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Assessment of the crude ethanol leaf extracts of *Momordica charantia* Linn., *Moringa oleifera* Lam., *Tabernaemontana pandacaqui* Poir. and *Mollugo oppositifolia* Linn. for its wound healing activity in castrated piglets

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Abstract

Botanicals possess healing and medicinal properties which could be an alternative to synthetic therapeutic drugs. The study evaluated the healing activity of selected botanicals on castration wounds of piglets. Crude ethanol leaf extracts of selected botanicals were subjected to wound healing activity assessment. Wound surface area, photography of wounds, epithelialization and scar appearance were assessed. Results showed that all plants contain reducing sugars, tannins, carbohydrates, polyphenols, and flavonoids which may have contributed to wound healing activity independently or synergistically. *Momordica charantia* Linn., *Moringa oleifera* Lam., *Tabernaemontana pandacaqui* Poir. and *Mollugo oppositifolia* Linn. crude ethanol leaf extracts exhibited pro-healing activity by affecting the various phases of healing process, wound closure, and epithelialization of castration wounds of piglets. *Tabernaemontana pandacaqui* Poir. and *Momordica charantia* Linn. crude ethanol leaf extracts have the best wound healing potential compared to the other extracts. The crude ethanol leaf extracts have properties that render them capable of promoting accelerated wound healing activity. The use of botanicals in the treatment of castration wounds of piglets is far more economical than using commercial preparations.

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Introduction

Castration of male piglets is a common practice in many countries. Castration is performed to avoid boar taint in the meat of sexually mature male pigs and to reduce aggression toward other pigs and caretakers. Boar taint is an accumulation of compounds, such as skatole and androstenone, in the meat of intact males that cause an unpleasant smell and taste that is released when pork is heated. Currently there are two methods of castrating male piglets: surgical castration and immunocastration. Surgical castration of piglets is carried out prior to weaning, most commonly within the first three days of life (AVMA, 2013).

In the Philippines, Section 6 of Department of Agriculture Administrative Order no. 41 series of 2000 otherwise known as the code of practice and minimum standards for the welfare of pigs states that castration is normally unnecessary in modern pig production systems. However, if castration is carried out, it shall be done as early as the management practice will allow (usually at 3-5 days) but not later than 14 days. It shall be performed using clean sharp instruments, with strict attention to hygiene.

Plants have been used as medicine since time immemorial for the treatment of various ailments of skin and dermatological disorders especially cuts, wounds and burns. They are in general far cheaper, and many can be gathered or home-grown virtually cost-free. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides, etc., which have been found in vitro to have antimicrobial properties (Dahanukar, 2000).

Many plants and various preparations have been used traditionally in relation to wound treatment, especially due to their immense potential to affect the wound healing process. Plant-derived extracts and/or isolates induce healing and tissue regeneration through multiple connected mechanisms, which often have a synergistic effect on the overall healing efficiency (Maver *et al.*, 2018).

There are plants found in the Philippines that have different uses such as in the treatment of diseases and illnesses. Examples of these plants are *Momordica charantia* Linn (*Ampalaya*), *Moringa oleifera* Lam. (*Malunggay*), *Tabernaemontana pandacaqui* Poir (*Pandakaki-puti*) and *Mollugo oppositifolia* Linn (*Papait*). These plants are being utilized by common folks in treating some illnesses.

These, among others like their abundance and other medical properties, prompted the researcher to explore more on the uses of these plants. Their healing and medicinal properties can serve as an alternative to therapeutic drugs which are expensive. Thus this study was conducted to evaluate the healing progress of castration wounds applied with *Momordica charantia* Linn., *Moringa oleifera* Lam., *Tabernaemontana pandacaqui Poir*. and *Mollugo oppositifolia* Linn ethanolic crude ethanol leaf extracts

Materials and methods

Wound healing activity test

Castration wounds of piglets were evaluated for healing activity. This was achieved by morphologic evaluation of the healing process, monitoring the progress of wound healing and determining the period of epithelialization.

Experimental animals

Piglets were identified based on their ear notch number and weighed before and after the study. The experimental animals were randomly housed in a farrowing crate with free access to commercial feeds and fresh water.

Surgical castration of piglets

Intact male piglets were properly restrained by suspension of the hind legs in a castration stand. A local anesthetic (2% Lidocaine HCl) was infiltrated on the proposed site of incision at a dose of 0.2mg/kg bodyweight. The scrotum and the surrounding area was cleaned with a cotton swab soaked in a mild disinfectant. Two horizontal incisions (~1.5cm) were made with a sharp scalpel on the skin of the scrotum over each testicle in the direction of the tail. The incisions were made low on the scrotal sac to allow for fluid drainage. The testicles were extracted by pushing through the incision.

The spermatic cord containing the ductus deferens, testicular artery, testicular veins, lymphatic vessels, nerves and the cremaster muscle was clamped with forceps then cut. The wound was applied with the different treatments and intentionally left open and undressed.

