Evaluation of Combined Herbal Extract Dressing Materials Effect on Open Wounds in Pig Model

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Background: *There are many herbal extracts available in Thailand claimed to be effective in wound healing and scar prevention. But very few wound dressing products are made from these ingredients. So this study evaluates the effectiveness of the combination of herbal extract dressing materials in animal models.*
Objective: *To compare the rate of wound size reduction and histological evaluation of 5 groups of dressing materials.*
Material and Method: *A porcine full-thickness wound model was used to compare 5 groups of dressing materials: normal saline (control), lipidocolloid (standard), combined herbal extract formula 1 (Allium cepa, Centella asiatica, Aloe vera), combined herbal extract formula 2 (Centella asiatica, Aloe vera) and placebo. The dressings were applied on full-thickness wounds on paraspinal region of 5 pigs (20 wounds per pig), 15 wounds per each group of dressing. The wounds were evaluated for the rate of epithelialization, healing time and histology.*
Results: *Dressing of combined herbal extract formula 2 showed significance in wound area reduction than the control group and placebo group in 12th and 15th day post-wounding. Dressing of herbal extract formula 1 showed significant in wound area reduction than control and placebo group in 15th day post wounding also. The wounds treated by both formulas of combined herbal extract dressing showed greater reduction in size than standard dressing, but not statistically significant. No dressings showed any statistical significant differences in the duration of healing time.*
Conclusion: *The Combined herbal extract dressing materials could provide wound-healing as the standard wound dressing material. These materials may reduce the cost of wound care in Thailand because the raw materials are available in the country. Further evaluation is needed in clinical settings before consideration for the treatment of the wounds.*

Keywords: *Scar, Herbal extract, Wound*

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Wound healing is the repairing process that follows injury to the skin and soft tissues. Wound healing occurs in three phases: inflammatory phase, proliferation phase, and remodeling phase[1]. In the proliferation phase, angiogenesis, collagen deposition, epithelization and wound contraction occur. In fibroplasia and granulation tissue formation, fibroblasts grow and form a new provisional extracellular matrix by excreting collagen and fibronectin. In epithelialization, epithelial cells proliferate and spread across the wound surface and wound contraction occurs as the myofibroblasts contract.

Many complementary and alternative products are used to treat wounds. There is a variety of forms such as gel, cream base, sheet, fiber. The main effect of these materials is to promote wound healing by maintaining moist environment.

There are herbal extracts which have been scientifically proven in wound healing promotion. The active ingredients of these natural plant extracts are Aloe vera (Aloe vera extract), onion extract (12% Allium cepa extract), Extract from Centella asiatica (Centella asiatica extract).

The most used part of Aloe vera is gel, which contain polysaccharide and glucomanine. These two substances are mainly used for emollientcy, protection against UV rays (antioxidation) and moisturization. There are studies show that extracts of Aloe vera has the ant-inflammatory effect on wound by the inhibitory action on the arachidonic acid pathway via cyclooxygenase[5]. Also, several studies[6-8] found that aloe could increase re-epithelialization in burn wounds significantly as compared with other cream-

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treated wounds.

Allium cepa is the formula extracted from onions; in Thai traditional medicine, it has been used as a remedy of flatulent, diuretic and a hair tonic for dandruff and hair fall prevention. In research reports, it is revealed that the onion extract could treat the hypertrophic scar due to its flavonoids[9] (quercetin and kaempferol) to reduce the production of the collagen fibrils within the scar dermis. Moreover, it also has an anti-inflammatory activity, an effect in the contracting ability and increase the rate of wound healing.

Many reports has published the effect of extract from Centella asiatica[10] that it increased cellular proliferation and type-1 collagen synthesis at the wound site[11], as evidenced by increase in DNA, protein and collagen content of granulation tissues. Quicker and better maturation and cross-linking of collagen was observed in the extract-treated rats. The extract treated wounds could promote epithelialization and the increase the rate of wound contraction. Animal studies have consistently shown topical application of Centella asiatica to a sutured wound significantly increased the breaking strength of the wound. Asiaticoside, a saponin extracted from Centella asiatica, is thought to be one of its active constituents. It was showed that a 0.2% asiaticoside solution applied topically twice daily for seven days to punch wounds in guinea pigs resulted in 56% increase in hydroxyproline, 57% increase in tensile strength, increased collagen content, and better epithelialization compared to control. Thus, selected triterpenes of Centella asiatica may help to improve wound repair with a better re-epithelialisation and a normalization of perivascular connective tissue allowing an improvement of the venous wall tone and elasticity.

