



The Wound Healing Action of Kakawati Gel from *Gliricidia sepium* (Jacques) Steudel (Family Fabaceae)

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Research Article

Please cite this paper as Rogie Royce Carandang^{*}, Kristal Cielo Buemio, Adelaine Lopez. The Wound Healing Action of Kakawati Gel from *Gliricidia sepium* (Jacques) Steudel (Family Fabaceae). IJPTP, 2015, 6(4), 2642-2649.

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Abstract

Objective: *Gliricidia sepium*, locally known as kakawati is recognized for its wound healing property. The study aimed to determine the wound healing action of a safe, stable, low cost formulation from the lyophilized sap of *G sepium*.

Methods: The organoleptic, physical, and chemical properties were determined. Short term stability testing, as well as compatibility study with common excipients was performed. Wound healing property was determined by three methods: (1) measurement of the rate of wound contraction, (2) tensile strength of the healed tissue using a tensiometer and (3) histological examination of collagen deposition. The 7.5% gel formulation was also tested for its efficacy with wound healing-impaired rats.

Results: The pilot-scale manufacture of a gel-based formulation using water extract of *G sepium* was feasible. The extract was compatible with glycerin, sodium CMC, sodium benzoate and sodium citrate. Stability testing with common excipients showed that the gel formulation was stable at a temperature of 30°C. It was stable at pH values of 5 to 10 and in natural and artificial light. The formulated product is a green gel of moderate consistency possessing a natural fragrance with faint fetid odor. The gel formulation was found to be effective at 7.5% concentration as a wound healing agent.

Conclusion: The gel formulation has been proven to have wound healing properties and was found to be safe, effective, stable and low cost.

Keywords: wound healing, kakawati, gel formulation, *Gliricidia sepium*, diabetes

Introduction

Expensive treatment of wound injuries creates a heavy burden to poor Filipinos. In line with this, the latter resorted to alternative herbal remedies that are not only tested to heal wounds but are also readily available. One of these herbal remedies is the plant *Gliricidia sepium* Jacques Steudel (Fam Fabaceae) that can be found almost everywhere. The sap of the bark, leaves and roots has been cited by folkloric literatures as a wound healing promoter^[1].

Many Filipinos suffer from diabetes and its complications. The long term effects of diabetes impair wound healing by diminishing sensation and arterial inflow. One of the manageable conditions is infection from unhealed wounds caused by impairment of the body's natural healing mechanism^[2].

The effects of diabetes on healing are diverse, multifactorial, complex and inter-related. It is one of the well-known intrinsic factors which affect wound healing. In fact, diabetes affects almost all stages of wound healing to some extent^[3].

With the knowledge that *G sepium* grows in abundance almost everywhere, the challenge was to develop the sap of the bark of the plant into a suitable, safe, and low cost dosage form as an alternative for the topical management of mild to severe wounds of normal as well as the wound healing-impaired individuals, which can be



brought about by diabetes complications, burns and other skin injuries^[4].

Material and Method

Collection of Plant Material

Fresh, matured barks of *G sepium* were collected from Lipa, Batangas. Collection may be done anytime of the year except when it rains in order to avoid fungal growth on the bark due to absorption of water.

Processing of Plant Material

Immediately after collection, the stem and trunks were washed thoroughly in running water and allowed to drip and air-dry. The bark was removed using a sharp knife. Undesirable plant parts and other extraneous matter were removed. The barks were subjected to mechanical extraction through expression using cheesecloth and purification of the sap extract was done by filtration. The 100% sap collected was then lyophilized to remove water and to ensure that the active principles were the only one present in the sample.

Physicochemical and Other Tests

The following tests were performed in the sample: (1) phytochemical screening, (2) organoleptic evaluation, (3) foreign organic matter, (4) moisture content determination and (5) extractives and ash determination.

Preformulation studies

The following tests were conducted: (1) hygroscopicity studies and (2) stability tests to heat, moisture, light and pH.

Compatibility Testing

Mixtures (1:1) of the lyophilized extract with an excipient placed in sealed and unsealed stoppered vials were stored at refrigerated temperature, ambient room temperature and in oven (Memmert) set at 50°C protected from light for four weeks. Three trials were performed for each condition and aqueous extract –excipient mixture. Their physical appearances were observed weekly and a thin layer chromatographic profile was done and compared to the control group.

Trial formulation

Three trials of 7.5% w/w kakawati gel were conducted following a basic gel formula consisting of the following: viscosity-enhancing agent, humectant, neutralizer, preservatives and appropriate solvent. The drug-excipient mixture was based on their respective compatibility.

