Mayana (*Coleus blumei*) Leaves Ointment in Wound Healing of Albino Rats (*Rattus albus*)

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***Abstract*—*Coleus blumei* has been known for its ornamental and potential medicinal uses. The colorful leaves are used for ornamental purposes. In a larger scale it also used for landscaping. There are studies that it can be used as antimicrobial agent but its wound healing potential is not yet explored. In this study, mayana leaves were air dried and turned to ointment form. The final product was applied to inflicted wounds of ten Albino rats which are assigned in the treatment group (treatment 2).Twenty Albino rats served as experimental animals. They are randomly assigned in two groups. The first group, or treatment 1, (10 animals) as the control. Inflicted wounds of Albino rats in treatment 1 was applied by the usual antiseptic (Betadine). The second group, treatment 2 served as the experimental animals where the ointment from mayana was applied. Initial wound size showed no significant result. The final wound size in millimeter after fifteen days showed significant result. The result revealed that the leaves of mayana has wound healing property in Albino Rats. The result of this study attested that the leaves of mayana can be utilized as a source of herbal plant, specifically to heal wounds.**

***Index Terms*— *Coleus, blumei,* wound, healing**

I. Introduction

The Philippines was endowed with indigenous herbal plants. Many of these plants have not yet been explored and some are neglected and remain underutilized *Coleus* is a genus of perennial plants native to tropical Africa, Asia, Australia and the Pacific Islands. It is composed of about 150 species under the Mint family of plants (*Lamiaceae*) and closely related to spearmint, peppermint, basil, thyme, oregano and salvia. *Coleus* is an erect and branched annual herb that grows to about 100 cm in height. Coleus stems are square while the leaves are blotched with toothed margins. Coleus leaves are ovate in shape about 5 to 10 cm in length. Coleus flowers bloom on terminal spiked stalks that are purplish, white, or bluish in color. Flowers are numerous, in simple or branched inflorescences, 15-30 cm long [1]. The coleus foliage is remarkably unique, exhibiting arrays of color combinations unmatched by any other plant species. Coleus has an aroma similar to camphor and extremely fragrant due to large amount of essential oils present in the plant. Unlike most of the mint family plants, coleus fibrous roots are used for medicinal purposes [2]. Medical Health Guide of 2011 reported that Mayana is used for immediate treatment of wounds, swelling, bruises, sprains, and cysts. Coleus use as medicine has ancient origins used in Ayurveda (Indian traditional medicine) with verifiable texts mentioned from ancient Sanskrit writings where it is used as a health tonic, treatment for heart problems, chest pains, lung ailments, digestive disorder and sleeplessness (insomnia). In the Philippines Mayana is grown for landscaping and ornamental purposes. Few studies was conducted on its medicinal use, hence this study.

This study determines the wound healing property of the mayana leaves, in terms of size of the wound until full epithelialization and the progress of the wound until final healing and contraction

II. Methodology

A. Materials

1) Experimental animals

Twenty Wistar Albino rats were used and were assigned in two groups. First group (10 rats-control), no ointment of *mayana leaves* in their wounds. Second group (10 rats-experimental group), with mayana leaves ointment in their wounds. Rat cages with water dispenser, rodent feeds, purified water, and animal house with proper temperature was provided.

2) Preparation of mayana leaves ointment

In the preparation of mayana leaves ointment, the following materials were used; Mayana leaves, canola oil, bees wax, digital balance, beaker. electric stove, saucepan, spatula, strainer, cheesecloth and ointment container.

3) For wound infliction.

For wound infliction, the following materials were used: Albino rats, laboratory gown, gloves, masks and surgical cap, holding tray, clean cloth, Ketamine Hydochloride (General Anesthesia), lidocaine (local

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anesthesia) shaver, surgical scissors, Betadine, forceps, syringe, alcohol and cotton balls.

B. Methods

1) Acclimatization of experimental animals

This study employed experimental research method, using twenty Wistar Albino rats, with an average weight of 120 grams. The rats were acclimatized for two weeks in an animal house. They were house individually, in standard cages for an acclimatization period of seven days or one week before the commencement of experiment. During this period the animals had free access to standard pellet diet and water in *adlibitum* in an ambient temperature of (24±2 ℃) and standard laboratory condition [3]. They were housed according to the experimental lay-out as shown in Table I.

