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THE WOUND HEALING AND ANTIMICROBIAL ACTIVITY OF CARABAO HORN (CORNU BUBALI) CREAM

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Abstract

The study was conducted to alleviate the problems in slaughterhouse wastes and promote the utilization of alternative cures for wounds in the absence of commercial ones. The present investigation reveals the wound-healing and antibacterial nature of carabao horn and these result justifies the folkloric claim of the community. The study aimed to determine if there was a significant difference between the four treatments in terms of wound size and determine its antimcorbial activity. The study was conducted to establish the scientific basis for the utilization of carabao horn as wound medicine. There were four treatments of the study and were tested using 12 male albino mice, comprising 3 mice per group. The experimented albino mice were firstly acclimatized to laboratory conditions for 5 days before the start of the experiment. The treatments were topically applied to the wound of the mice until healed. Results of the study revealed that Treatment 1 has the highest percentage of wound closure (HPWC) 83% higher than the positive control, erythromycin with 64% HPWC. The wound sizes in the animals treated with the positive control, erythromycin showed by the 17th day, indicating that treatment 1, at that given concentration, had a better wound healing property than the positive control. Result of the antimicrobial activity of the carabao horn cream showed that the cream was partially active against *Staphyloccus aureus* and *Escherichia coli* and inhibited greater ZOI (zone of inhibition) than the positive control. The antimicrobial property of the carabao horn investigated aids in the wound healing promotion of the sample excuted among the test specimens. The study further suggests looking into the Physico-chemical properties and other microbiological activities of the carabao horn.

Discipline (s): Natural Product, Waste Utilization, and Wound-Healing Activity

1. INTRODUCTION

1.1 Rationale

Herbs and horns are increasingly regarded as substitutes for pharmaceuticals because of their medicinal properties that can treat such illnesses, due to the growth of traditional medicine. According to the Philippine Traditional Medicine Journal, the government has recognized the value of traditional medicine in people's critical health care. Herbs were beneficial as a traditional treatment for a variety of diseases. Herbal plants are being employed as the foundation for cosmetics, supplemental vitamins, and substitutes for processed medicinal drugs whenever the need arises. This indicates that the value of herbs is critical in the production of medicines and vitamins.

Horn is well-known in the Philippines and abroad for its technical and technological use. Amulets, beads, bracelets, knife handles, tuning pins for string instruments, and other things fashioned of horn are described in "the technology of skeletal materials since the Roman period." Due to its odourlessness, the horn has also been used for snuff bottles and tops of snuff bottles made of other materials.

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Water Buffalo is the largest member of the Bovini tribe and a very valuable domestic animal. It is a significant animal in our life since it provides us with milk that is higher in fat than cow's milk. Leather is made from its skin, while buttons are made from its horns. It can also be used to transport humans. Buffalo is beneficial both during and after its death.

In the Northern Philippines, traditional "quack" doctors utilized horns as medicine to extract the toxins from snake and dog bites. As disclosed in the Chinese Traditional Medicines, the Chinese have found medicinal uses for buffalo horns. The horns are halved, soaked in hot water, scooped out, sliced, dried in the sun, scratched, and pounded into a fine powder to be used as medicine.

According to the study of Rui Liu et al Am J Chin Med., 2010, titled "Antipyretic and antioxidant activities of the aqueous extract of *Cornu bubali* (water buffalo horn)". The researchers stated that the results suggest that *Cornu Bubali* exhibits antipyretic activity in both infectious and noninfectious hyperthermia. The antipyretic activity of WBH may be due to the effects on enhancing antioxidation enzyme activities, decreasing dinoprostone (PGE2) production, and protecting the rCMECs against hydrogen peroxide-induced injury.

The Natural Products Journal claimed that the horn of the saiga antelope is often used in Traditional Chinese medicine (TCM). It is a famous and valuable musk, pilose antler (Lurong), and rhino, the most-renowned medical material of animal origin used in TCM. However, the saiga has also joined the rhinoceros on the list of endangered species. The main beneficial effects of the horn, which could be described in scientific terms, are mostly antipyretic and anti-ulcerative. Despite some indication of its efficacy, no specific compound from the saiga horn has been isolated or scientifically described. It can be assumed that the amino acid composition of the horns of the various animals: the buffalo, the saiga, the ox, and the goats are almost the same in terms of nature and composition when they only differ in their ratios.

The similarity of amino acid and peptide compounds of these horns has confirmed the similarity of anti-inflammatory and anti ulcerative physiological actions. Thus, we can interchange the ability of the horns.