Application of the crude leaf extracts

Crude ethanol leaf extracts of *Momordica charantia* Linn., *Moringa oleifera* Lam., *Tabernaemontana pandacaqui* Poir. and *Mollugo oppositifolia* Linn. were applied topically by spraying on the edges of the castration wound once daily, starting from day 0 to day 7. The day of the wound creation was considered as day zero. Control animals did not receive any treatment while piglets in Treatment II were treated with 0.2% Nitrofurazone. Nitrofurazone is an antimicrobial organic compound belonging to the nitrofuran class. It is most commonly used as a topical antibiotic ointment for superficial wounds, burns, ulcers, and skin infections. It is effective against gram-positive bacteria and gram-negative bacteria (Drugs.com, 2018). Animals in Treatments III, IV, V and VI were treated with crude ethanol leaf extracts of the selected botanicals.

Ethical consideration

The research was carried out in strict accordance to the rules governing the use of laboratory animals. The experimental protocol statement was approved and a certification was issued by the Institutional Animal Care and Use Committee (IACUC) of the Don Mariano Marcos Memorial State University.

Data gathered

Progress of healing

Wound healing progress was assessed dimensionally, visually and physiologically. Progress of healing was monitored at different time intervals on days 0, 2, 4, 6, 8, 10 and 12 until the wound had completely healed. The following parameters were used:

Table 1. Photographic wound assessment tool

Domain	Assessment	Score
Edges	0 - Wound is closed (skin intact) or nearly closed 1 - Majority (>50%) of edges are attached with an advancing border of epithelium 2 - Majority of edges are attached even with wound base 3 - Majority of wound edges are unattached 4 - Majority of wound edges are rolled, thickened or fibrotic	
Necrotic Tissue Type	0 - None visible or wound are closed (skin intact) or nearly closed 1 - Majority of necrotic tissue are thin white/grey or yellow slough 2 - Majority of necrotic tissue are thick, adherent white/yellow slough or fibrin 3 - Majority of necrotic tissue are white/grey devitalized tissue or eschar 4 - Majority of necrotic tissue are hard grey to black eschar	
Total Amount of Necrotic Tissue	0 - None visible or open wound or wound is closed (skin intact) or nearly closed 1 - Less than 25% of wound bed covered 2 - 25% to 50% of wound covered 3 - More than 50% and less than 75% of wound covered 4 - 75% to 100% of wound covered	
Skin Color Surround-ing Wound	0 - Pink or normal 1 - Bright red 2 - White or gray pallor 3 - Dark red or purple 4 - Black	
Total Amount Granula tion Tissue	0 - wound is closed (skin intact) or nearly closed 1 - 75% to 100% of wound is covered with granulation tissue 2 - More than 50% and less than 75% of open wound is covered with granulation tissue 3 - 25% to 50% of wound bed is covered with granulation tissue 4 - Less than 25% of wound bed is covered with granulation tissue	
Epithelia-lization	0 - 100% wound covered, surface intact 1 - 75% to less than 100% wound covered 2 - 50% to less than 75% wound covered 3 - 25% to less than 50% wound covered 4 - Less than 25% wound covered	
Total Score		

Wound surface area (mm)

This was obtained by direct measurement of wound dimensions. The parameters used were the outer dimensions of the wound: length and width (in millimeters). These parameters were measured directly on the wound using a digital caliper. Wound surface area was calculated from these dimensions following the formula used for elliptic, shallow and regularly shaped wounds as described by Humbert *et al.* (2004).

Wound surface area =
0.785 × Longest length (mm) × Greatest width (mm)

Wound status

This was assessed through photography of the wound. Wound photographs were rated using the photographic wound assessment tool (PWAT) developed by Houghton *et al.* (2000). Six domains were used in the PWAT. These six domains include wound edges, necrotic tissue type and amount, skin color surrounding wound, amount of granulation tissue, and epithelialization (Table 1).

A number from 0 to 4 was assigned for each of the six domains

The total PWAT score for each wound photograph was calculated by summing the scores assigned to each of the six domains. Thus, the range of possible total PWAT scores was between 0 and 24 with zero representing a completely healed wound.

Images were acquired by means of a digital photographic camera

An image acquisition protocol was used in the study such as: the distance between camera and animal should be about 20-30cm in order to have a global vision of the wound, the background should be green color, the flash must be on and the camera should be placed parallel to the wound (Acha *et al.*, 2005). The parameters of the camera were set to: ISO speed 400, exposure time 1/60s and aperture F 5.6.

Epithelialization period

This was determined by noting the number of days required for the scab to fall from the wound surface without any residual raw wound (Mechesso *et al.*, 2016). This was taken as end point of complete epithelialization.

Scar appearance

Scars were assessed at different time intervals on 6, 8 and 10 days after castration using the Stony Brook Scar Evaluation Scale (Table 2). The Stony Brook Scar Evaluation Scale is a wound evaluation scale developed by Singer *et al.* (2007) to measure short-term cosmetic outcome of wounds 5 to 10 days after injury.

Table 2. The Stony Brook scar evaluation scale

Parameter	Scar category	Points
Width	>2 mm	0
	≤ 2 mm	1
Height	Scar elevation present	0
	No scar elevation	1
Color (redness)	Scar more red than the surrounding skin	0
	Scar of the same color or lighter than surrounding skin	1
Incision line	Present	0
	Absent	1
Overall appearance	Poor	0
	Good	1

Note: Mark (1) if affirmative for positive categories only; otherwise, mark (0)

Scars were assigned 0 or 1 point for the presence or absence of the following: width greater than 2 mm at any point of the scar, raised or depressed scar, a darker coloration than the surrounding skin, incision line and overall poor appearance. A total cosmetic score was then calculated by adding the individual scores on each of the five categories from 0 (poor) to 5 (best). A five-point rating scale used for Stony Brook Scar Evaluation Scale (Table 3) served as criteria for evaluating scar integrity and overall physical appearance.

