

**WOUND HEALING POTENTIAL OF THE ETHANOLIC EXTRACT OF BANANA FLOWER (*Musa sapientum*, BBB ‘Saba’, Family Musaceae)**Rufo S. Calixtro, Jr.^{1,*}, Analiza P. Malalay¹, Ponciano B. Epino² and Lazaro E. Avelino³¹College of Pharmacy, University of Perpetual Help-Dr. Jose G. Tamayo Medical University, Biñan, Laguna, Philippines 4024²College of Occupational Therapy, University of Perpetual Help-Dr. Jose G. Tamayo Medical University, Biñan, Laguna, Philippines 4024³General Education Department, University of Perpetual Help-Dr. Jose G. Tamayo Medical University, Biñan, Laguna, Philippines 4024***Corresponding author e-mail:** calixtro_2005@yahoo.com**ABSTRACT**

The banana flower (*Musa sapientum*, BBB ‘saba’) ethanolic crude extract was investigated for its wound healing potential in vivo. Circular excision and linear incision wounds were inflicted on male Swiss mice, after which, they were treated with different concentration of banana extract. Betadine was used as the standard treatment. Significantly higher wound contraction is obtained when ethanolic extract was applied on the wound than the control. At $\geq 10\%$ ethanolic extract, significant reduction in wound size is observed than betadine treated wounds. Similarly, a significantly higher tensile strength of the wounded skin of mice is obtained on ethanolic extract treated mice than the control. Tensile strength is significantly better at $\geq 15\%$ ethanolic extract than betadine treated animals. Results indicate that banana flower ethanolic extract has a potential for use in healing wound.

Key words: Banana flower, ethanolic extract, excision wound, incision wound, wound healing potential**INTRODUCTION**

A wound is an injury in which the skin or another external surface is torn, pierced, cut, or otherwise^[1]. It can be internal or external in origin. Wound healing is a natural process of the body where dermal and epidermal tissues are regenerated through continuous cell-to-cell and cell-to-matrix interactions. It involves three overlapping phases namely, inflammation, proliferation, and remodelling^[2]. Inflammation occurs between 0-3 days after injury wherein the neutrophils migrate around the incision area. During the proliferation phase, the incisional area is filled with granulation tissue within 3 to 12 days. Remodelling phase sets in, 3-6 months after where collagen fibers are synthesized leading to an increase in tensile strength of the skin^[3]. Many antiseptic drugs are available for treating wound injuries; however, they may cause adverse reactions

when used for a long period^[4]. Thus, healing of wounds still remains a challenge to modern medicine. Traditionally, large number of plants/plant extracts/decoctions or pastes are widely used for the treatment of cuts, burns, and wounds^[5].

In the Philippines, banana is an important crop mainly used as food. One of the more popular varieties widely cultivated in the country is ‘saba’, *Musa sapientum*, Saba (BBB) L., Musaceae^[6]. Aside from its value as a food, banana is widely used in traditional medicine. Young leaves were used for cool dressing of inflamed and blistered surfaces and as cool application for headaches while juice from flowers mixed with curds are used for dysmenorrhea and menorrhagia^[7]. Flowers are also used as cardialgic, and when cooked, are used for diabetes. According to a recent study, the aqueous and methanolic extracts of plaintain banana showed

wound healing properties through increased wound breaking strength, reduced glutathione, decrease percentage of wound area, scar area and lipid peroxidation^[8]. They concluded that the wound healing was probably through antioxidant effect and various biochemical parameters.

The present study was undertaken to investigate if the banana flower extract of 'saba' variety exhibits wound healing activity. Results of this study will not only widen our knowledge about the diverse pharmacologic action of the banana plant, but may also provide an alternative safe and effective therapy for wound healing. The banana flower extract of 'saba' variety was investigated for its wound healing activity for the first time.

MATERIALS AND METHODS

Plant Material

The banana flower was obtained from the Science and Technology-based farm for banana (Saba) production in Maningit, Sagpangan, Aborlan, Palawan. This is the main cultivation site in the country for it provides a well-drained fertilized soil and full sun exposure required for growing this variety of banana^[9]. The plant was duly authenticated by the curator of botanical herbarium of the Museum of Natural History at the University of the Philippines in Los Baños, Laguna. The fresh flower was washed with 1% sodium hypochlorite for 15 minutes, then washed in running water, cut into small pieces, and air-dried at 50°C in a hot air oven for 24 hours. Dried samples were ground to powder using a mechanical grinder, and kept separately until use.

Extraction

One hundred grams of powdered sample were subjected to extraction with 200 mL ethyl alcohol by maceration with constant stirring overnight. The extracts were filtered through sterile Whatman number 1 filter paper and then centrifuged at 8000 rpm for 10 minutes^[10]. The residue obtained was weighed and the percentage yield was computed. The average yield of extract was 73.25%.

