Original Article

The efficiency of herbal on blood clotting and wound healing of ear notched pigs

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Abstract

This study aimed to determine the efficiency of *Jatropha podagrica* Hook. f., *Chromolaena odorata* (L.) and *Centella asiatica* (L.) on the reduction of blood clotting and promoting wound healing in notched piglets. Thirty-four notched piglets aged 2–3 days were selected for applying fresh exudes of *J. podagrica* Hook. f. to the right ear and povidone iodine to the left ear as a control. In the experiment of *C. odorata* (L.) and *C. asiatica* (L.), fifty notched piglets were divided into five groups and 10, 20, 30, 40 and 50% each of herbal gel was applied to the right ear and pure gel applied to the left. Bleeding time and wound-healing scores were recorded. When applying fresh exudes of *J. podagrica* Hook. f., bleeding time was 11.73 seconds while the control group was 35.45 seconds. The groups which applied 30% and 40% of *C. odorata* (L.) gel stopped bleeding at 28.46 and 43.62 seconds, respectively. 50% of *C. asiatica* (L.) gels showed a significant reduction of bleeding time (15 seconds) when compared to the control group (34 seconds) ($P < 0.01$). The wound-healing score using fresh exudes *J. podagrica* Hook. f. from day 1 to day 3 (2.07, 3.17 and 3.97, respectively) was significantly higher than the control (1.53, 2.34, 3.06, respectively) ($P < 0.05$). 50% gel *C. odorata* (L.) showed a higher score of wound healing from day 1 to day 4 (2.0, 2.80, 3.60 and 4.0, respectively) than the control group (1.20, 1.86, 2.84 and 3.74, respectively) ($P < 0.05$). While, 50% gel *C. asiatica* (L.) gel showed a higher score from day 2 to day 5 (2.60, 3.44, 4.33 and 4.90, respectively) compared to the control group (1.86, 2.70, 3.60 and 4.60, respectively) ($P < 0.01$). This experiment indicated that fresh exudes of *J. podagrica* Hook. f., 50% gel *C. asiatica* (L.) and 50% gel *C. odorata* (L.) reduced the time of blood clotting and promoted wound healing. Therefore, these herbals may use as a potential choice for stopping bleeding and inducing wound healing in notched piglets in order to reduce stress and the chance of secondary infection as well as to improve animal welfare.

Keywords: Efficiency, *C. asiatica* (L.), *C. odorata* (L.), *J. podagrica* Hook. f., notched pigs

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Introduction

Ear notching is carried out on new-born baby pigs for the purpose of identification. All pigs in the same litter should have the same ear notch in the right ear. They will have a different notch number in the left ear. Ear notches are read with the litter number (pig's right ear) first followed by the individual identification number (pig's left ear) read second. There are five designated zones (1, 3, 9, 27, and 81) in the Litter Ear and three designated zones (1, 3, and 9) in the individual pig ear. When reading, look the pig in the face then read left-to-right, it will always be litter number then pig number (Fisher, 2020). Ear notching is definitely important because if there is a disease going around it will be easy to track which pigs have it and where they came from. A common practice is to cut approximately 6.5-mm (1/4-in) deep notches into the ears of piglets using specifically made notchers (Brady, 1993). In about 3 months, the wound is 80% as strong in its repair as it was before the injury but the wound area will never reach 100% of its original strength. Depending on the size and the severity of the wound, the entire healing process may take up to a couple of years to complete (Gosain and DiPietro, 2004, Mathieu *et al*., 2006).

The piglet may suffer from stress resulting from pain and the attraction of other piglets to the resulting bloody ears. A previous study determined that ear notching evoked calls with higher peak frequencies and cortisol concentrations at 4 h tended to be greater ($P < 0.10$) than the control treatments (Marchant-Forde *et al*, 2009). It is assumed that it will improve welfare if the ears and notchers are properly disinfected and notching is performed on young piglets (<3 days). Bleeding and trauma from ear notching may result in colonisation by bacteria and the development of necrosis or induce secondary infection.