Table 3. Five-point rating scale used for Stony Brook scar evaluation scale

Point scale	Range	Descriptive rating
5	4.20-5.00	Best
4	3.40-4.19	Good
3	2.60-3.39	Average
2	1.80- 2.59	Fair
1	1.00-1.79	Poor

Weight gain of piglets

Piglets were weighed (in kilograms) before the start of the study and on the 12th day after castration. Weight gain was calculated using the formula:

Weight Gain= Weight at 12 days after castration – Weight before castration

Total Cost of 7-days Treatment of Castration Wound Per Piglet. This was computed by adding all the factors of input (raw materials, labor, etc.) used.

Data analysis

All data were expressed as mean ± standard error of the mean (SEM). The statistical analysis was carried out by one-way analysis of variance (ANOVA) test at different time intervals employing the statistical program, Statistical Tool for Agricultural Research (STAR). Significance of the difference between means was determined by Tukey's honestly significant difference (HSD) test and least significant difference (LSD) test. Differences between groups were considered statistically significant at P value < 0.05 levels.

Results and discussion

Wound surface area

The assessment of wound status based on measurements of wound surface area (mm²) is presented in Table 4. The results showed that after 2 days' post-castration, *Tabernaemontana pandacaqui* Poir. crude ethanol leaf extract treated piglets obtained the lowest wound surface area measurement with a mean of 17.79 ± 1.73 mm² followed by *Momordica charantia* Linn. crude ethanol leaf extract, 0.2% Nitrofurazone, *Mollugo oppositifolia* Linn. crude ethanol leaf extract treated group and control group with wound surface area measurements of 19.10 ± 0.35 mm², 20.28 ± 2.83 mm², 24.34 ± 2.95 mm² and 24.60 ± 0.94 mm² respectively. Piglets treated with *Moringa oleifera* Lam. crude ethanol leaf extract recorded the highest wound surface area of 26.04 ± 4.07 mm². However, there was no statistically significant difference among the treatments. The result denoted that the wound is undergoing an initial process of healing response. Wound repair is at the defensive/inflammatory phase (second stage of wound repair). The animal's body focuses on destroying bacteria and removing debris essentially preparing the wound bed for the growth of new tissue. During Phase 2, neutrophils enter the wound to destroy bacteria and remove debris. These cells often reach their peak population between 24 and 48 hours after injury, reducing greatly in number after three days. As the neutrophils leave, macrophages arrive to continue clearing debris. These cells also secrete growth factors and proteins that attract immune system cells to the wound to facilitate tissue repair. This phase often lasts four to six days after wounding and is often associated with edema, erythema (reddening of the skin), heat and pain (Maynard, 2015).

Table 4. Wound Surface Area (mm²) Measurement of the Different Treatments at 2, 4, 6, 8, 10 and 12 days' Post-castration

Treatment	Day					
	2	4	6	8	10	12
Control	24.60 ± 0.94	12.90 ± 1.59[a]	4.01 ± 0.11	2.81 ± 0.12[a]	1.95 ± 0.04[a]	1.06 ± 0.11[a]
0.2% Nitrofurazone	20.28 ± 2.83	7.77 ± 1.16[b]	2.87 ± 0.12	1.82 ± 0.08[b]	1.41 ± 0.04[b]	0.68 ± 0.05[b]
M. charantia	19.10 ± 0.35	9.58 ± 0.30[ab]	3.03 ± 0.28	2.00 ± 0.10[bc]	1.60 ± 0.11[ab]	0.72 ± 0.04[b]
M. oleifera	26.04 ± 4.07	11.28 ± 0.42[ab]	3.94 ± 0.12	2.47 ± 0.08[ac]	1.90 ± 0.09[a]	0.86 ± 0.08[ab]
T. pandacaqui	17.79 ± 1.73	8.79 ± 0.24[ab]	3.10 ± 0.34	1.86 ± 0.16[b]	1.23 ± 0.10[b]	0.72 ± 0.04[b]
M. oppositifolia	24.34 ± 2.95	10.40 ± 1.49[ab]	3.89 ± 0.48	2.25 ± 0.15[abc]	1.87 ± 0.11[a]	0.89 ± 0.03[ab]

At four days after castration, the wound surface area of 0.2% Nitrofurazone treated group registered the lowest measurement with a mean of 7.77 ± 1.16 mm². *Tabernaemontana pandacaqui* Poir. had a wound surface area of 8.79 ± 0.24 mm², while *M. charantia* Linn, *M. oppositifolia* Linn. and *M. oleifera* Lam. crude ethanol leaf extract treated animals had 9.58 ± 0.30 mm², 10.40 ± 1.49 mm², and 11.28 ± 0.42 mm² wound surface areas correspondingly. Results disclosed that there were significant differences in the wound surface area measurement between the 0.2% Nitrofurazone treated group and the control (untreated) group at day 4 post-castration. On the other hand, there was no measurable difference between the crude ethanol leaf extracts treated groups and the 0.2% Nitrofurazone treated group. The result suggests that 0.2% Nitrofurazone and the crude ethanol leaf extracts were able to exhibit their antibacterial and anti-inflammatory activities thereby enhancing wound repair.