Phytochemical Analysis

Three hundred grams of ethanolic extracts were subjected to phytochemical and Fourier Transform Infrared (FTIR – Model ABB MB 3000) - Attenuated Total Reflectance analyses at the Standard and Testing Division of the Department of Science and Technology (DOST) Industrial Technology Development Institute in Bicutan, Taguig City, Metro Manila. These tests were done to determine if the

ethanolic extracts contain active constituents that may be responsible for its medicinal activities.

Animals

Male Swiss mice weighing 3 to 3.5 grams were purchased from the animal breeding division of the Food and Drug Administration in Alabang, Muntinlupa City, Metro Manila. They were housed under standard environmental conditions of 25±0.5°C, 50- 60% humidity and 12 hours light/dark cycle. The animals were fed with standard pellet diet and provided with sufficient water.

Acute Toxicity Study

Acute toxicity study was performed according to Organization for Economic Cooperation and Development (OECD) guidelines No. 423 (OECD, 2000)^[11]. The mice were kept overnight fasting prior to drug administration. The mice were divided into 2 groups with six animals each, one control group and a treated group that received a single oral dose (2000 mg/kg, b.w.) of ethanolic crude extract. After the administration of extract, food was withheld for further 3 to 4 hours. Animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours with special attention during the first 4 hours and daily thereafter for a period of 14 days for the symptoms of toxicity and death.

Acute Dermal Toxicity

The acute dermal toxicity test was carried out to determine the therapeutic dose of ethanolic crude extracts. The Organization for Economic Cooperation and Development (OECD) guidelines No. 402 (1987) was followed for this study^[11]. Twenty percent concentration of the extract was applied on the shaved back of 6 mice. Observation was done daily for 14 days for severe signs of distress, pain and death.

Wound Healing Activity

The animals were grouped (6 animals per group) into standard, control, and four groups for testing wound healing activity of ethanolic crude extract. The control group was treated with sterile water and the standard was treated with 10% Betadine povidone iodine solution. The test groups were treated with 5%, 10%, 15% and 20% (w/v) of extract respectively. These concentrations of the extract were used in the succeeding studies.

Excision Wound Healing Activity

The mice were anesthetized by administering pentobarbital (50-60 mg/kg b.w., i.p.) A full thickness of excision wound of circular area (approx.

78.5 mm² and 1 mm depth) was inflicted on the shaved back of the mice 30 minutes after pentobarbital injection. The wounding day was considered as day zero. The extract was topically applied on the wound till it was completely healed. The wounds were monitored and the area was measured on 4, 8, 12, 16 post-wounding days and the mean percent wound closure was computed^[12].

Incision Wound Healing Activity

The mice were anesthetized as in the above after which, incision wounds of about 2 cm in length and 1 mm depth were inflicted using a sterile scalpel on the shaved back of the mice 30 minutes after pentobarbital injection. The wound was stitched at 0.5 cm intervals using sterile 75 cm black silk suture and ½ circle curve cutting no. 3/0 needle. The serrated stitch on both wound edges was tightened for good closure of the wounds. The extract was topically applied on the wound for 10 days. The wounding day was considered as day zero. When wounds were cured thoroughly (about 8 days after treatment), the sutures were removed and the tensile strength of the skin was measured using a tensiometer on the 10th day^[12].

After conducting the experiment, all test animals used were euthanized by overdosing them with potassium chloride and were buried afterwards. All the procedures done and handling of the test animals were approved by the Institutional Animal Ethics Committee (approval No. 04 S-2013).

Statistical Analysis

The determination of the most effective concentration of the crude extract was done by subjecting the results of biological tests to statistical analysis. A one-way analysis of variance was utilized to determine the most effective banana extract concentration.

T-test was done to determine if there is significant difference between the most effective extract concentration and 10.0% Betadine povidone iodine standard.

RESULTS AND DISCUSSION

Phytochemical Tests

The extract obtained was dark brown, ground and fibrous sample. Saponins, tannins, glycosides, sterols and triterpenes were the active constituents present. An Attenuated Total Reflectance Fourier Transform infrared spectroscopy (FTIR) was done on the *Musa sapientum* ('Saba') extract using ABB MB3000 spectrophotometer. The FTIR reflectance spectrum of 'Saba' is shown in Figure 1. The peaks found in the

spectrum indicated the bonds present and its functional group. The broad band region centered at 3286 cm⁻¹ can be assigned to hydrogen bonding attributed to the alcohol group. The band in the region of 2924 and 2854 cm⁻¹ can be assigned C-H stretching of alkenes. The band in the region of 1704 and 1635 cm⁻¹ can be assigned to the C=O stretching for carboxylic acid groups. Betadine is composed of Povidone Iodine, Glycerine (alcohol group), Citric acid (carboxylic acid group), Sodium Phosphate and Sulphate (ammonium salt). The bands located in the region of 3286 cm⁻¹; 1704 and 1635 cm⁻¹ indicate presence of alcohol group and carboxylic acid group in the 'Saba' extract. This makes the 'saba' extract a viable wound healing substance since the composition of that of the leading antiseptic brand Betadine includes these functional groups.