There have been no evidential studies on the combination of these natural plant extracts and its effectiveness in wound healing activity on open wounds. If we can find an herbal extract dressing material that can treat wounds the same as or better than the imported dressing materials, it should be beneficial to patients. The objective of this study is to compare the efficacy of the combination of herbal extract dressing materials in the treatment of open wounds to the standard dressing materials.

Objective

Primary objective

To compare rate of healing between each group. The wound area (mm^2) will be measured in 5 different treatments i.e., control (NSS), lipidocolloid dressing (standard), combined herbal extract formula 1, formula 2 and formula 3.

Secondary objective

Day that the wound has complete epithelialization.

Compare histopathology of wounds.

Material and Method

Materials

Animals

5 Thai-bred pigs from the Faculty of Veterinary Science, Mahidol University, weighed 28 to 32 kg were selected to use in the experiment. After arrival, the animals were housed at the laboratory animal housing unit at the faculty of Veterinary Science for a minimal acclimation periods 7 days before experiment. The animals were housed one per cage. Cages and feeders will be sanitized on a daily basis. They were maintained according to the "Guide for the care and Use of Laboratory Animals" established by National Laboratory Animal Center, Mahidol University, Thailand. After an acclimation period, the wound creation was performed randomly into 5 groups.

Group I (control group):

These wounds did not receive any treatment after injury, only cleaning with normal, sterile saline solution and covered with Tegaderm™ (hydrocolloid adhesive with an outer clear adhesive cover film that is impermeable to liquids, bacteria and viruses). The wounds were changed every day.

Group II (standard group):

These wounds were treated with lipidocolloids which were changed every day after cleaning with normal, sterile saline solution.

Group III (test group-formula 1):

These wounds were treated with formula 1 by applying it on the wound after cleaning with normal, sterile saline solution and covered with sterile gauze, change once a day.

Formula 1: Allium Cepa Extract 12%, Centella Asiatica Extract 5%, Aloe vera Extract 2.5%.

Group IV (test group-formula 2):

These wounds will be treated with formula 2 by applying it on the wound after cleaning with normal sterile saline solution and covered with sterile gauze, change once a day.

Formula 2: Centella asiatica extract 5%, Aloe vera extract 2.5%.

Group V (test group-formula 3):

These wounds will be treated with formula 3

by applying it on the wound after cleaning with normal, sterile saline and covered with sterile gauze, change once a day.

Formula 3: Base solution consist of solvent (water), thickener (Acrylates), neutralizer (triethanolamide), solubilizer (polysorbate), skin conditioning (allantoin), preservatives (phenoxy-ethanol, methylparaben, butylparaben, ethylparaben) Fig. 1.

Method

The animals were sedated, intubated, prepared anesthetized with azaperone (2 mg/kg, intramuscular, zoletil 5 mg/kg intramuscular and isoflurane inhalation) prior to and during infliction of the experimental wounds. The surgical interventions were carried out under sterile conditions using anesthesia drug. All of the animals received prophylactic antibiotics treatment; enrofloxacin (5 mg/kg, subcutaneous) also inject, and Analgesics drugs; carpofen (4 mg/kg, subcutaneous), all of these procedures were done by attending veterinarian. 20 full thickness wounds 1.5x1.5 cm in size per each pig were designed. After dorsal hair was shaved, a template was used to define the wound sites, which were organized in two rows on the lateral paraspinal areas, 10 wounds per each row. The application field was outlined with a marking pen. The skin was cleaned with chlorhexidine and povidone solution and draped with sterile cloths just prior to skin excision. A No. 15 blade was used to excise a 2.25 cm^2 full thickness wound (1.5x1.5 cm). The full thickness of skin and underlying subcutaneous layers was removed taking care not to injure the underlying musculature (Fig. 2). The wounds were covered with moist gauze during the operation to avoid desiccation. All wounds were cleaned with normal, sterile saline solution following the program for each group. During the wound healing, animals' general condition and skin irritation was observed daily.

Animal ethical committee approval

The study was approved by Animal Care and Use Committee, Faculty of Veterinary Science, Mahidol University (FVS-ACUC).

Measurement and data collection

All wounds were measured every 3 days until complete epithelialization. The wound area (cm^2) was recorded by using a Visitrak (Trademarks of Smith & Nephew); a portable digital device. Day of complete epithelialization was recorded.