In countries like China and Vietnam, rhino horn has long been utilized as a traditional medicinal component. Traditional Chinese medicine has used rhino horn in combination with other natural components to treat fevers and relieve the symptoms of arthritis and gout in the past. Headaches, hallucinations, high blood pressure, typhoid, snakebite, food poisoning, and even possession by spirits are among the historical applications. According to Lixin Huang, president of the American College of Traditional Chinese Medicine, "every historical documented usage of rhino horn in traditional Chinese medicine was for treating illnesses such as fever and infection." In 1990, Hong Kong researchers reported a set of tests in which they found water buffalo horn to be just as effective at reducing fever in mice at large doses.

1.2 State of the art

In Kalinga and Apayao, butchers tend to throw the horns of the carabao, goats, and so on after they slaughter these animals. Instead of throwing, the horns can be used as a traditional wound

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healing medicine. Also, this connects that people don't know that horns can be used as an alternative treatment for wounds. However, there's no scientific basis but it was practiced or established by the elders in Kalinga and Apayao. (Carbonel, et.al, 2013)

1.3 Analysis of the Problem

Although carabao horn have long been used for therapeutic purposes, there are few published studies on its utilization in medicine. Considering its value and availability, this prompts the proponent to establish the wound-healing and antimicrobial activity of Carabao Horn.

A. Significance of the Study

The traditional practices of Yapayaos and Ykalingas have included the utilization of carabao cream in wound healing, however, there is no scientific data on the use of this horn in wound healing and antimicrobial activity. The success of the study would help alleviate the problems in slaughterhouse wastes and promote the utilization of alternative cures for the wound in the absence of commercial one. The study could establish as well the scientific basis of utiliozation of carabao horn as traditional medicine.

B. Scope and Delimitation of the Study

The carabao horn came from Luna, Apayao, and Tabuk City, Kalinga and were collected from one of its slaughterhouses. All necessary procedures for sample preparation were conducted at Kalinga State University. This study focused on converting processed carabao horn into a topical cream. Its wound-healing activity was determined using experimental procedures similar to the study of S. Magewari, et. Al in 2015. The Antimicrobial activity was conducted at Reshional Standards and Testing Laboratory of Department of Sceince and Technology, Region 2, Tuguegarao City, Cagayan. The researchers used the outer part of the carabao horn as the active component of the cream. Wound healing activity procedure and cream preparation were conducted and approved by the Philippine Institute of Traditional and Alternative Health Care (PITAHC) -Cagayan Valley Herbal Processing plant, Tuguegarao City, Cagayan. The treatment 2 was subjected for the antimicrobial activity.

2. REVIEW OF LITERATURE

As indicated by the latest projections in product creation for human consumption, the value of traditional medicines in people's lives is now being carried in the industry mission. The majority of new product development is now centered on combinations of natural flavors derived from fruits and/or herbs. This is a hint that it is time to consider the benefits of using the traditional way of curing such ailments because being traditional is proven good and effective.

Traditional Medicines are now being promoted and accepted as alternative medications for some illnesses, especially in the countryside. In the Philippines, it has been a practice among different ethnics like the Ilocanos in the Cordillera who are among those who tend to doctor themselves whenever they are in times of crisis. They believed that the traditional way of medication could also be an effective remedy to prevent or cure such illnesses due to the fact

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that the use of herbs and other source of medicine like the parts of animals such as the snake bile, bones, and horns have Traditional medicines are gradually being promoted and acknowledged as an alternative treatment for certain disorders, particularly in rural areas. It has been a practice among various ethnic groups in the Philippines, such as the Ilocanos in the Cordillera, who are among those who tend to self-medicate whenever they are in a crisis. They believed that traditional medicine, which includes the use of herbs and other sources of medicine such as snake bile, bones, and horns, may be a useful approach to prevent or cure such conditions. Horn was utilized by the Ilocanos in Kalinga and Apayao to treat skin ailments and wounds.

The horn may be used to detect poisoned beverages, creating bubbles in the presence of some poisons, according to Greek and European tradition from the 5th through the 19th centuries.

The keratin contents of the horn combats fatigue and skin regeneration. Its amino acids serve as building blocks for the skin. It also contains trace elememts such as potassium which soothes skin irritation. The carabao horn is used to eliminate toxic substances from poisoning, it is used to lower body temperature during convulsive period, and used as anti-inflammatory, anti-bleeding such as nose-bleeding and delirium.

Due to its high quality protein content, horns have a variety of purposes; for example, if it is in decoct form, the usage of the horns can clear heat from the body and blood, as well as eliminate poisonous compounds from patients, based from Medical Records of Famous Physicians, 2004. It can also be used to treat a case of blood vomiting or a nosebleed. It has convulsion-relieving, anti-inflammatory, anti-infection, bleeding-time shortening, and can also treat a case of high temperature and coma with delirium.

Furthermore, according to the information found in the Medical book, the buffalo horn contains four key components: cholesterol, peptides, proteins, and amino acids. Amino acids are the body's "building blocks." When protein is digested, it is broken down into 22 different amino acids. The remaining eight are non-essential (cannot be manufactured by the body) and eight are essential (cannot be manufactured by the body) (can be manufactured by the body with proper nutrition). Non-essentials should occasionally be supplied to ensure the best available supply.