Toxicity Study

None of the 6 male Swiss mice died 14 days after oral dose administration of the ethanolic crude extract. The same result was obtained after the applying 20% w/v of the ethanolic crude extract to the shaved back of the mice.

Excision Wound Study

The healing activity of banana flower ethanolic extract on excised wound in mice is presented in **table 1**. Significant difference is observed when the wound is treated with the extract. Significantly, higher wound contraction is obtained when ethanolic extract was applied on the wound than the control. At ≥ 10% ethanolic extract, a significantly higher reduction in wound size is observed than betadine suggesting that at these concentrations, the banana flower ethanolic extract is more effective in healing wound than the standard chemical.

Incision Wound Study

Table 2 summarizes the effect of banana ethanolic extract on the tensile strength of healed incised wound. The table shows that tensile strength of the wounded skin of mice is significantly higher on ethanolic extract treated mice than the control. This suggests that ethanolic extract is more effective in improving skin integrity of mice. Significantly, tensile strength is better at ≥ 15% ethanolic extract than the standard chemical betadine.

Results of the study show that the banana ethanolic extract has wound healing potential. The effect is comparable with that of betadine. However, at 15% ethanolic extract concentration, the extract is significantly more effective in wound healing and improving tensile strength of wounded skin (**Table 3**).

CONCLUSION

Based on the results of the study, banana flower ethanolic extract has a potential for use in healing wound. There is a need to purify the extract and

identify which among the constituents identified in phytochemical analysis are responsible for the wound healing property of the extract. The extract can be used as an alternative compound in wound healing.

Figure 1. FTIR Spectra of the banana ethanolic crude extract

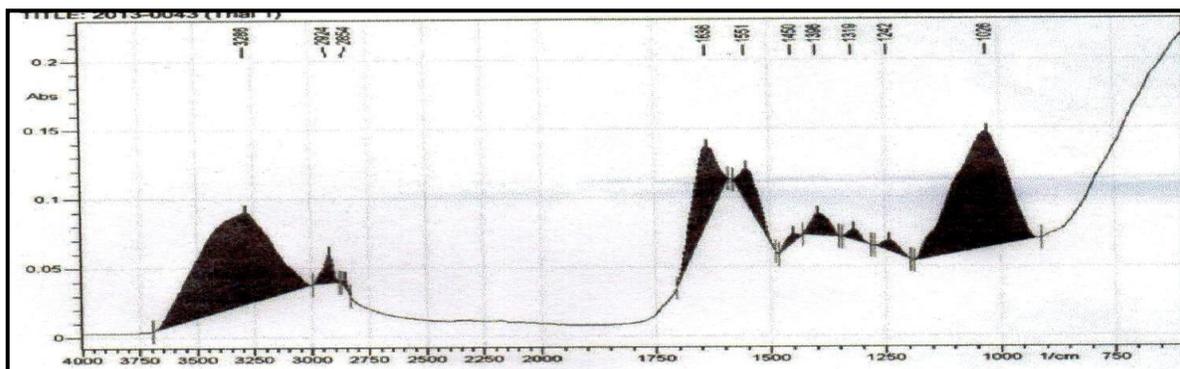


Table 1. Effect of banana flower ethanolic extract on healing of excised wound in mice

Treatment (%)	Percent wound healing contraction (mm ²) ^a
Betadine	71.08 a
0	50.00 b
5	81.16 a c
10	92.63 c d
15	98.51 d
20	99.84 d

^aBased on the mean of 6 replicates. Means followed by the same letter are not significantly different at 5% level of significance. Wound healing contraction was measured 16 days after wound infliction.

Table 2. Effect of banana flower ethanolic extract on tensile strength of healed incised wound

Treatment (%)	Tensile strength at 10 post wounding day (g/mm ²) ^a
Betadine	4.54 a
0	2.73 b
5	4.33 a c
10	4.74 a c d
15	5.27 d
20	5.90 e

^aBased on the mean of 6 replicates. Means followed by the same letter are not significantly different at 5% level of significance.

Table 3. Comparison of the wound healing activity between 15% banana flower ethanolic extract and betadine (standard)

Ethanolic extract effect	T-value ^a
Wound contraction	9.20*
Tensile strength	3.35*

^aTabular t-value (0.05) = 2.22

* Significant at 5% level of significance

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