Fine-resolution digital photographs (1,280 x960 pixels) of the wounds were taken after wound cleaning with normal sterile saline solution. A digital camera (RICOH CX5, Ricoh Co., Ltd., Japan) was used, facing down, 10 cm above the wound, permitting direct comparison between individual wounds.

To avoid human bias, the person who performed the wound measurements was blinded.

At day 3rd, and day 14th, biopsy was done at wound edge using disposable biopsy punches, 5 wounds per each pig. The biopsy wounds were not included in the wound area measurement. The tissue will take and fix in 10% buffered formalin. Each specimen was embedded in a paraffin block and a thin section (3 μm) was prepared and stained with Hematoxylin Eosin method. After that, the slides were examined histologically under a light microscope to evaluate the amount and type of inflammatory cells, quantity and characteristic of collagen fibers. The pathologist was blinded.

Statistical analyses

Difference in wound area and day of epithelialization among the five treatment groups will be compared using one-way analysis of variance with ANOVA. All statistical data analyses will be performed using SPSS. A p-value ≤ 0.05 was considered statistically significant.

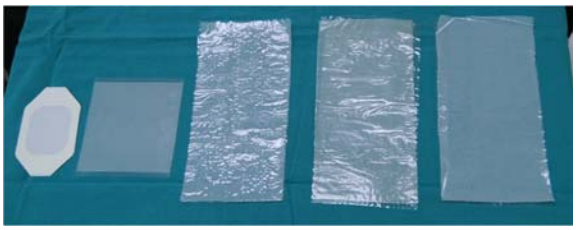
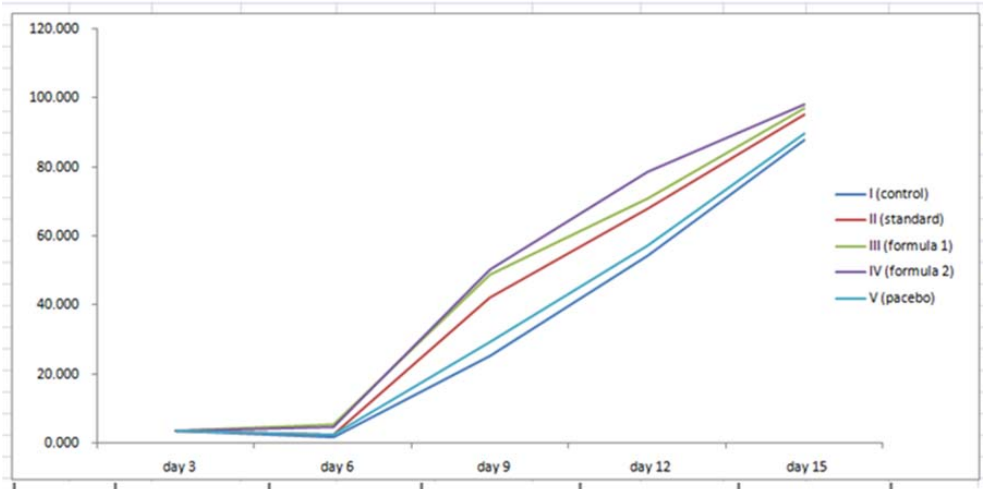


Fig. 1 Dressing materials.

Fig. 2 Wound creation.



I	3.577 ± 2.214	1.788 ± 3.548	25.227±17.474	54.486±11.461	87.968±7.781
II	3.577±2.214	2.495±5.592	42.365±17.830	67.933±13.090	95.179±4.410
III	3.604±2.229	5.419±3.042	48.766±20.429	71.017±11.381	97.018±3.468
IV	3.517±2.173	4.627±5.487	50.154±15.418	78.639±5.267	98.212±2.471
V	3.636±2.253	2.443±4.460	29.432±12.798	57.273±5.239	89.773±4.847

Fig. 3 Mean of percent reduction in wound area in each group.

Results

Wound area measurement

Combined herbal therapy appeared to accelerate the wound healing of the full thickness skin defect at 12th and 15th day after injury compared with that of the control group and placebo group. At 12th day after injury, mean of percent wound size reduction was significantly faster in group IV than in the control group ($p = 0.002$) and placebo group ($p = 0.009$). At 15th day after injury, the mean of percent wound size reduction was also significantly faster in group III and IV than in the control ($p = 0.007$, 0.002 respectively) and placebo group ($p = 0.043$, 0.013 respectively). The standard group also showed faster wound size reduction than the control group ($p = 0.045$). The trend of group III and IV showed smaller in size than the standard dressing group but there was no statistical significance (Fig. 3, Fig. 4 and Table 1).