Aside from forming antibodies to fight invading germs and viruses, amino acids are involved in the enzyme and hormone systems, they make nucleoproteins (RNA and DNA), they transport oxygen throughout the body, and they are involved in all muscular movement.

For thousands of years, *Cornu bubali* (water buffalo horn, WBH) has been utilized in Traditional Chinese Medicine (TCM). From the study of Rui Liu, Jin-ao Duan, Hao Wu, Pei Liu, Er-xin Shang, Da-wei Qian ,three peptides with antioxidant properties were purified from aqueous extract of *Cornu bubali* by consecutive chromatographic methods including gel filtration chromatography, ion-exchange chromatography, and high performance liquid chromatography.

The sequences of the three peptides were identified to be Gln-Tyr-Asp-Gln-Gly-Val(WBH-

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1,708Da), Tyr-Glu-Asp-Cys-Thr-Asp-Cys-Gly-Asn (WBH-2, 1018Da) and Ala-Ala-Asp-Asn-Ala-Asn-Glu-Leu-Phe-Pro-Pro-Asn (WBH-3, 1271Da) by matrix assisted laser desorption ionization time-of-flight/time-of-flight mass spectrometry (MALDI-LIFT-TOF/TOF MS). These peptides were found to diminish the DPPH radical and protect rat cerebral microvascular endothelial cells (rCMECs) from H (2) O (2)-induced damage, suggesting that they have antioxidant action. These findings imply that WBH-1, WBH-2, and WBH-3, obtained from water buffalo horn aqueous extract, are natural antioxidants that may contribute to WBH effectiveness.

The study analyzed and identified the water-soluble components of water buffalo horn (*Cornu bubali*, WBH), and also established a method for investigating these components. Shotgun proteomic analysis identified proteins in WBH aqueous extraction: keratin, collagen, desmoglein, etc. (Yao Xue Xue Bao, 2015).

As reported by the study entitled, "Medicinal Use and Legalized Trade of Rhinoceros Horn from the Perspective of Traditional Chinese Medicine Practitioners in Hong Kong", rhino horn is one such illegally traded substance that is consumed for cultural, medical, and social reasons (But, Lung, & Tam, 1990; Gao, Stoner, Lee, & Clark, 2016; Milliken & Shaw, 2012; Truong, Dang, & Hall, 2016). Rhino horn is traditionally used to dissipate heat and cleanse impurities imprisoned deep within the body (But et al., 1990), but its use has expanded in recent years to encompass cancer treatment, hangover cures, and social consumption (Milliken & Shaw, 2012; Truong et al., 2016).

The inquirer in the study surveyed 15 interviewees that were between the age of 41 and 78 years with 3 to 45 years of clinical experience. During their official TCM study, ten interviewees learned about rhino horn. Seven had their own practices, while the other eight worked for companies that provided TCM services. All of the 15 interviewees agreed that rhino horn had medicinal characteristics that are relevant to TCM, with seven emphasizing that it should only be used to treat serious ailments.

Although it was collectively decided that it should be used in decoctions with other medicinal substances, only seven interviewees acknowledged to independent intake.

TCM practitioners were mainly in agreement that rhino horn is used to dissipate heat, detoxify blood, and treat wen bing (warm disease), but they were divided on other claims, such as its potential to treat cancer. Only six of the interviewees were aware that rhino horns continue to grow throughout their lives. Eight interviewers were unable to identify its chemical composition; six named keratin as a component, and four named different minerals. Only two interviewers were able to identify the active substance, both of whom mentioned minerals.

The study, conducted by the Chinese University of Hong Kong, discovered that rhino horn, as well as the horns of the saiga antelope, water buffalo, and ordinary cow, lowered fever in rats given a high dose of the substance (But et al., 1990). Rhino and saiga horn were remained effective at lowering temperatures at modest doses. Because rhino horn is rarely given as a single substance in treatment (Patton, 2011), the researcher looked for study on rhino horn used alone as well as in traditional medicine prescriptions, which are often decoctions comprising

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herbs and other substances. In December 2019, a literature search was undertaken in online databases such as Scopus, Web of Science, and Google Scholar. Using terms like "rhino horn" and "xi jiao" as keywords (which means rhino horn in Mandarine). Liu, Wang, et al., (2016), compared the antipyretic, sedative, and procoagulant effects of seven different species of animal horn.

Nhat Linh Dao (2014), rhino horn is a type of drug that can be used in conjunction with other medical materials to lower high fevers, improve blood quality, treat haemorrhage, convulsions, and coma, and improve sexual performance and detoxification. Rhinos are now listed as an endangered species in the Red Book, and hunting them is prohibited. Because using rhino horn as a treatment is no longer possible, buffalo horn is used instead.