TABLE I. EXPERIMENTAL LAYOUT

T1-control	T2- experimental
T1S1	T2S1
T1S2	T2S2
T1S3	T2S3
T1S4	T2S4
T1S5	T2S5
T1S6	T2S6
T1S7	T2S7
T1S8	T2S8
T1S9	T2S9
T1S10	T2S10

2) Preparation of mayana leaves ointment

Mayana leaves were collected locally. Dirt was removed from the leaves by rinsing with clean water. The leaves were air-dried for five (5) days (without direct sunlight exposure) and cut into 1-inch pieces. Using a beaker and digital balance the air-dried leaves and oil were measured. Fourteen (14) grams of dried mayana leaves and four hundred fourteen (414) canola oil were mixed. Following double boiler method of heating, the mixture was heated over a low heat for 45 minutes to one (1) hour until the oil changed its color. The oil was strained using cheesecloth and the infused oil was heated again with the bees wax until melted as shown in Figures 1a, and 1b. The final product (ointment) was transferred in a glass storing bottle while still warm [4] as shown in Figure 1c.

Figure 1 a. Boiling of *Mayana leaves* and oil

Figure 1 b. Strained M*ayana leaves* and oil mixture

Figure 1 c. Ointment in glass bottle for storage

3) Wound infliction

Each Albino rat was anesthesized with ketamine hydrochloride intraperitoneal (60mg/kg) before wound infliction (see Figure 2a.). This was done with closed supervision of a licensed veterinarian. The dorsal skin was shaved and cleaned with Betadine prior to the experiment (see Figure 2b.).To assure of equal size of the wound per animal and to restrain the animal during wound infliction Lidocaine (.05 ml/rat) was administered subcutaneously prior to wound infliction at the dorsal thoracic region (See Figure 2c.). Wound size was measured before infliction using plastic ruler and erasable marker (see Figure 2d.).The wound was made on the back, dorsal thoracic region using a surgical scissors. An open full-thickness wound that was 15 millimeters long was cut up to the level of subcutaneous adipose tissue by means of surgical blade and surgical scissors [3] as shown in Figure 2e. After the wounding process, each Albino rats was caged individually and wrapped with sterilized cloth to avoid hypothermia due to anesthesia.

All wounds were left uncovered in treatment 1. Treatment Group 2 (experimental) was treated with prepared mayana leaves ointment daily at a dose of.15 mg/animal using sterile cotton tipped applicator that lasted for 1 minute [5].

Figure 2 a. Intraperitoneal administration of Ketamine hydrochloride

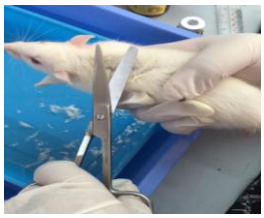


Figure 2 b. Shaving and cleaning prior to wound infliction

Figure 2 c. Lidocaine administration (subcut) at the dorsal thoracic region

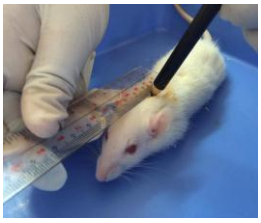


Figure 2 d. Measurement of the dorsal thoracic region before wound infliction

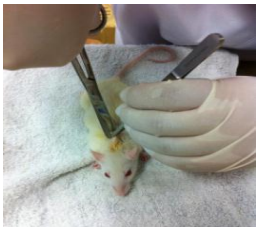


Figure 2 e. Wound infliction

4) Data gathered and statistical analysis

Initial wound size in millimeter was measured before the start of the study and the final wound size after 13 days when the wound already healed using a caliper. Likewise the difference in initial and final size of the wound in experimental group (Treatment 2) was computed. The data gathered was statistically evaluated through standard T-test. A statistical analysis was performed using Microsoft Excel, version 2013. A significant difference was achieved when the value of t-statistics is greater than t-critical value. On the other hand the progress of wound healing was observed by taking pictures every five days interval.

III. Results and Discussion

A. Initial Wound Size of the Experimental Animals in Millimeters

Table II shows the initial wound size of the experimental animals. Animals in treatments 1 and 2 had a mean wound size of 15 mm. T-test was used to evaluate the significant difference among the treatment groups and it showed no significant difference on the wound size of the experimental animals, which implies, that the sizes of the wounds of all experimental animals were almost equal on the start of the study to avoid bias on the result.

B. Final Wound Size of the Experimental Animals in Millimeters

Table III displays the wound size of the experimental animals on the 13th day of experimentation. Treatment 1 had a mean wound size of 3.022 mm while Treatment 2 had a mean wound size of 1.219 mm. T-test was used to evaluate the significant difference among the treatment groups and it showed a significant difference on the wound size of the experimental animals. At this point of the experimental period, wounds in treatment 1 are still in healing process while in treatment 2, the wounds were already healed as evident by epithelialized tissues. It further shows that rats in treatment 2 showed more progressive epithelization than treatment 1 animals making mayana ointment effective as a healing agent.