Boakye *et al.* (2018) mentioned in their study that wound healing agents are generally classified as agents that are either able to stimulate fibroblast proliferation, induce keratinocyte cell proliferation and differentiation, increase collagen formation, exhibit antimicrobial, antioxidant and anti-inflammatory activities. The ability of an agent to possess two or more of these biological properties suggest that the agent is potentially a good wound-healing agent.

By day six post-castration, the mean wound surface area of 0.2% Nitrofurazone treated animals was reduced to 2.87 ± 0.12 mm², whereas *M. charantia* Linn, *T. pandacaqui* Poir., *M. oppositifolia* Linn. and *M. oleifera* Lam. crude ethanol extract treated piglets had mean wound surface areas of 3.03 ± 0.28 mm², 3.10 ± 0.34 mm², 3.89 ± 0.48 mm² and 3.94 ± 0.12 mm² respectively. The control group recorded the highest mean wound surface area of 4.01 ± 0.11 mm². However, analysis of variance revealed no significant difference between and among the treatments. The result inferred that the crude ethanol leaf extracts promoted healing of epithelial wounds and enhanced wound closure. Topical administration of the ethanol leaf extracts is effective in faster wound healing due to the larger availability at the wound site. Likewise, the usage of herbal extracts accelerates wound healing by inducing the migration of fibroblasts (Thakur *et al.*, 2011). In the initial stages of wound healing, fibroblasts play a vital role by actively proliferating, migrating to wound area and inducing the synthesis of new extracellular matrix. Fibroblasts are most abundant cells in skin tissue and the major functions of these cells during wound healing include, rupturing of fibrin clots, generation of extracellular matrix components and collagen structures that support the tissue homeostasis. Collagen synthesis and granulation tissue formation play critical role in wound contraction (Bolla *et al.*, 2019).

The mean wound surface area on day eight after castration in the 0.2% Nitrofurazone treated group was 1.82 ± 0.08 mm². The groups treated with *T. pandacaqui* Poir. crude ethanol leaf extract had a wound surface area of 1.86 ± 0.16 mm². *Momordica charantia* Linn. had 2.00 ± 0.10 mm², *M. oppositifolia* Linn. had 2.25 ± 0.15 mm² whereas *M. oleifera* Lam. crude ethanol leaf extract treated piglets had a wound surface area of 2.47 ± 0.08 mm². The results further revealed that the mean surface wound areas of piglets treated with 0.2% Nitrofurazone, were comparable to that of *M. charantia* Linn., *T. pandacaqui* Poir. and *M. oppositifolia* Linn. crude ethanol extract treated group but was significantly different from the mean wound surface areas of the control and the *M. oleifera* Lam. crude ethanol leaf extract treated groups. Additionally, the wound surface areas of *M. charantia* Linn., *M. oleifera* Lam. and *M. oppositifolia* Linn. crude ethanol leaf extract treated animals were comparable but significantly different to the control and the 0.2% Nitrofurazone treated groups. The results implied that 0.2% Nitrofurazone and the application of the crude ethanol leaf extracts has enhanced closure of wounds at day eight after castration. The enhanced wound closures suggest that the crude ethanol leaf extracts has potential in the management of wound healing. This potency may be associated with the individual or synergistic effects of phytoconstituents that hasten the process of wound healing. A completely healed wound is defined as one that has returned to its normal anatomical structure, function and appearance within a reasonable period of time. Wounds that fail to heal in a timely and orderly manner results in chronic, non-healing wounds (Enoch and Price, 2004). In general, smaller wounds heal more quickly, while large, deep wounds tend to take longer (Advanced Tissue, 2014).

At day ten after castration, the lowest wound surface area was detected in the animals treated with *T. pandacaqui* Poir. crude ethanol leaf extract having a mean of 1.23 ± 0.10 mm². Piglets treated with 0.2% Nitrofurazone had a reduced wound surface area of 1.41 ± 0.04 mm² while *M. charantia* Linn., *M. oppositifolia* Linn. and *M. oleifera* Lam. crude ethanol leaf extract treated groups had mean wound surface areas of 1.60 ± 0.11 mm², 1.87 ± 0.11 mm² and 1.9 ± 0.09 mm² respectively. Analysis of variance revealed that the mean wound surface area of the 0.2% Nitrofurazone and the *T. pandacaqui* Poir. crude ethanol leaf extract treated groups were comparable with each other but was significantly different from the rest of the treatments. Moreover, no significant difference was noted in the mean wound surface area of *M. charantia* Linn. crude ethanol leaf extract treated piglets compared to the other treatments. The results signify that 0.2% Nitrofurazone and the crude ethanol leaf extracts had accelerated the healing process as evidenced by significant improvement of wound closure.