Buffalo horn has been used in the countryside for thousands of years to treat headaches caused by weather changes, high temperature, and detoxification. Many current medical studies have demonstrated the health benefits of buffalo horns. Many studies in Shenghai, Beijing, and other Chinese cities have found that both rhino and buffalo horns contain 17acid amines, and their organic and inorganic compositions are nearly identical.

According to clinical research findings in 3270 patients at 50 research centers in Beijing, Shenghai, Tianjin, and Guangzhou, scientists concluded that using buffalo horn and rhino horn has similar effects for 30 diseases such as epidemic encephalitis, high fever, thrombocytopenia-related haemorrhage, schizophrenia, and other conditions. Furthermore, buffalo horn has no negative effects; although, in rare situations, nausea, stomach distension, and other mild digestive issues may occur. Buffalo horn has some health benefits, according to Quach Lan Trung's "modern Chinese practical medicine," such as lowering blood pressure and heart rate, reducing leucocyte quantity, reducing blood clotting time, preventing the development of colibacillus, beta hemolytic streptococcus, and infection, lowering convulsion intensity and death rate in laboratory animals, and lowering cholesterol in blood.

Conforming to Emily Pierce (2019), water buffalo horns are high in protein and low in fat. An individual’s body needs protein to help build and repair muscle, skin, and other body tissues. Protein also helps fight infection, balance body fluids, and carry oxygen through the body (Healthwise Staff, 2019).

History of Wound Healing

The history of wound healing is the history of humankind. A clay tablet from 2200 B.C. is one of the oldest medical writings known to man. The "three healing gestures"—washing the wounds, preparing plasters, and bandaging the wounds—are described for the first time on this tablet.1, 2, 3.

Plasters, as used by the ancients and early moderns, are today's equivalent of wound dressings. These plasters were made up of a variety of ingredients such as mud or clay, plants, and herbs. Plasters were used to cover wounds while also absorbing exudate. Oil is a frequent ingredient in plasters; it may have given some protection from infection because bacteria do not grow well in oil, and it would have prevented the bandage from sticking to the wound as a nonadherent

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dressing. Because beer was the first recognized wound treatment product, the Sumerians made at least 19 different types of beer. Aside from being the first one to use adhesive bandages, the Egyptians were also the first to apply honey to wounds and to paint them with green paint, which signifies life and includes copper, which is harmful to bacteria. The Greeks recommended that the wound be washed with clean water that has been boiled first, vinegar (acetic acid), and wine. Surgery was not recognized as a distinct and respectable area of medicine until the 18th century. The antiseptic procedure was a great breakthrough in the nineteenth century. Antibiotics were introduced to help control infections and reduce mortality. Modern wound healing emerged in the 20th century, and there are now over 5,000 wound care products available. The majority of current dressings contain extremely absorbent components like alginates, foam, or carboxymethylcellulose. There are two types of dressings: occlusive and semi occlusive. Growth factors, sophisticated honey-based dressings, and hypochlorous acid–based cleansers are some of the options. Bioengineered tissue, negative pressure therapy, and hyperbaric oxygen therapy have revolutionized the treatment of chronic wounds.

The first wound treatments will be described 5 millennia ago. Various wound-care principles have been passed down from generation to generation since then. Progress beyond historical wound care procedures is a new event, in comparison to a significant number of general technology innovations over the last 100 years. To maintain this progress and provide future direction, it is critical to understand the historical aspects of wound therapy (both successes and failures).

Antibiotics provide the main basis for the therapy of bacterial infections. However, the high genetic variability of bacteria enables them to rapidly evade the action of antibiotics by developing antibiotic resistance. Thus there has been a continuing search for new and more potent antibiotics. Due to the cost effectiveness, safety, increasing failure of chemotherapy and antibiotic resistance exhibited by pathogenic microbial agents; search for plant products has increased for their potential antimicrobial activity.

3. RESEARCH PARADIGM

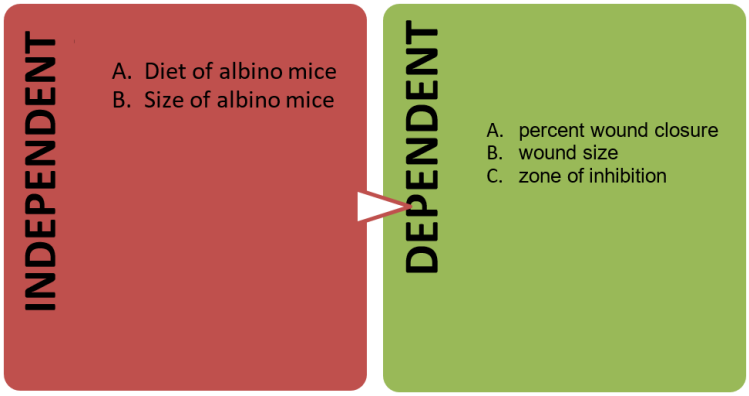

Figure 1: Research Paradigm

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The study used the above research paradigm to scientifically established the wound healing and antimicrobial activity of carabao horn. The size and diet of the albino mice were the independent variables while the percent wound closure (PWC), wound size and the zone of inhibition depend on the different treatments, which contains the different concentration of carabao Horn including the positive control.