Final formulation

The formulation with the best physical attributes was selected to be the prototype formula. It was validated using a larger batch size. In-process quality control tests for the aqueous extract were performed. The amount of viscosity-enhancing agent and water phase that had to be stirred and homogenized necessitated the use of Heidolph mixer. The finished product was evaluated based on the following parameters: color, appearance, consistency, pH, microbial content, and TLC.

Validation of Wound Healing Activity.

A. Measurement of Wound Contraction

After surgery, the excision wound margin was traced at 3-day intervals and the area measured by the aid of a caliper. Measurements were to be continued to the 15th day. Wound contraction percentage was determined from the measurement using the formula:

$$\% \text{ Wound Contraction} = (\text{Healed Area} / \text{Total Area}) \times 100$$

B. Measurement of Tensile Strength

Tensile is the resistance to breaking under tension. It indicates how much the repaired tissue resists breaking under tension and may indicate in part the quality of the repaired tissue. The instrument used for measurement is called a tensiometer. For the quantitation, one of the edges of the wound was fixed while applying a measurable force to the other one. The load or weights in grams required to disrupt



the wound is determined after complete healing of the wound.

C. Histological examination

The skin including the wounds was removed from each rat for histological examination. The wounded area was fixed with 10% formaldehyde. Dehydrated samples were embedded into paraffin and cut into thin sections. These sections were stained with eosin and examined under light microscope. The thickness of collagen layer deposited on the tissue was measured.

D. Data analysis

One-way ANOVA was used to determine if there was a significant difference between treated groups at different time intervals. Least Square Difference (LSD) was used to know which doses have significant difference. The data were analyzed by considering the following: percent of wound contraction, tensile strength, and histological examination of collagen formation.

Stability Testing and Expiration Dating of Kakawati Gel

To determine the products stability to heat, the product was stored in its final packaging at ambient room temperature for reference, and in ovens (Memmert) set at accelerated temperatures of 40, 50 and 60°C. Physical changes were observed visually and assayed by TLC.

Results and Discussion

Table-1 presents the summary of the physicochemical and other tests performed in the sample.

Phytochemical screening was conducted to determine what groups of plant constituents are present and could be extracted from the plant material [5]. It was found that tannins, glycosides, reducing substances, plant acids and flavonoids were present in the samples. Saponins, particularly the steroidal type were also detected. The presence of alkaloidal substances was not observed

The sap extract obtained from the bark of *G sepium* by mechanical expression was green in color but upon standing, separation of green sediments and yellowish-brown solution was noted. This can be

homogenized through agitation. It had an herbal characteristic odor. However, a distinct fetid odor of the plant, though very faint, could still be detected. The sap extract was acidic, with a pH of 5.45, specific gravity of 1.0271 and relative viscosity of 1.4334 cps. The pH of the sap is said to be preferable because the pH of the normal human skin is also slightly acidic. This is significant since higher acidity or alkalinity may cause irritation or could even worsen the dermatologic condition. The color and odor imparted by the sap extract were somehow expected to affect the color and odor of the gel since it will comprise 7.5% of the formulation.

Table-1: Summary of the Physicochemical and other tests

A. Phytochemical Screening	Results
1. Litmus Paper	Slightly acidic
2. Tannins	Present
3. Glycosides	Present
4. Reducing Substances	Present
5. Alkaloids	Absent
6. Plant Acids	Present
7. Saponins	Present
8. Flavanoids	Present
B. Tests performed	Mean*
1. pH	5.45
2. specific gravity	1.0271
3. viscosity	1.4334
4. foreign organic matter	0.05%
5. moisture content	5.86%
6. water-soluble extractives (hot)	1.85%
7. water-soluble extractives (cold)	1.47%
8. alcohol-soluble extractives (hot)	0.86%
9. alcohol-soluble extractives (cold)	1.13%
10. total ash	13.73%
11. acid-insoluble ash	0.06%
12. water-soluble ash	0.14%

* Three trials were performed

The determination of foreign organic matter was performed. The determination may be used as a measure of purity and can



serve as a standard for the plant material [6]. Foreign organic matter found in the samples includes soil, dead insects and decayed matter. These were removed from the samples. The mean percentage foreign organic matter was 0.05%, which was considered low, indicating that the samples were of high purity prior to use.