By day 12 after castration, the lowest mean wound surface areas was observed in the 0.2% Nitrofurazone treated piglets (0.68 ± 0.05 mm²). The mean wound surface area in piglets treated with crude ethanol leaf extracts of *M. charantia* Linn. and *T. pandacaqui* Poir. was identical (0.72 ± 0.04 mm²). *Moringa oleifera* Lam. crude ethanol leaf extract treated animals had a mean wound surface area of 0.86 ± 0.08 mm² whereas the mean wound surface area in the *M. oppositifolia* Linn. treated piglets was 0.89 ± 0.03 mm². The highest mean wound surface area was seen in the control or untreated group (1.06 ± 0.11 mm²). The above results indicate that 0.2% Nitrofurazone and the crude ethanol leaf extracts significantly improved wound healing owing to its positive influences on the various of stages of the healing process.

Photographic wound assessment

The mean photographic wound assessment tool (PWAT) scores of the castration wounds of piglets at 2, 4, 6, 8, 10 and 12 days after castration is presented in Table 5. Extracts were found to be significantly different from those treated with *M. oppositifolia* Linn. (15.22 ± 0.40), *Moringa oleifera* Lam. (15.56 ± 0.48) crude ethanol leaf extracts and the control group (16.89 ± 0.40).

Table 5. Mean Photographic Wound Assessment Tool (PWAT) Scores of the Castration Wounds of Piglets at 2, 4, 6, 8, 10 and 12 days' Post-castration

Treatment	Day					
	2	4	6	8	10	12
Control	16.89 ± 0.40[a]	9.45 ± 0.22[a]	5.11 ± 0.11[a]	1.67 ± 0.00[a]	0.90 ± 0.19	0.78 ± 0.11
0.2% Nitrofurazone	11.11 ± 0.59[b]	6.11 ± 0.56[b]	3.11 ± 0.11[b]	1.00 ± 0.00[b]	0.84 ± 0.11	0.71 ± 0.00
M. charantia	12.67 ± 0.38[b]	6.78 ± 0.29[b]	3.33 ± 0.00[bc]	1.00 ± 0.19 [b]	0.84 ± 0.11	0.71 ± 0.00
M. oleifera	15.56 ± 0.48[a]	9.11 ± 0.11[a]	3.78 ± 0.11[c]	1.11 ± 0.22 [ab]	0.84 ± 0.11	0.71 ± 0.00
T. pandacaqui	11.78 ± 0.62[b]	6.33 ± 0.38[b]	3.00 ± 0.00[b]	1.00 ± 0.00[b]	0.84 ± 0.11	0.71 ± 0.00
M. oppositifolia	15.22 ± 0.40[a]	6.67 ± 0.19[b]	3.67 ± 0.19[c]	1.11 ± 0.11[ab]	0.84 ± 0.11	0.71 ± 0.00

The results imply that wound healing in animals treated with 0.2% Nitrofurazone *T. pandacaqui* Poir. and *M. charantia* Linn. crude ethanol leaf extracts have progressed in a timely and orderly fashion through the normal stages of wound healing as demonstrated by the morphological features and the lower healing scores which indicates better wound healing.

Castration wound of piglets treated with 0.2% Nitrofurazone, *T. pandacaqui* Poir. and *M. charantia* Linn. crude ethanol leaf extracts showed similar healing features such as majority of wound edges are attached even with the wound base, majority of the necrotic tissue is thin white/grey slough, < 25% of necrotic tissue covered the wound bed, skin color surrounding the wound is bright red in color, > 50% and <725% of open wound is covered with granulation tissue and 50% to < 75% of the wound is covered with epithelial tissue. Piglets treated with *M. oppositifolia* Linn., *Moringa oleifera* Lam. crude ethanol leaf extracts and the control group displayed the following features: majority of the wound edges are attached even with wound base, majority of necrotic tissue is thick, adherent white The accumulation of necrotic tissue and slough in the wounds might have resulted from inadequate local blood supply. Necrotic tissue contains dead cells and debris that are a consequence of the fragmentation of dying cells. Slough or fibrin, > 50% and < 75% of wound is covered with necrotic tissue, skin color surrounding the wound is bright red in color, > 50% and <725% of open wound is covered with granulation tissue and 25% to < 50% of the wound is covered with epithelial tissue.

Slough is a yellow fibrinous tissue that consists of fibrin, pus and proteinaceous material.

The accumulation of necrotic tissue or slough in wounds promotes bacterial colonization and prevents complete repair of the wound. Necrotic burden is an all-encompassing term that describes necrotic tissue, slough, excess exudate and high levels of bacteria present in the wound environment. If the necrotic burden is allowed to accumulate in a wound it can prolong the inflammatory response, mechanically obstruct the process of wound contraction and impede re-epithelialization (Enoch and Price, 2004).

Angiogenesis, also known as neovascularization, is the development of new capillaries from existing and/or pre-existing vascular networks, and it plays an integral part in the repair system. Angiogenesis is a crucial step of wound healing, and some botanical extracts have been shown to modulate angiogenesis and accelerate wound healing. Angiogenesis provides the nutritional supply to maintain cell metabolism and to form an intact delivery system. The regeneration (angiogenesis) occurs by forming the vascular conduits that supply nutrients, the building blocks to the cellular elements of the granulation tissue, and the overlying epidermal cells begin to migrate across the tissue defect to restore the epithelial barrier function of the skin (Thangapazham *et al.* , 2016).