4. OBJECTIVES

Generally, this study aimed to produce alternative healing for wounds. Specifically, it sought to answer the following questions:

1. What is the percent wound closure per treatment?
2. Is there a significant difference between the percent wound closure of the four treatments?
3. What is the antimicrobial activity of carabao horn cream against *Staphyloccous aureus and Escherichia coli?*

5. MATERIALS AND METHODS

5.1 Locale of the Study

The carabao horn was collected from Tabuk City, Kalinga, and Luna, Apayao slaughterhouses. All necessary procedures for sample preparation were conducted at Kalinga State University. This study focused on converting processed carabao horn into a topical cream. Its wound-healing activity was determined using experimental procedures similar to the study of S. Magewari, et. Al in 2015. The researchers used the outer part of the carabao horn as the active component of the cream. The Antimicrobial activity was conducted at Regional Standards and Testing Laboratory of Department of Sceince and Technology, Region 2, Tuguegarao City, Cagayan. Wound healing activity procedure and cream preparation were conducted and approved by the Philippine Institute of Traditional and Alternative Health Care (PITAHC) - Cagayan Valley Herbal Processing plant, Tuguegarao City, Cagayan. AM

5.2 Research Design

The researchers used the experimental research design. Healthy albino mice of either sex of four months approximately weighing 25 to 40gms were used as experimental animals. No prior drug treatment was employed. The animals were housed in polypropylene cages under standard environmental conditions of temperature (25±3°C) with relative humidity (63±2%) under 12h light/dark cycles. Standard laboratory animal feed and water were fed with ad libitum. The animals were allowed to acclimatize to laboratory conditions for 5 days before starting the experiment. The experimental protocols and procedures were approved by the Bureau of Animal Industry. The study was carried out in Cagayan Valley Herbal Processing Plant, Tuguegarao City after getting the approval from Bureau of Animal Industry.

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5.3 Data Gathering

To come up with the product, powdered carabao horn, Beeswax, virgin coconut oil (VCO), and Sunflower Oil were used. The ratio of the raw materials used is described in table 1 for the different treatments. The wound healing activity was conducted using the following materials: 12 male Sprague Dawley Albino Mice bought and verified at the CVHPP, Local Anesthesia (bought and used at the CVHPP as well), and 2.5 cm x 7 cm x 7 cm container, Laboratory Scissors, and Kelly Forceps. In addition, the following materials were utilized as well; 3 plastic containers, a Laboratory blender/Grinder, Strainer, an Analytical balance, a 15ml beaker, Dropper, Laboratory thermometers, Masking tape, and Blade. The drugs were topically applied daily until the formation of complete epithelial layer, starting from the first day of wound excision.

Table 1: Documented wound sizes in mm of test subjects

Treatment	Days of repeated wound size measurements in mm														
	29(April)			5 (May)			9 (May)			12 (May)			16 (May)		
1	21	21	30	8	8	8	5	5	4	2	3	2	1	1	1
2	17	21	18	8	8	9	6	6	6	2	4	2	1	2	1
3	21	24	24	8	8	10	7	6	9	4	4	4	1	1	1
4	18	18	20	7	7	8	5	4	5	1	1	1	0	0	0

Table 1 showed the decrease in wound size of the albino mice.

Legend:

Treatment 1 (T1) - Pure powdered carabao horn

Treatment 2 (T2) - 5g Powdered carabao horn cream

Treatment 3 (T3) - 2.5g Powdered carabao horn cream

Treatment 4 (T4) - Erythromycin cream (positive control)

All the animals were monitored daily and observed for any wound fluid, evidence of infection and any other abnormalities. The diameters of the wound were measured immediately by using a ruler. The wound closure was measured at 4-6 days intervals to see the percentage of wound closure and epithelization time that indicated the formation of new epithelial tissue to cover the wound. The number of days required for falling of the scar without any residual of the raw wound gave the period of epithelization.

The Antimicrobial, activity was investigated using the procedure of Guevarra, 2005 and further described below.

5.4 General Procedure

5.4.1 Preparation for the powdered carabao horn product:

The tube-like part of the horn was sharpened and washed with hot water. The horn was properly sun-dried, then scratched with a clean sharp knife or with a cutter to obtain thin layers of the

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horn until enough amount was taken. After which, pound the thin scratched parts of the horn with the mortar and pestle and the grinder until it became a fine powder, and strain it. Then, place the powdered carabao horn in a clean container. When enough powdered horns were produced, measure the needed powdered carabao horn and the other compounds needed.