The result of the study can be supported by numerous studies as follows; Del Fierro and Nolasco (2013) reported that traditional healers in Southwest Cebu, Philippines, used mayana for immediate treatment of wounds, swelling, bruises, sprains, and cysts. Rosmarinic acid is one of the main active components of *Coleus blumei* and is known to have numerous health benefit [6]. Rosmarinic acid, a caffeic acid ester and a component of several members of the Lamiaceae family including *Rosmarinus officinalis* [7].

TABLE II. Initial Wound Size in Millimeters

Treatment	Animal number											T-test
	1	2	2	4	5	6	7	8	9	10	Mean	
1 (control)	15	15	15	15	15	15	15	15	15	15	15	T-stat – .65535< T-crit- 1.73 Not Significant
2 (withmayana ointment)	15	15	15	15	15	15	15	15	15	15	15	

TABLE III. Final Wound Size in Millimeters after 13 Days

Treatment	Animal number											T-test
	1	2	2	4	5	6	7	8	9	10	Mean	
1 (control)	0	6.29	2.93	3.85	4.83	6.57	0	3.1	0	2.65	3.22	t-Stat - 1.75 >t-Crit- 1.73
2	0	0	0	3.51	5.98	0	2.7	0	0	0	1.219	Significant

C. Difference in Final Mean Size and Initial Mean Size of Treatment 2 (mm)

Table IV shows the difference in final mean size and initial mean size of Treatment 2. Final wound size had a mean of 1.219 mm., and initial wound size had a mean size of 15 mm. T-test was used to evaluate the significant difference of final and initial wound size and it showed a significant difference of wound size in Treatment 2, which further shows the effective wound healing property of *Mayana leaves* in Albino rats. The results revealed that there is a significant difference between the wound length of the experimental animals before and after the treatment of mayana leaves ointment. Studies showed that qualitative analysis by phytochemical screening of P. scutellarioides (L.) (synonym with *Coleus blumei*) leaves extracts revealed the presence of polyphenols, flavonoids, saponins, and quinones [8]. Flavonoids are wide range of phytochemical with various pharmacological effects

including antioxidant, anti-inflammation, anti-platelet, anti-allergic, cytotoxicity, reduce risk for heart disease or cancer etc [9]. Particularly, mayana contain rosmarinic acid and studies in rats, has been reported that Rosmarinic acid. It displays general anti-oxidant and anti-inflammatory effects, and may protect against various forms of cancers. Additionally, it can be absorbed through the skin when in an ethanol base (typically perillyl alcohol). It can ease hemolysis, the breaking of red blood cells, via C3-convertase inhibition [10].

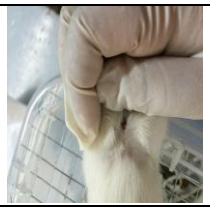
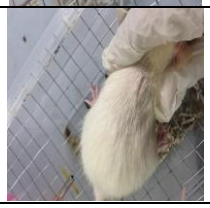
TABLE IV. DIFFERENCE IN FINAL MEAN SIZE AND INITIAL MEAN SIZE OF TREATMENT 2 (MM)

Treatment	Animal number											T-testt
	1	2	2	4	5	6	7	8	9	10	Mean	
2 (initial wound size)	15	15	15	15	15	15	15	15	15	15	15	t-Stat - 20.54 >t-Crit- 1.73 Significant
(final wound size)	0	0	0	3.51	5.98	0	2.7	0	0	0	1.219	

D. *Pictures of Progress of Wound Healing*

Table V shows the progress of wound healing every 5 days interval. Visually, Albino rats in treatment 2 exhibited a higher wound contraction compared to the Albino rats in the control group (treatment 1). Mayana contain plant phenolics, a significant group of secondary metabolites which have diverse medicinal applications. Among various phenolics, rosmarinic acid is an important and has proven for its medicinal properties and well-characterized physiological functions as concluded by Shetty in 2008. Rosmarinic acid, which is reported to have astringent, antibacterial, antiviral and antioxidant activities, is one of the most prominent secondary compounds in *Coleus blumei* (Lamiaceae) [11].

TABLE V. PROGRESS OF WOUND HEALING

Day	Treatment	
	1 (control)	2 with Mayana Leaves ointment)
1		
5		
11		
13		

IV. CONCLUSION AND RECOMMENDATION

Ornamental plants like mayana are commonly known to beautify our surroundings, due to its colorful leaves. Looking through its medicinal value, studies have shown that it has antimicrobial properties, however findings of this study revealed that *mayana leaves* has wound healing property.

Further studies should be explored on the possibilities of using other methods on turning the product into cream or lotion in different concentrations and different types of administration to heal burn and other skin injuries. Its anti-hemorrhagic potential can also be explored.

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