Wound infection occurs when microorganisms, such as bacteria, grow within the damaged skin of a wound. If a wound becomes infected, it can get worse instead of better. Symptoms can include increasing pain, swelling, and redness (Leonard, 2019). The existence of microorganisms within a wound lead to delayed healing. However, the relative number of micro-organisms (usually more than 10^5 per gram of tissue can cause clinical infection) and their pathogenicity dictate whether a wound becomes infected or shows signs of delayed healing. Bowler (2003) claimed that the presence of 100,000 or more bacterial cells per gram of wound tissue is a key determinant in delayed wound healing and infection. In addition, the continued presence of bacteria in a wound leads to endotoxin production and prolongs the inflammatory phase of wound healing. Although inflammation is part of normal wound healing, the repair process may be prolonged if the inflammation is excessive (Enoch and Price, 2004).

At four days' post-castration, the mean PWAT score of piglets treated with 0.2% Nitrofurazone (6.11 ± 0.56), *T. pandacaqui* Poir. *(6.33* ± 0.38*), M. charantia* Linn. (6.78 ±0.29) *and M. oppositifolia* Linn. (6.67 ± 0.19) crude ethanol leaf extracts were significantly lower than those treated with *Moringa oleifera* Lam. (9.11 ± 0.11) crude ethanol leaf extracts and the control group (9.45 ± 0.22). The 0.2% Nitrofurazone, *T. pandacaqui* Poir.*, M. charantia* Linn. *and M. oppositifolia* Linn. crude ethanol leaf extract treated groups showed similar morphological features such as >50% of the wound edges are attached to the wound bed with an advancing border of epithelium, majority of necrotic tissue is thin white/grey slough, no visible necrotic tissue or wound is closed, skin color surrounding the wound is pink or normal, 75% to 100% of the wound is covered with granulation tissue and 75% to < 100% of the wound is covered with epithelial tissue. Whereas, those treated with *Moringa oleifera* Lam. crude ethanol leaf extracts and the control group exhibited the following healing features: majority of wound edges are attached even with the wound base, necrotic tissue is thin white/grey slough, < 25% of wound bed is covered with necrotic tissue, skin color surrounding wound is bright red, 75% to 100% of the wound is covered with granulation tissue and 75% to < 100% of the wound is covered by epithelial tissue.

The findings revealed that the use of 0.2% Nitrofurazone, *and* the selected botanical crude ethanol leaf extracts accelerated the wound healing process. Complete healing of the wound might be ascribed to its anti-inflammatory property and the absence of infection. Ranade and Collins (2014) stated that botanicals help reduce the risk of delayed wound healing, enhance the healing process, and decrease incidence of infections. Plant chemical constituents are classified into broad categories that assist with skin integrity such as antimicrobial, anti-inflammatory, antioxidant, cytotoxic, and immune stimulant. For support of circulation, herbs stimulate blood flow to better deliver oxygen and nutrients to the wound area as well as provide cleansing to prevent contamination. Botanicals fight specific wound-compromising bacteria.

Shah and Amini-Nik (2017) cited in their study that the most common mechanisms behind phytochemical-mediated enhanced wound healing are their antioxidant, anti-inflammatory, and anti-microbial effects.

According to Aslam *et al.* (2018), the anti-inflammatory property of *T. pandacaqui* Poir. crude ethanol leaf extract may be attributed to the presence of flavonoids and other bioactive compounds that work in combination to heal the wound. Catechin, apigenin, luteolin and rutin are classes of flavonoids found in many medicinal plants that possess wound healing activities. Taesotikul *et al.* (2003) also mentioned that alcoholic extracts of *T. pandacaqui* Poir. has significant anti-inflammatory, antipyretic and anti-nociceptive activities. These activities are due to its alkaloidal components.

The PWAT scores of piglets treated with 0.2% Nitrofurazone (3.11 ± 0.11), crude ethanol leaf extracts of the selected botanicals and the control group (5.11 ± 0.11) produced a statistically significant difference at day six after castration. However, PWAT scores for piglets treated with 0.2% Nitrofurazone, *T. pandacaqui* Poir. *(3.00 ±* 0.00*) and M. charantia* Linn. (3.33 ± 0.00) crude ethanol leaf extracts were comparable but significantly different to the PWAT scores of piglets treated with *M. oppositifolia* Linn. (3.67 ± 0.19) and *M. oleifera* Lam. (3.78 ± 0.11) crude ethanol leaf extracts. The results demonstrated the efficient wound healing activity of 0.2% Nitrofurazone and the crude ethanol leaf extracts. The extracts might have played a critical role in the wound healing process by increasing the rate of wound closure, epithelialization and prevention of secondary bacterial infections that would have complicated and delayed wound healing (Zeng *et al.*, 2016).

The animals treated with 0.2% Nitrofurazone, and crude ethanol leaf extract of the selected botanicals have wound edges that are completely closed at day six after castration while the control group have >50% of wound edges attached to the wound bed with an advancing border of epithelium. No visible necrotic tissue was observed in all the treatments. The color of skin surrounding the wound was pink or normal except for the control which has a bright red color. The piglets treated with 0.2% Nitrofurazone, and crude ethanol leaf extract of the selected botanicals have wounds completely (100%) covered with granulation and epithelial tissue.