5.4.2 Preparation of Cream formulation:

Two treatments were used for the carabao horn product cream which was prepared by fusion method. In this method, the constituents of the base were placed together in a rice cooker and allowed to melt together at 70°C. After melting, the ingredients were stirred gently maintaining the temperature of 70°C for about 5 min, and then cooled with continuous stirring to 40°C.

5.4.3 Wound healing Activity

The excision wound healing activity was studied by the method described by Luisa A DiPietro and Farahpour and Habibi. The skin area on the dorsal thoracic region of the mice was removed using a microblade one day before the experiment. The surgical procedures were carried out under sterile conditions. The experimental animals were anesthetized with anesthetic ether. After the successful anesthesia, the mice were fixed in a dorsal posture on a surgery table. Circular, full-thickness surgical wounds with diameters of 17mm-30mm, 1 cm away from the backbone were made using kelly forceps and laboratory scissors. Using this excision wound method, the epidermal, dermal, hypodermal, and panniculus carnosus layers were removed completely. After making surgical wounds, all mice were marked using a non-toxic colour. The animals were divided into the following four groups of three animals each (male) and were treated by the 4 treatments.

The drugs were topically applied daily until the formation of complete epithelial layer, starting from the first day of wound excision.

All the animals were monitored daily and observed for any wound fluid, evidence of infection and any other abnormalities. The diameters of the wound were measured immediately by using a ruler. The wound closure was measured at 4-6 days intervals to see the percentage of wound closure and epithelization time that indicated the formation of new epithelial tissue to cover the wound.

The percentage of wound contraction was determined using the following formula:

$$\text{Percentage of wound contraction} = \frac{(\textbf{Initial day wound size} - \textbf{Specific day wound size})}{\text{Initial day wound size}} \times 100$$

The number of days required for falling of the scar without any residual of the raw wound gave the period of epithelization.

5.4.4. Detection of Antibacterial Activity

The antibacterial activity of the carabao horn cream was evaluated against the reference strain *Staphyloccous aureus*, a gram positive organism and *Escherichia coli* a gram negative organism. The disc diffusion method (Guevara, 2005) was used in the microbial evaluation. Bacterial cultures maintained on nutrient agar slants were taken and aseptically inoculated into

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10 ml of sterile broth. Then broth containing the bacteria were incubated at 37 o C for 24 hours, and designated as the working stocks used for antibacterial studies. Small autoclaved discs about 6 mm diameter size of Whatmann filter paper (No.41) were treated by the carabao horn cream then these saturated paper discs were inoculated equidistantly. These set up were incubated at 37o C for 24 hours. In the whole investigation, paper disc impregnated with 85% ethanol was taken as control. The zone of inhibition (ZOI) around each disc indicative of the sensitivity at that concentration was observed and measured using a sterilized micro caliper. Activity of the extract was compared with the corresponding references (Guevara, 2005) [8]:

ZOI value of <10 mm, maybe expressed as inactive

ZOI value of 10-13 mm, partially active

ZOI value of 14-19 mm, active

ZOI value of 19 mm, very active

5.5 Statistical analysis

Statistical analysis performed to analyze the data was one-way ANOVA followed by the Duncan Multiple Range Test (DMRT) to compare the mean values of each treatment among groups. Significant differences between the means of parameters were determined by using DMRT. All statistical analysis was performed using SPSS Statistical version 18.0 software packages.

6. RESULTS AND DISCUSSION

The following tables showed the results of the treatments of the study.

Table 1 showed the decrease in wound size of the albino mice. There was a progressive decrease in wound area with time, indicating the efficacy of the treatments in healing the induced wounds.

The wound sizes in the animals treated with the positive control, erythromycin showed by the 17th day, indicating that treatment 1, at that given concentration, had a better wound healing property than the positive control.

The table 2 shows the percentage wound closure of the powder (T1), cream (T2 & T3), and positive control (T4). 83 percent for Treatment 1, 59 percent for Treatment 2, 74 percent for Treatment 3, and 64 % for Treatment 4. It also shows that Treatment 1 was the most effective among the other three treatments.

Table 2: Percentage Wound Closure of albino mice using four treatments

	Treatment 1	**Treatment 2**	**Treatment 3**	**Treatment 4**
Initial Area	24	19	23	19
Final Area	4	5	5	3
% Wound closure	**83%**	**59%**	**74%**	**64%**

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The table shows the percentage wound closure of the powder (T1), cream (T2 & T3), and positive control (T4). 83 percent for Treatment 1, 59 percent for Treatment 2, 74 percent for Treatment 3, and 64 % for Treatment 4. It also shows that Treatment 1 was the most effective among the other three treatments.