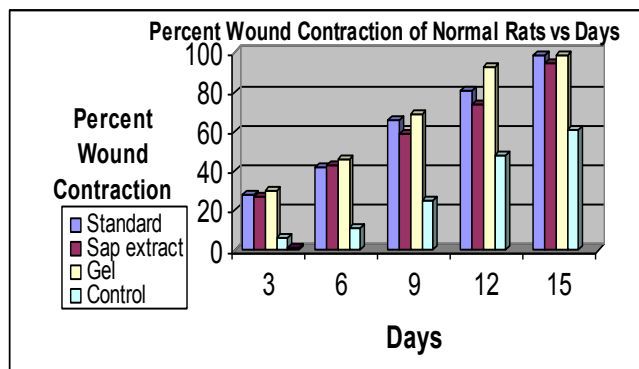


Figure 1: Percent Wound Contraction vs. Days of Normal Rats

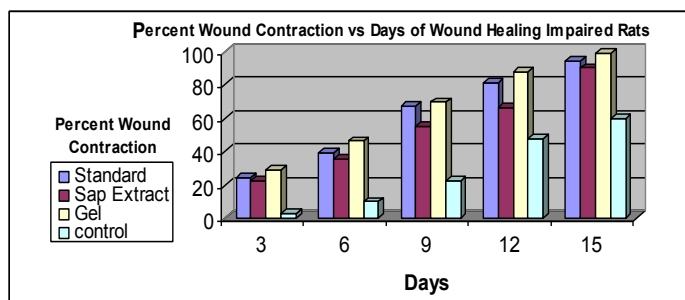


Figure 2: Percent Wound Contraction vs. Days of Wound healing Impaired Rats

Varying quantities of moisture are usually present in drugs. With natural products, absorbed moisture is of great concern. The presence of moisture in the drug could affect its stability in storage as moisture promotes mold growth. Moisture is also an important consideration in formulation since the amount of the active constituent in a formulation is usually based on the moisture-free drug. The mean percent moisture content of the samples was determined to be 5.86%. It was expected that a low moisture content should be obtained since the sap was been lyophilized. However, the lyophilized sap tends to absorb moisture upon exposure to air, thus, proper storage should be considered.

In the determination of extractives, the hot and cold method of water and alcohol soluble extractives were done. These measures can be used as a basis for the identity and purity of the plant material and as such, may be set as standards. They are approximate measures of the amount of a certain constituents or a group of related constituents the drug contains. In the samples, the mean percent water-soluble extractive for hot and cold method was determined to be 1.85 and 1.47% respectively, while the mean percent alcohol soluble extractive for hot and cold method was determined to be 0.86 and 1.13%.



Figure 3: Examination of the excised wound under the light microscope treated with *G sepium* gel formulation

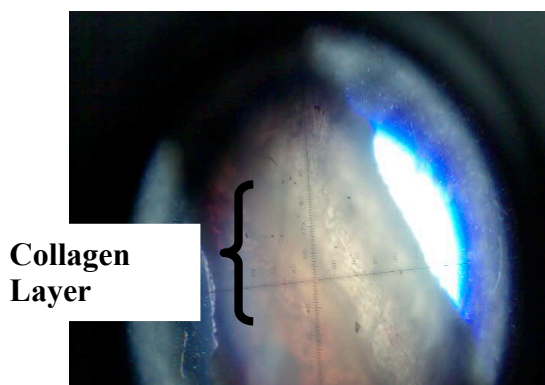


Figure 4: Examination of the excised wound under the light microscope treated with Solcoseryl® Jelly

Ash determinations were also conducted. The total ash, acid-insoluble ash and water



soluble ash contents were determined. The mean percent total ash content of the samples was found to be 13.73%, mean percent acid-insoluble ash content was 0.06%, and the mean percent water soluble ash, 0.14%. These represent the inorganic salts naturally occurring in the drug and adhering to it. However, in some cases, these may include inorganic matter added for the purpose of adulteration. Therefore, it may be used as a basis for cleanliness and purity of the plant material, aside from serving as a basis for judging its identity [7].

