3	Significant

Determination of healing duration

A direct planimetric study was used to evaluate wound healing kinetics [46]. Wound contraction, a key aspect of wound healing, was assessed by measuring the length and width of excision wounds in different groups daily using a caliper. The surface area of the wounds was calculated using the formula developed by Malachowa *et al*. (2013) [47]. Then, analysis of variance (ANOVA) was used to compare means and standard deviations of different areas [48, 49]. The re-epithelialization period was estimated by counting the number of days it took for the scab to fall off and for the wound to close completely without leaving an open wound behind [50, 51].

Statistical analysis

The data generated by the study were summarized into tables and graphs. Then, they were analyzed using ANOVA, means, standard deviations, 99.5% confidence intervals, and p values.

III. Results

Extraction result

After evaporation, a yield of 2.99 % dry extract was obtained from the fresh leaves of *Gaertnera phanerophlebia* ($ST380RF_{Dec}$). This resulting extract was a dark brown, slightly fragrant powder.

Free radical scavenging capacity

The aqueous extract ($ST380RF_{Dec}$) of the twigs leaves of *Gaertnera phanerophlebia* Baker exhibited antioxidant activity (Table. 5).

TLC -bioautography (DCM-Methanol (95 :5)) on aqueous extract revealed the formation of an intense yellow spot (Fig. 4). The yellow color change in the DPPH assay indicates a reduction in the DPPH radical, a key indicator of antioxidant power in plant extracts. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical that appears purple in its radical form. When antioxidants are present in a sample, they react with the DPPH radical, reducing it to a colorless or yellow compound called diphenyl picryl hydrazine.

These results show that the IC_{50} value of 8.56 ± 0.3 µg/mL for the $ST380RF_{Dec}$ extract is lower than the value of 11.43 ± 0.1 µg/mL for α-tocopherol, a standard antioxidant. This suggests that the $ST380RF_{Dec}$ extract is more effective at inhibiting free radicals. According to Abas *et al*. (2006) and Ahmad *et al*. (2010), the antiradical property of *G. phanerophlebia* is powerful when values are lower than 30 µg/ml. The comparisons above indicate that the aqueous extract exhibits strong antioxidant activity. Specifically, this high antioxidant activity suggests potential health benefits associated with the extract's ability to scavenge free radicals.

Table no 5 : DPPH scavenging activity of the aqueous extract from twings leaves of *G. phanerophlebia*

DPPH scavenging activity at 1 mg /ml		
Parameters	Aqueous extract ($ST380RF_{Dec}$)	α- tocophérol
TLC – DPPH test	+++	+++

DPPH RSA (%)	92.04	93.023
IC_{50} (µg/ ml)	8.56 ± 0.3	11.43

RSA : Radical Scavenging Activity ; IC_{50} : Inhibitory Concentration 50 % ; +++ : high intensity;

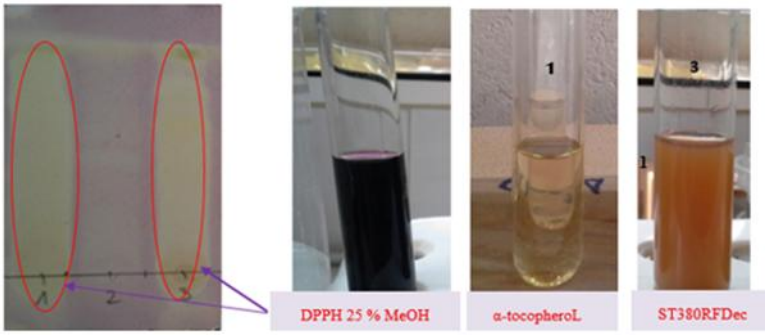

Fig. 4: DPPH scavenging activity of aqueous extract of *G. phanerophlebia*

Ointment formulation

The herbal ointment (Tsitsiromade 5 %) from aqueous extract of *G. phanerophlebia* ($ST380RF_{Dec}$) was successfully formulated, packaged in 30 g aluminum tubes, and labeled. This ointment contains several excipients of natural origin that are known for their skin-healing, soothing, and regenerative properties including beeswax, white petrolatum, glycerol, and zinc oxide (Fig. 5). These ingredients are intended to promote rapid and lasting skin repair. The product contains no synthetic preservatives, artificial fragrances, or animal-derived ingredients.