Towards the eight day following castration, the mean PWAT scores of piglets treated with *M. oppositifolia* Linn. (1.11 ± 0.11) and *Moringa oleifera* Lam. (1.11 ± 0.22) crude ethanol leaf extracts were comparable to that of the control group (1.67 ± 0.00) but significantly different from the PWAT scores of piglets treated with 0.2% Nitrofurazone (1.00 ± 0.00), *T. pandacaqui* Poir. (1.00 ± 0.00) *and M. charantia* Linn. (1.00 ± 0.19) crude ethanol leaf extracts. There was no measurable difference on the mean PWAT scores of *M. oppositifolia* Linn. and *Moringa oleifera* Lam. crude ethanol leaf extract treated group compared to the 0.2% Nitrofurazone, *T. pandacaqui* Poir. *and M. charantia* Linn. crude ethanol leaf extract treated groups. All the treatments displayed the following morphological features: wound edges were closed and no visible necrotic tissue was present, the color of the skin surrounding the wound was pink and the wounds were totally covered with granulation and epithelial tissues. Results implied that after eight days there was still a physiological progression of wound healing. The main mechanisms of skin wound healing might be due to migration and proliferation of cells with re-epithelialization and synthesis of granulation tissue (Gushiken *et al.*, 2017).

Photographic evaluation of the castration wounds showed similar morphologic healing features at ten and twelve days' post-castration including a total closure of the wounds, no visible necrotic tissue, normal coloration of the skin and complete epithelialization. No significant differences were observed among the treatments on days 10 and 12.

A visual comparison of the healing progress of castration wound of piglets at 2^{nd}, 4^{th}, 6^{th}, 8^{th}, 10^{th} and 12^{th} day post-treatment with 0.2% Nitrofurazone and crude ethanol leaf extracts of *Momordica charantia* Linn., *Moringa oleifera* Lam., *Tabernaemontana pandacaqui* Poir. and *Mollugo oppositifolia* Linn. is shown in Fig. 1 & 2.

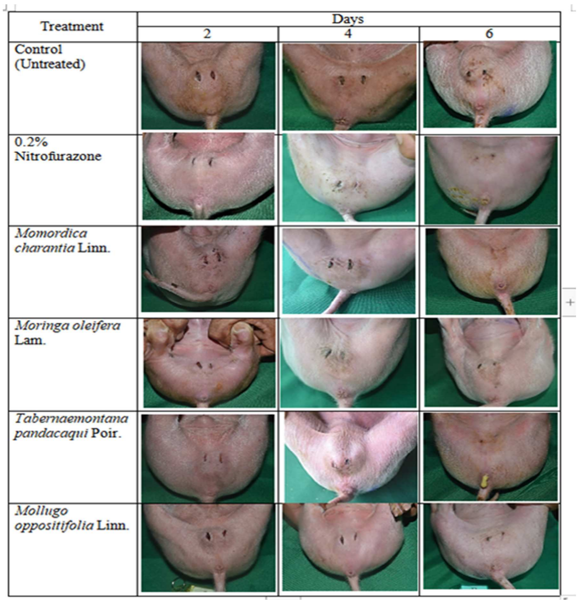

Fig. 1. Morphological representation of healing progress in castration wounds of piglets treated with 2.0% Nitrofurazone and crude ethanol extracts of selected botanicals at 2,4 and 6 days after castration.

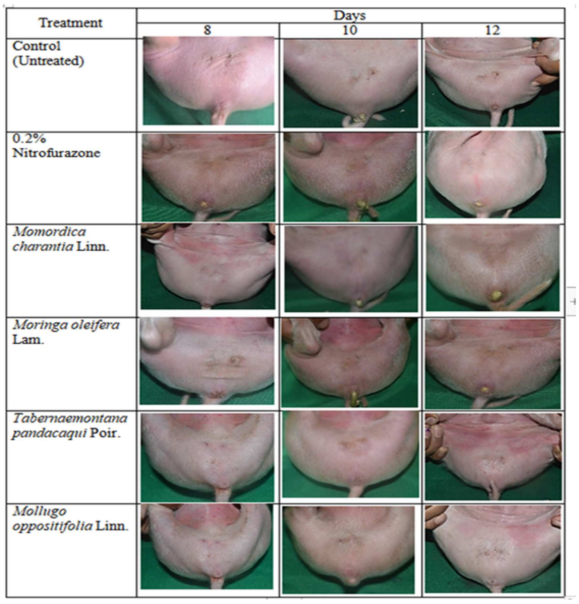

Fig. 2. Morphological representation of healing progress in castration wounds of piglets treated with 2.0% Nitrofurazone and crude ethanol extracts of selected botanicals at 8,10 and 12 days after castration.

The results showed progressive changes in wound healing in the test groups as compared with the control (untreated) group. Treatment of animals with the standard drug (0.2% Nitrofurazone) and crude ethanol leaf extracts of the selected botanicals showed significant healing as compared to the untreated group.

Period of epithelialization

The period of epithelialization (in days) exhibited by the crude ethanol leaf extracts of the selected botanicals on castration wound of piglets is depicted in Fig. 3. Epithelialization is defined as the reconstitution of the cells of the epidermis in order to cover the injured site and restore barrier function (Padgett *et al.* , 2007) Epithelialization is essential for successful closure of the wound. The time for complete epithelialization was short in the crude ethanol leaf extracts and 0.2% Nitrofurazone (Treatment II) treated groups as compared to the control.