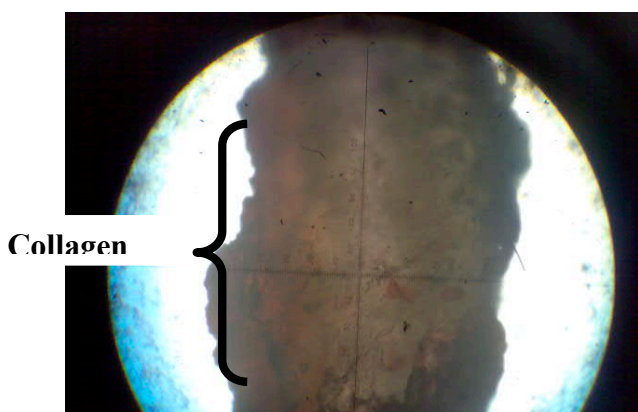


Figure 5: Examination of the excised wound under the light microscope using *G. sepium* extract

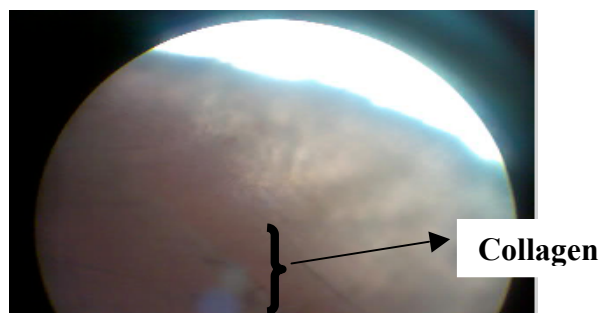


Figure 6: Examination of the excised wound under the light microscope (Control)

Based on the compatibility testing and trial formulations done, final formulation of the gel was accomplished using the following excipients: sodium CMC, glycerin, sodium citrate, sodium benzoate and deionized water. The adjustment in the amount of excipients was based on the objective that the right pH, consistency and gel stability be met.

As part of the stability studies, the formulation was subjected to varying degrees of temperature to see how heat affected the integrity of the active

ingredient, as well as the excipients. The gel formulation was stored in its final packaging at an ambient temperature (30°C) and accelerated conditions of 40, 50 and 60°C. No changes were observed for those samples stored at a room temperature (30°C). Liquefaction, darkening and increased in pH were noted at 40°C after 1 month and at 50 and 60°C after 15 days and 10 days respectively. TLC results showed no changes in the R_f values of spots produced when compared with the standard chromatogram of *G. sepium* and neither there were new spots produced. However, at temperatures 40°C, 50°C, 60°C, it was observed that during the later part of the stability period, liquefaction occurred. Degradation was fastest at 60°C. TLC showed disappearance of 3 spots at 40°C after 1 month. At 50°C, disappearance of 3 spots was seen on the 15th day. The same was true at 60°C, wherein there was disappearance of 3 spots on the 5th day. It can be seen that there was decomposition of the active ingredient since other markers can no longer be detected. TLC profiles of samples stored at temperatures 40°C, 50°C and 60°C were noted. As expected, an increase in temperature would cause an increase in the rate of liquefaction, owing to a fall in apparent viscosity and consistency. The results showed a trend that more adverse storage conditions caused earlier signs of instability in the gel formulation.

Moisture has an adverse effect on drug stability, as well. When a drug is exposed to an environment having a considerably high relative humidity (RH), the drug can take up moisture that could result in degradation particularly if the drug is prone to hydrolysis. Moreover, it can hasten possible interaction with the other excipients, particularly those that are easily oxidized. Stability to moisture of the lyophilized sap extract showed no significant changes in samples stored in stoppered vials, except for slight darkening in a sample exposed at



90% RH (KNO_3). On the other hand, samples stored in unstoppered vials showed slight darkening even at 75% RH (NaCl). TLC of the lyophilized sap extract revealed that those with stoppered vials were stable up to 75% RH after 2 weeks while those with unstoppered vials were found to be unstable even at 60% RH. Thus, it follows that an increase in RH favors decomposition of the active constituents particularly with unstoppered vials.

Hygroscopicity study of the gel formulation stored at 45% RH absorbed a mean amount of moisture 0.04% of its initial weight; 0.04% at 60% RH; 0.13% at 75% RH; and 0.27% at 90% RH. These data suggest that the container-closure system of the gel formulation was tightly fitted and impervious to moisture, thus, the affinity to moisture was lesser although there is increasing relative humidity.

Instability due to light or photodegradation is dependent on two factors: (1) intensity and (2) wavelength. Sunlight encompasses the wavelength range of 290-780 nm, however, only the higher UV range of 290-390 nm can cause photodegradation. Conversely, light from fluorescent light bulbs includes visible and potentially degrading UV radiation, which includes the wavelength range of 320-380 nm. On the other hand, light from conventional tungsten filament light bulbs, discharge radiation of >390 nm wavelength. This causes little or no harm to drug products^[8]. In the study, the samples of the lyophilized sap extract and formulated gel were stored under sunlight and fluorescent light bulbs. No significant changes were observed in all of the samples after two weeks of exposure at different conditions. Nonetheless, it is important that the lyophilized sap extract be stored in amber-colored bottles which have the highest capacity to adsorb radiation. The final packaging container which is an Aluminum collapsible tube for the gel formulation prevents and delays photolysis.