Fig. 5: Composition, labelle and packaging of the herbal ointment Tsitsiromade 5 %

Quality parameters of Tsitsiromade 5 %

The quality, physicochemical, organoleptic, skin toxicity, and stability parameters of the developed herbal ointment (Tsitsiromade 5 %) are presented in the following table.

Table no 6 : Quality parameters of the herbal ointment Tsitsiromade 5 %

Herbal ointment (Tsitsiromade 5 %) parameters				
Appearnace	**Consistancy**	**Spreadability**	**Whashability**	**Homogeneity**
Odor : Pleasant Color : Brown Texture : Smooth	Semi-solid	Uniform (No clumps or particles)	Good	Good
Phase separation	**pH**	**Sterility**	**Toxicity**	**Stability**
Nothing	Compliant (5.01 - 6. 32)	The samples are free of any living microorganisms: 0 UFC/g (bacteria, yeasts, and other germs)	Erythema : 0 Oedema : 0 PII = 0 No cutaneous changes were observed within 72 hours Non-toxic for skin (PII < 0.5)	No change after 3 months at 20° C

All of these parameters appear to comply with the European Pharmacopoeia guidelines.

The pH of the herbal ointment is close to that of the skin (5.01 to 5.26), which is a positive attribute for topical preparations. Its similarity to the natural pH of skin (typically between 4.1 and 5.8) is beneficial for several reasons. The smooth formulation indicates uniform mixing of the contents and the absence of grittiness.

The absence of germs in ointments is strongly linked to the use of high-quality raw materials and strict adherence to Good Manufacturing Practices (GMP). Tsitsiromade 5 % has been found to be safe for skin application with no adverse reactions observed. This suggests that the preparation does not cause skin irritation or other harmful effects. Furthermore, the preparation exhibited no signs of deterioration or breakdown during testing or storage. It has the ability to provide a pleasant, effective moisturizing effect. This indicates that the ointments maintained their intended physical and chemical properties, such as consistency, and appearance, over the observed period. Additionally, the ointments are quickly absorbed by the skin, leaving it soft and hydrated.

Wound repair efficacy of Tsitsiromade 5 %

This study examined the effects of applying the herbal ointment Tsitsiromade 5 %, which is formulated from an aqueous extract of *G. phanerophlebia*, to excision wounds on rats. The measurements taken were recorded on days 1, 3, 6, 9, 11, 13, 15, 18 and 21, then the surface averages and standard deviations were calculated. Figure no 6 illustrates the change in wound size over time, specifically showing the percentage decrease in the average wound area throughout the experimental period. This visualization allows for a clear understanding of how quickly wounds are healing by tracking the reduction in their size.

Both the aqueous extract of *G. phanerophlebia* ($ST380RF_{Dec}$) and Brulex® have been found to accelerate wound closure compared to other treatments. This effect is observed in the speed of wound contraction and the overall healing process. However, the developed ointment significantly increased the rate of wound healing and reduced the epithelialization period. Compared to the group treated with aqueous extract and Brulex®, the ointment (Tsitsiromade 5 %) resulted in a 2-day faster healing time, and compared to the untreated group, it resulted in a 7-day faster healing time. These results suggest that the ointment is more effective than the other treatments at promoting wound healing.

Unlike the untreated group (Fig. 7), the treated wounds did not exhibit signs of inflammation, redness, or infection during the treatment period. These results suggest that the treatment was effective in preventing or managing these typical wound complications.

The provided information suggests that the Tsitsiromade 5 % ointment made from the aqueous extract of twings leaves has a wound-healing effect, specifically accelerating the rate of wound contraction, and that this activity may be comparable to that of Brulex®.

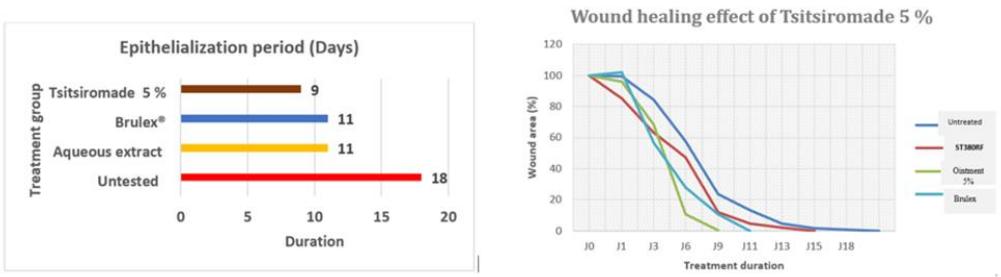

Fig. 6: Effect of Tsitsiromade 5 % ointment from *G. phanerophlebia* on excision wound healing in rats.