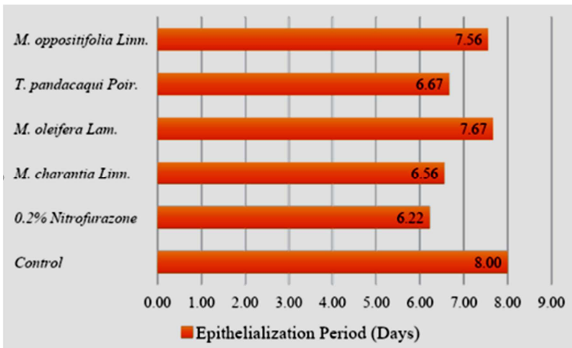

Fig. 3. Period of epithelialization (number of days) exhibited by the crude ethanol leaf extracts of the selected botanicals on castration wound of piglets.

On average, the period of epithelialization for the control group was 8 days. The 0.2% Nitrofurazone treated group showed the shortest epithelialization time of 6.22 days whereas *Momordica charantia* Linn. (*Ampalaya*), *Tabernaemontana pandacaqui* Poir. (*Pandakaki-puti*), *Mollugo oppositifolia* Linn. (*Papait*) and *Moringa oleifera* Lam. (*Malunggay*) had a mean epithelialization period of 6.56, 6.67, 7.56 and 7.66 days respectively.

The results showed that the crude ethanol leaf extracts possess a definite pro-healing action as demonstrated by enhanced epithelialization. The shorter period of epithelialization could be due to the ability of the crude ethanol leaf extracts to enhance collagen synthesis, induce cell proliferation, and antimicrobial activities of bioactive constituents (Wang, *et al.*, 2011)

Comparison among treatment means (Table 6) revealed that the 0.2% Nitrofurazone, *Momordica charantia* Linn. and *Tabernaemontana pandacaqui* Poir. treated groups, with means of 6.22 ± 0.22, 6.56 ± 0.11, and 6.67 ± 0.38, showed a shorter epithelialization period compared to the control group (untreated) having a mean of 8 days. Similarly, *Momordica charantia* Linn. crude ethanol leaf extract showed significant difference of epithelialization period as compared to *Moringa oleifera* Lam. (7.67 ± 0.19) and *Mollugo oppositifolia* Linn. (7.56 ± 0.11) crude ethanol leaf extracts. Moreover, *Moringa oleifera* Lam. and *Mollugo oppositifolia* Linn. crude ethanol leaf extracts showed a shorter epithelialization period than the control but failed to reach statistical significance.

Table 6. Epithelialization period (days) of castration wounds of piglets in the different treatments

Treatment	Mean
Control	8.00 ± 0.19[a]
0.2% Nitrofurazone	6.22 ± 0.22[d]
Momordica charantia	6.56 ± 0.11[cd]
Moringa oleifera	7.67 ± 0.19[ab]
Tabernaemontana pandacaqui	6.67 ± 0.38[bcd]
Treatment	7.56 ± 0.11[abc]

Results indicate that treatments with the crude ethanol leaf extracts as well as those treated with 0.2% Nitrofurazone were superior to the control group which received no treatment. Likewise, the results show that the crude ethanol leaf extracts possess a definite pro-healing action as demonstrated by advanced epithelialization. Epithelialization is characterized by replication and migration of epithelial cells across the skin edges in response to growth factors. Cell migration may begin from any site that contains living keratinocytes, including remnants of hair follicles, sebaceous glands, islands of living epidermis, or the normal wound edge. In acute wounds that are primarily closed, epithelization is normally completed in 1 to 3 days. In open wounds, including chronic wounds, healing by secondary intention cannot progress until the wound bed is fully granulated (Hopf *et al.*, 2008).

The presence of flavonoids and tannins in the crude ethanol leaf extracts might have promoted the healing process and increased the rate of epithelialization. Flavonoids and tannins are known to promote wound healing process by their astringent and antioxidant properties. They play a very important role in cutaneous tissue repair as they prevent tissue damage that stimulates wound healing process. Flavonoids are identified to reduce lipid peroxidation not only by preventing or slowing the onset of cell necrosis but also by improving vascularity. Lipid peroxidation refers to the oxidative degradation of lipids. The process of lipid peroxidation is important in skin wounds. (Barku *et al.*, 2013; Mohan *et al.*, 2014). A drug that inhibits lipid peroxidation is believed to increase the viability and strength of collagen fibers and prevents cell damage by promoting DNA synthesis. Flavonoids prevent or delay the onset of cell necrosis and also improve vascularity to the wounded area (Agyare *et al.*, 2016).

Conclusion

The results of the study indicated that (a) *M. charantia* Linn., *M. oleifera* Lam., *T. pandacaqui* Poir. and *M. oppositifolia* Linn. crude ethanol leaf extracts exhibited pro-healing activity by affecting the various phases of healing process, wound closure, and epithelialization of castration wounds of piglets and *T. pandacaqui* Poir. and *M. charantia* Linn. crude ethanol leaf extracts have the best wound healing potential compared to the other extracts.

Based on the findings of the study, the crude ethanol leaf extracts have properties that accelerate the wound healing process, lower the incidence of infection in castration wounds and improve weight gain of piglets. And the crude ethanol leaf extracts have properties that render them capable of promoting enhanced wound healing activity.

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