As stated above, pH is a critical factor that should be considered in the formulation of topical preparations. Imbalance in the pH of the skin results in irritation or subsequent aggravation of any dermatologic condition present. Thus, the pH of the preparation should match or at least deviate slightly from that of the skin. However, changes in the pH of the drug as a result of addition of certain excipients, can significantly affect its stability. The lyophilized

sap extract were dissolved in different mediums whose pH were adjusted that is pH 1.0, 3.0, 5.0, 7.0 and 10.0. At pH 1.0, the solution initially turned brownish colored for both stoppered and unstoppered vials. At pH 3.0 and 5.0, those with unstoppered vials showed mold growth after 2 weeks while with stoppered vials showed turbidity. Result from the study showed that *G. sepium* lyophilized sap extract was highly unstable at pH less than 5.0 as signified by darkening, turbidity and mold growth formation. The lyophilized sap extract was found to be most stable at pH 5.0 to pH 10.0. However, TLC showed that at pH 10.0 there was decomposition of the lyophilized sap extract since only one spot was detected. Only at pH 5.0 and pH 7.0 gave the same number of consistent markers. This is deemed favorable since the pH of normal human skin is slightly acidic.

Part of the objective of this study was to produce a suitable gel formulation that would be beneficial not only for individuals with normal wound healing response but also to wound healing impaired patients such as diabetics. In line with this, two treatment groups were devised. The first treatment group consists of 10 male diabetes-induced rats while the other group consists of 10 normal male albino rats. Four wounds were also excised but this time were treated with Solcoseryl Jelly as the standard drug, pure sap extract of *G. sepium*, 7.5% gel formulation and control. Three tests were also conducted among the treatment groups.

In terms of wound contraction (Figures 1-2), wound healing impaired rats showed slower wound contraction as compared to normal rats. The 7.5% gel formulation exhibited faster rate of wound contraction on wound-healing impaired rats as compared to the standard drug, thus showing a significant difference. On the other hand, Solcoseryl[®] Jelly exhibited the same response with that of the gel formulation on normal rats. There was also



a significant difference between the rate of wound contraction of Solcoseryl jelly and the pure sap extract in both treatment groups. The same is true with the 7.5% gel formulation having significant difference with that of the sap extract. The three aforementioned treatments showed significant difference with that of the control.

Tensile strength on wound healing-impaired rats and normal rats revealed that there was no significant difference between the standard (Solcoseryl jelly), pure sap extract and gel formulation. However, a significant difference was obtained between the three treatments and the control.

In terms on the thickness of collagen deposition, wound healing-impaired rats showed significant difference between the standard and pure sap extract. Collagen layers (Figures 3-6) formed by the used of the standard drug were thicker as compared with that collagen formed by the pure sap extract. No significant difference on thickness of collagen layers was revealed between the standard and the formulated gel and the latter with the pure sap extract. Likewise, normal rats exhibited no significant difference between the standard, formulated gel and the pure sap extract. The three aforementioned treatment groups showed significant difference with that of the control.

Results showed that the 7.5% formulated gel had comparable efficacy as compared with that of the standard. The pure sap extract also had the nature to promote wound healing. However, a formulated gel was found to be more effective in terms of the rate of wound contraction in both treatment groups.

Conclusion

The pilot-scale manufacture of a gel-based formulation using water extract of *G sepium* is feasible. The extract is compatible with glycerin, sodium CMC, sodium benzoate and sodium citrate. It was proven to have wound healing properties. Stability testing with common excipients showed that the gel formulation was stable at temperatures of 30°C. It was stable at pH values of 5 to 10 and in natural and artificial light. The formulated product is a green gel of moderate consistency possessing a natural fragrance with faint fetid odor. The gel

formulation was found to be effective at 7.5% concentration. The gel formulation costs about Php 20.98 per unit based on the product costing. The gel was proven to be at par with the standard product, Solcoseryl gel, and more effective than the control, in wound healing activity for both normal and wound healing-impaired rats.

Acknowledgement

The researchers would like to acknowledge Prof. Jocelyn Bautista-Palacpac for her invaluable support in pursuing this research.

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AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

PEER REVIEW

Not commissioned; externally peer reviewed.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.