The progression of healthy wound healing from the initial application (J0) of the products to day 11 (J11) are summarizes as follow (Fig. 7).

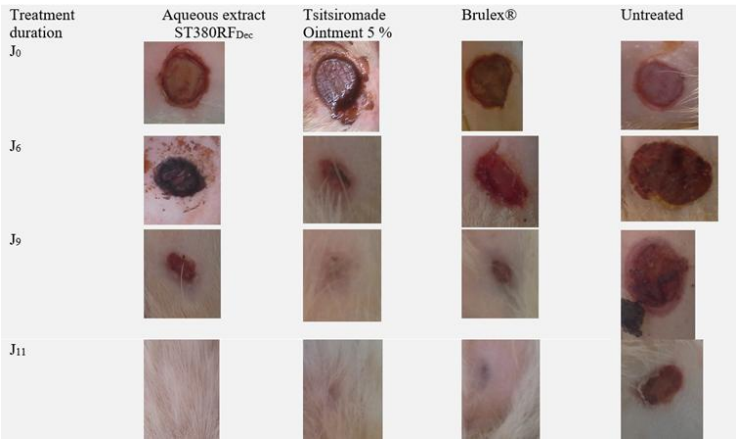

Fig. 7: Healing progression of wound treated with Tsitsiromade 5 %

These results suggest that the formulated ointment may lead to quicker wound closure and scab formation. They also suggest that the excipient enhances the healing process by facilitating the extract's action or having its own beneficial effects on wound healing.

IV. Discussion

Wound closure is a multi-stage process that culminates in the final remodeling phase. During this phase, myofibroblasts contract the wound edges. Contraction, collagen realignment, and apoptosis of unnecessary cells all contribute to wound closure.

This study highlighted the plant extract's excellent ability to scavenge free radicals, suggesting that it could act as an antioxidant and offer potential health benefits by neutralizing harmful free radicals. Antioxidants, like those in this extract, achieve this by donating electrons to unstable free radicals, which prevents cellular damage and wound formation.

The results of the wound healing test suggest that this herbal ointment (Tsitsiromade 5 %), formulated with an aqueous extract of *G. phanerophlebia* may accelerate the wound healing process compared to an untreated wound. Many traditional remedies use herbal ointments to promote wound healing. However, some commercial products make unsubstantiated claims about their efficacy. Therefore, scientific research is necessary to validate these claims and understand how the ointments work, which could lead to more effective and reliable wound care treatments.

This study validates the effectiveness of ointment formulated with *G. phanerophlebia* extracts in promoting wound healing without inducing adverse reactions. Numerous studies have demonstrated that topical therapies containing antioxidants can help manage inflammation and promote wound healing by regulating the redox balance. These therapies aim to reduce the excess reactive oxygen species (ROS) that can hinder the healing process. Antioxidants can create a favorable environment for cell proliferation and tissue repair by neutralizing ROS and decreasing inflammation [52].

Additionally, the present research suggests that *G. phanerophlebia* extracts could be used to develop commercial dermatological products. This finding lends support to the traditional use of the plant for wound treatment and encourages further exploration of its medicinal properties.

V. Conclusion

In conclusion, this review provides an overview of the research on some of medicinal RUBIACEAE species with potential applications in traditional medicine that have not yet been exploited.

Tsitsiromade 5 %, an herbal ointment made from *G. phanerophlebia* extract endemic to Madagascar, is considered effective for wound healing and has been shown to produce better results than other treatments. Tsitsiromade 5 % had the fastest rate of wound reduction and the shortest epithelialization and healing times compared to the other treatments. While it shows promise, improvements are needed, such as reducing staining, for better cosmetic acceptance. This new product could be an alternative for wound management.

This study provides therefore the first scientific evidence of the effectiveness of *G. phanerophlebia*'s in promoting skin repair. The study underscores the need to further explore this plant's biochemical composition and biological activities, particularly in the context of modern health challenges. And the last but not the least, this research highlights the importance of culturally sensitive health interventions and collaboration between traditional healers and modern health professionals.

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