Healing time

With regard to the duration to complete epithelialization, there were no statistical significant differences among any group (Table 2 and Table 3).

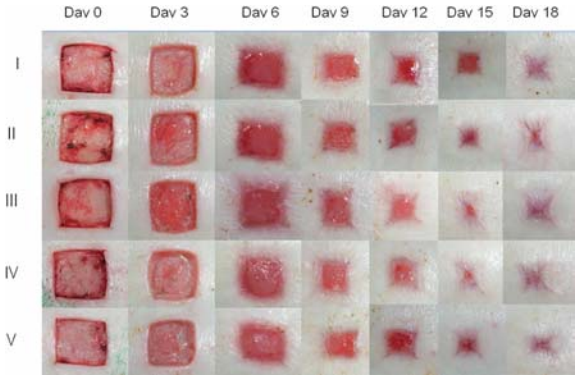


Fig. 4 Photo record of the wound at the day of wound measurement.

Histological evaluation

Histological studies of the tissue that obtained from wound edge at 3rd day showed that in group IV had an decrease in inflammatory cells especially neutrophils, more than other groups (Table 4, Fig. 5); but there was no significant difference between collagen and fibroblast and among any groups at 21 day (Fig. 6).

Discussion

Herbal extracts have been proved to have benefit in the wound management for many previous studies[5,6,9,10]. But there has been no study of the benefits of the dressing material that composed of the combination of herbal extract in the wound before. In this study the combined herbal extract groups showed significant effect on the wound area reduction compared to the control group (normal saline) and placebo group (base formula) but not significant in the standard group (lipidocolloid). The total healing time was not difference among the groups. Histological results also support the evidence of improvement in wound healing, tissue that was obtained from combined herbal extract groups had a decreased inflammatory cell more than other groups. But no difference in collagen and fibroblast level between any groups.

It seems that the combined herbal extract in group 4 (formula 2) has the better effect in decreasing inflammatory process and healing than group 3 (formula 1). For the ingredients of group 4, Centella asiatica extract 5% and Aloe vera extract 2.5% compared to ingredients in group 3, Allium cepa extract 12%, Centella asiatica extract 5%, Aloe vera Extract 2.5%. The difference between the two groups was Allium cepa extract in group 3. This might be the anti-proliferative effect of the Allium cepa extract on the fibroblasts and epithelial cells that leads to slower wound area reduction. There are many studies that revealed anti-

Table 1. Multiple comparison of percent reduction in wound area among 5 groups

Day 3	I	II	III	IV	V
I	1	1	1	1	1
II		1	1	1	1
III			1	1	1
IV				1	1
V					1
Day 6	I	II	III	IV	V
I	1	0.998	0.508	0.723	0.998
II		1	0.7	0.88	1
III			1	0.997	0.686
IV				1	0.87
V					1
Day 9	I	II	III	IV	V
I	1	0.617	0.093	0.067	0.99
II		1	0.767	0.678	0.87
III			1	1	0.229
IV				1	0.173
V					1
Day 12	I	II	III	IV	
V					
I	1	0.732	0.065	0.002	0.99
II		1	0.551	0.059	0.938
III			1	0.709	0.17
IV				1	0.009
V					1
Day 15	I	II	III	IV	V
I	1	0.045	0.007	0.002	0.947
II		1	0.944	0.734	0.206
III			1	0.989	0.043
IV				1	0.013
V					1

* *p*-value <0.05

Table 2. Mean healing time of each group

Group	I	II	III	IV	V
Days	17.62	16.87	16.50	16.12	17.62
SD	1.06	1.55	1.60	1.52	1.06

Table 3. Multiple comparison of healing time

Group	*p*-value				
	I	II	III	IV	V
I	1	0.931	0.652	0.303	1
II		1	1	0.973	0.931
III			1	1	0.652
IV				1	0.303
V					1

Table 4. Cell counts from histological examination

Group	Inflammatory cell	Neutrophil	Fibroblast	Collagen
I	++++	++	++++	+++
II	+++	+++	+++	+++
III	++++	++	+++	+++
IV	+++	+	++++	+++
V	+++	+++	++++	+++