This study was congruent with the study of Luo, J., Yan, D., Zhang, D., Feng X., Yan, Y., Dong, X., & Xiao, X. (2011, June 12), entitled "Substitutes for endangered medicinal animal horns and shells exposed by antithrombotic and anticoagulation effects". The result showed that, the 12 frequently used medicinal animal horns and shells (MAHS) including the *Cornu bubali* horn. All the MAHS contain keratoprotein, and they were reported with many bioactivities, such as anticoagulation effect (Kim et al., 2004), antithrombotic effect (Kim et al., 2004), anti-fever effect (Hu et al., 2006), and anti-inflammatory effect (Sun, 2004).

Table 3 shows that there were no significant differences among the four treatments of this study. Since the *F critical value* was greater than the *F-value*. Results implied that there is no significant difference among the treatments and the Treatments 1-3 are as effective as the positive control, commercially utilized erythromycin.

Table 3: ANOVA: Single Factor of the Four Treatments

ANOVA							
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***	**Remarks**
Between Groups	13.5	3	4.5	0.030612245	0.991707239	6.591382116	*Not Significant*
Within Groups	588	4	147				
Total	601.5	7					

Table 3, the between-group differences in the four treatments are not statistically significant (p-value > 0.05). The observed F-value is lower than the critical F-value, suggesting that there is no evidence to reject the null hypothesis of no significant difference between the treatments.

Carabao (buffalo) horn has a high keratin concentration, making it useful for treating skin conditions and wounds (L. Carbonel, et.al.2013). It was also noted that the skin regions treated with the formulations had higher initial water absorption values and have been proven to control important biological functions, including cell proliferation and protein synthesis, improved hydration and elasticity after treatment with the samples of keratin peptide as discussed by Britanicca and the group of Barba in 2008.

As gleaned on the table 4, result showed that carabao horn cream is partially active considering the 12 mm ZOI (zone of inhibition) measured. It was greater that the ZOI of the 85% ethanol.

Table 4: Antimicrobial Activity of Carabao Horn against *Staphyloccous Aureus*

Zone of inhibition (mm)	***Staphyloccous Aureus***				
Carabao Horn Cream	**R1**	**R2**	**R3**	**Mean**	+
	13	12	11	12	8

Table 4 presents the results of the antimicrobial activity of Carabao Horn against Staphylococcus aureus. The table shows the zone of inhibition in millimeters (mm) for three replicates (R1, R2, and R3) of Carabao Horn Cream tested against Staphylococcus aureus.

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Gram negative bacteria inhibits a mean of 12 mm when the antimicrobial activity of Carabao horn cream was investigated against *Escherichia coli.* Result of the study have shown that ZOI measured inhibited by the sample was greater than the positive control.

Result of the antimicrobial activity suggested that presence of antimicrobial property of carabao horn aids in the promotion of wound healing. Along with its antimicrobial activity, studies have shown that carabao horn has antipyretic activity that may be due to amino acid metabolism, arachidonic acid, or oxidative stress. (Liu, et.al, 2010 and 2016)

Table 5: Antimicrobial Activity of Carabao Horn against *Escherichia coli*

Zone of inhibition (mm)	***Escherichia coli***				
Carabao Horn Cream	**R1**	**R2**	**R3**	**Mean**	+
	12	13	13	12	8

Table 5 presents the results of the antimicrobial activity of Carabao Horn against Escherichia coli. The table displays the zone of inhibition in millimeters (mm) for three replicates (R1, R2, and R3) of Carabao Horn Cream tested against Escherichia coli.

7. SUMMARY AND CONCLUSION

This study investigated the scientific basis of the utilization of carabao horn as wound healing cream. Its wound-healing activity was determined using experimental procedures similar to the study of S. Magewari, et. Al in 2015. The researchers used the outer part of the carabao horn as the active component of the cream. Four treatments were used to investigate the wound healing activity.

The wound sizes in the animals treated with the positive control, erythromycin showed by the 17th day, indicating that treatment 1, at that given concentration, had a better wound healing property than the positive control.

The result shows the percentage wound closure of the powder (T1), cream (T2 & T3), and positive control (T4). 83 percent for Treatment 1, 59 percent for Treatment 2, 74 percent for Treatment 3, and 64 % for Treatment 4. The result implied that Treatment 1 was the most effective among the other three treatments, better than the positive control, erythromycin. Results further revealed that there are no significant differences among the treatment in terms of wound size and percent wound closure.

The antimicrobial activity of the carabao horn cream revealed that the product is partially active based from the zone of inhibition against *Staphyloccocus aureus and Escherichia coli* and is higher than the positive control.

The antimicrobial activity revealed the antimicrobial property of carabao horn that aid in the cell proliferation and protein synthesis, improved hydration and elasticity thus promoted wound healing.

Moreover, the present investigation reveals the wound-healing and antibacterial nature of carabao horn and these result justifies the folkloric claim of the community.