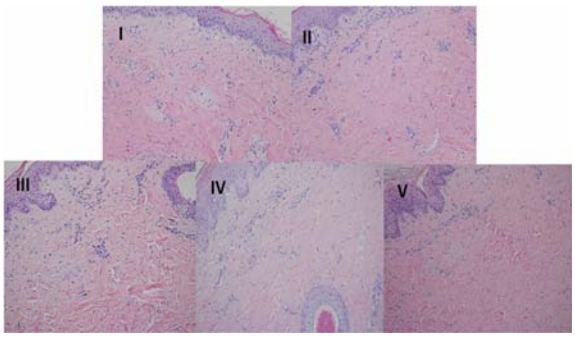


Fig. 5 Histological examination at 3rd day post wounding from wound edge of group I-V.

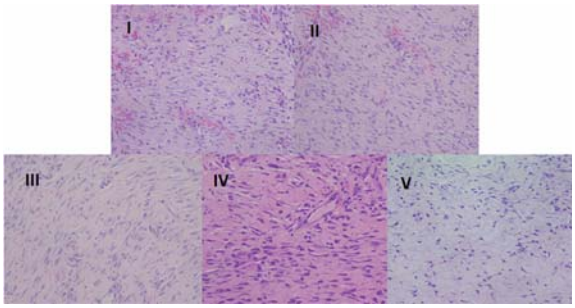


Fig. 6 Histological examination at 21st day post wounding from wound edge of group I-V.

inflammatory effect of Allium cepa but we could not observe such effect in this study.

From this study, the effectiveness of combined herbal extract in wound healing activity support the production of new dressing material impregnated with this formula. The materials are available in Thailand. There is a possibility to produce the new effective dressing material for wound care with lower price compared to the imported ones. Further evaluation is needed in clinical settings before consideration for treatment of wounds.

Conclusion

This study might be the first study to demonstrate the wound healing effect of the combined herbal extract dressing material. The combined formula of 5% Centella asiatica and 2.5% Aloe vera has an effective wound healing activity. It has potential to be a dressing material with affordable price. However, further evaluation is needed in clinical settings before consideration for the treatment of these wounds.

What is already known on this topic?

Herbal extracts such as Aloe Vera and Centella asiatica have wound healing effect and have been used for wound care for a long time.

What this study adds?

This study showed the efficacy of the combination of the herbal extract in dressing materials and was comparable to the commercial lipidocolloide dressing in wound treatment. This knowledge should be applied in clinical situation to the benefit of patients.

Potential conflicts of interest

None.

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การประเมินการหายของบาดแผลจากการรักษาด้วยส่วนผสมสมุนไพรสกัดในหมูทดลองเพื่อขอรับการพิจารณาจากคณะกรรมการจรรยาบรรณการใช้สัตว์ทดลอง ศูนย์สัตว์ทดลองแห่งชาติ มหาวิทยาลัยมหิดล

ณัฐชา วามวาณิชย์, อภิรักษ์ ชวงสุวนิช

ภูมิหลัง: *ประเทศไทยมีสารสกัดจากพืชหลายชนิดที่ใช้ในการรักษาบาดแผลในการทดลองนี้จะทำการทดลอง ดูการผสมของสารสกัดหลายชนิด เพื่อรักษาบาดแผลในหมู*

วัตถุประสงค์: *เพื่อเปรียบเทียบผลการลดขนาดของบาดแผลและผลทางพยาธิสภาพของแผล โดยสารสกัดสมุนไพร จากพืชผสมในความเข้มข้นต่างกัน*

วัสดุและวิธีการ: *แบ่งบาดแผลชนิดลึกในหมูเป็น 5 กลุ่ม คือ กลุ่มที่ใช้วัสดุน้ำเกลือ, ปิดแผลมาตรฐาน, สารสกัดสมุนไพรสูตร 1, สูตร 2 และสูตรหลอก ติดตามการหดตัวของแผล, การหายของแผลและผลทางพยาธิวิทยา*

ผลการศึกษา: *สารสกัดทั้งสูตร 1 และ 2 ให้ผลการลดขนาดของแผลดีกว่ากลุ่มควบคุมวันที่ 12 และ 15 แผลในทุกกลุ่ม หายในระยะเวลาที่ไม่แตกต่างกันทางสถิติ*

สรุป: *สารสกัดจากพืชมีผลในการกระตุ้นการหดตัวของแผลดีกว่าการใช้สารปิดแผลมาตรฐานแต่ควรมีการศึกษาเพิ่มเติมในมนุษย์ต่อไป*