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8. IMPLICATIONS AND RECOMMENDATIONS

Although carabao horn has been used in traditional medicine to aid in the healing of wounds, there is no scientific evidence to support this practice. Results of the study has revealed that concentrated or powdered carabao horn alone is enough to promote wound healing activity as effective as the commercial one. Success in the project can help address the issues with slaughterhouse wastes and encourage the adoption of alternate wound treatments in the absence of marketable ones. It is further recommended that the carabao horn be subjected to in-depth Physico-chemical analyses and microbiological activities of the carabao horn for future utilization and natural product development. Moreover, studies to isolate and characterize the bioactive constituents of the carabao horn are suggested. It may also be used to search for bioactive lead agents that could be used in the partial synthesis of some useful drugs.

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Above all, to Almighty God for His strength and favors.

Declaration of Interest Statement

The authors have no relevant interest(s) to disclose.

FIGURES

Wound Healing Activity

Figure 2: Feeding of the test animals

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Figure 3: Marking of test animals

Figure 4: Removal of the fur

Figure 5: Injection of anesthetic by Dr. Bas-ong and the keeper

Figure 6: Wound infliction to test animals

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Figure 7: Observation of the researchers to the behaviour and wound size of the test animals

Application of the different treatments to the test animals.

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MAY 17, 2022 PHOTO OF THE ALBINO MICE

T1. 1 (powdered horn)

T2 cream with horn powder)

T3 (cream with horn powder)

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T4 (erythromycin cream)

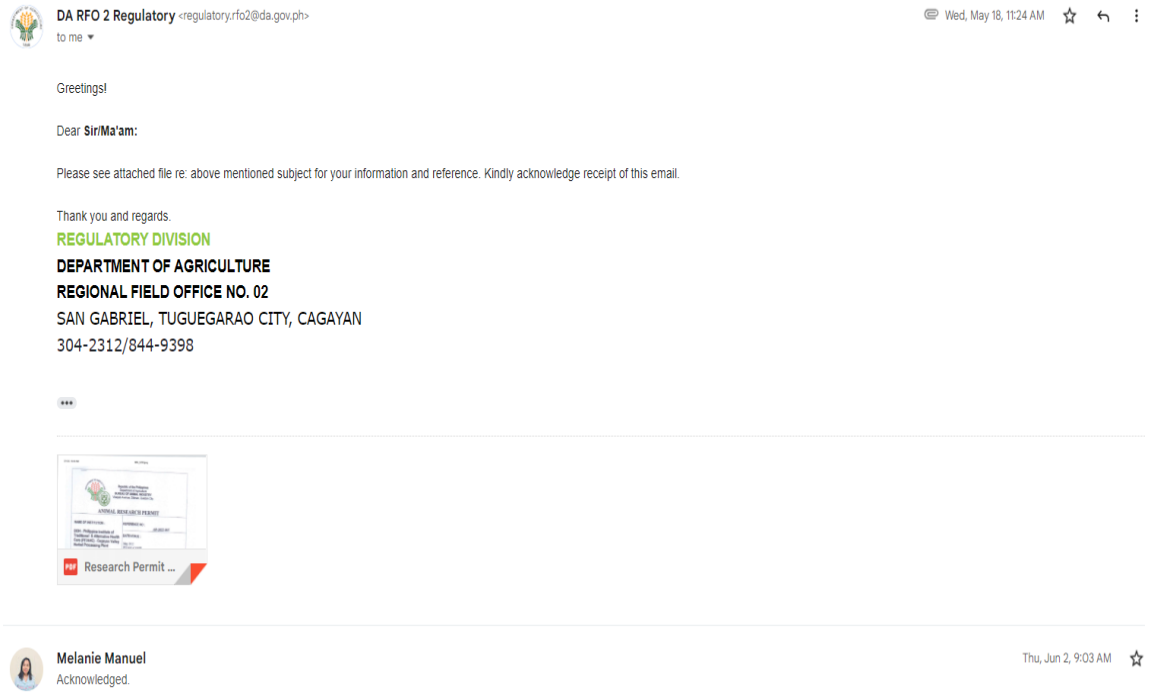

Figure 8: Online Application of Bureau of Animal Industry Permit to conduct the study

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DEPARTMENT OF SCIENCE AND TECHNOLOGY
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ANTIBACTERIAL ANALYSIS
WORKSHEET

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Date Reported : May 03, 2022
Submitted by : Melanie Manuel
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Page : 1 of 1

ANTIBACTERIAL ANALYSIS									
Sample Number	Sample Description	ZONE OF INHIBITION (mm)							
		Staphylococcus aureus				*Escherichia coli*			
		R1	R2	R3	MEAN	R1	R2	R3	MEAN
MIC-0399	Carabao Hand Cream	13	12	11	12	12	13	13	12

Zone of Inhibition	Inferences:
<10 mm	Inactive
10-13 mm	Partially active
14-19 mm	Active
>19 mm	Very active

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