Normally, after notching the farmers normally apply povidone iodine then let the pigs gather in the same pen. Notched pigs are attracted by other pigs because of bleeding which may induce a secondary infection and late wound healing. Therefore, this research was interested in using herbal extracts to stop bleeding in ear-notched pigs faster than normal as well as to reduce wound-healing time. There are many types of herbals that have medicinal properties for wound healing and stopping bleeding in animals, including *Chromolaena odorata* (L.), *Centella asiatica* (L.) and *J. podagrica* Hook. f., *C. odorata* is a traditional plant widely used for its wound-healing properties (Vijayaraghavan *et al*., 2017). The phytochemical properties of *C. odorata* are antibacterial (Vijayaraghavan *et al*., 2018), anticancer (Suksamrarn *et al*., 2004), anti-inflammatory (Owoyele *et al*., 2005) and antioxidant (Bhargava et al., 2013). *C. asiatica* is a herb that is known for treating many types of disease, such as gastrointestinal diseases, gastric ulcers, asthma, wound healing and eczema (Brinkhaus *et al*., 2000). The fresh exudate of *J. podagrica* Hook. f. has properties to stop bleeding of wounds and abrasions. Research has shown that *J. podagrica* Hook. f. has antimicrobial activity against some gram-positive bacteria and multidrug-resistant strains of microorganisms (Aiyelaagbe *et al*., 2007; Bhaskarwar *et al*., 2008).

This research was designed to study the efficiency of three kind of herbals; *J. podagrica Hook.* (F.), *C. odorata* (L.) and *C. asiatica* (L.). on reducing bleeding time and promoting wound healing in ear notched pig. The anticipated results were to discover alternative herbal extracts for wound care and for improving the welfare of piglets.

Materials and Methods

Ethics statement: The study was submitted to and approved by the ethics committee and decision board of Maejo University Animal Care and Use Committee (MACUC 027A/2020).

Herbal extract preparation: The three kinds of herbal plants used for the experiment were *J. podagrica Hook.* (F.), *C. odorata* (L.) and *C. asiatica* (L.) (Figure 1). The green leaves were collected from young plants for herbal extract. The *J. podagrica* Hook. f. fresh exudate of copious sticky sap was collected from the tree for 15–30 minutes directly after picking the leaves. The fresh exudate was kept in a sterile plastic bottle and used for the experiment within the day. *C. odorata* and *C. asiatica* crude extracts were prepared by mashing the leaves, which were then weighed and kept at 4 °C in a glass bottle until use (Neelapong *et al*., 2019). Gelling agent was prepared using 98.5 mL of distilled water 1 gram of aristo flex AVC gel (© Sinthai Chemicals & Trading Ltd.,Part) and 0.5 g of preservative (DMDM Hydantoin and iodopropynyl butylcarbamate; Bio Nature Plus CO., LTD). Then *C. odorata* and *C. asiatica* crude extracts were mixed with gelling agent in percentages of 10, 20, 30, 40 and 50% (v/v).

Animal selection: Three crossbred piglets, Landrace × Large white × Duroc aged 1 to 3 days were selected for experiments of herbal efficiency on blood clotting and wound healing. Thirty-four piglets were used for testing the efficiency of *J. podagrica* Hook. f. The testing of *C. asiatica* (L.) and *C. odorata* (L.) efficiency used 50 piglets for each, divided into five groups of 10. In all experiments the right ear of the piglets was used for applying the herbal exudate and herbal gel, while the left ear was set as the control.

Ear notching: To show the litter number, V-shape cutting, size 1/4-inch-deep, was done using a stone ear notcher on each ear of the piglets. The piglets were cut on both the bottom and top edges of the right ear and the top of the left ear. The individual pig number was cut on the bottom of the left ear. To notch the ear, farmers use an appropriate size notcher, which it was important to keep clean with disinfectant (Aaron, 2020).

Efficiency of herbal extract on blood clotting and wound healing: After ear notching, the testing of the efficiency of *J. podagrica* Hook. f. was carried out by applying 0.5 mL fresh exudate to the wound on the right ear and applying povidone iodine (10% W/V) to the wound on the left ear. The efficiencies of *C. asiatica* (L.) and *C. odorata* (L.) were tested by applying pure gel to the left ear wound (the negative control) and 10, 20, 30, 40 and 50% (v/v) herbal gel to the right ear wound.

The time of blood clotting was recorded after notching the ear until bleeding stopped in both the control and experimental groups. The wounds were observed every day to define a healing score from 1 to 5, score 1; the wound was red, score 2; dry sores around the wound, score 3; dry and black around the wound, score 4; dry wound/scab and score 5; the wound completely healed (Figure 2). Traditional criteria to assist in the identification of wound infection were observed such as abscess, cellulitis, and discharge.

Statistical analysis: The data was tested for normal distribution by Skewness and Kurtosis. Mean values were compare by paired t-test analysed using ANOVA to measure the difference between the means of the control and experimental groups using t- test and Duncan in SPSS (V $_{26.0)}$ for Windows program.

Figure 1 The herbals applied to ear-notched piglet, A: *Jatropha podagrica* Hook. f., B: *Centella asiatica* (L.) and C: *Chromolaena odorata* (L.)

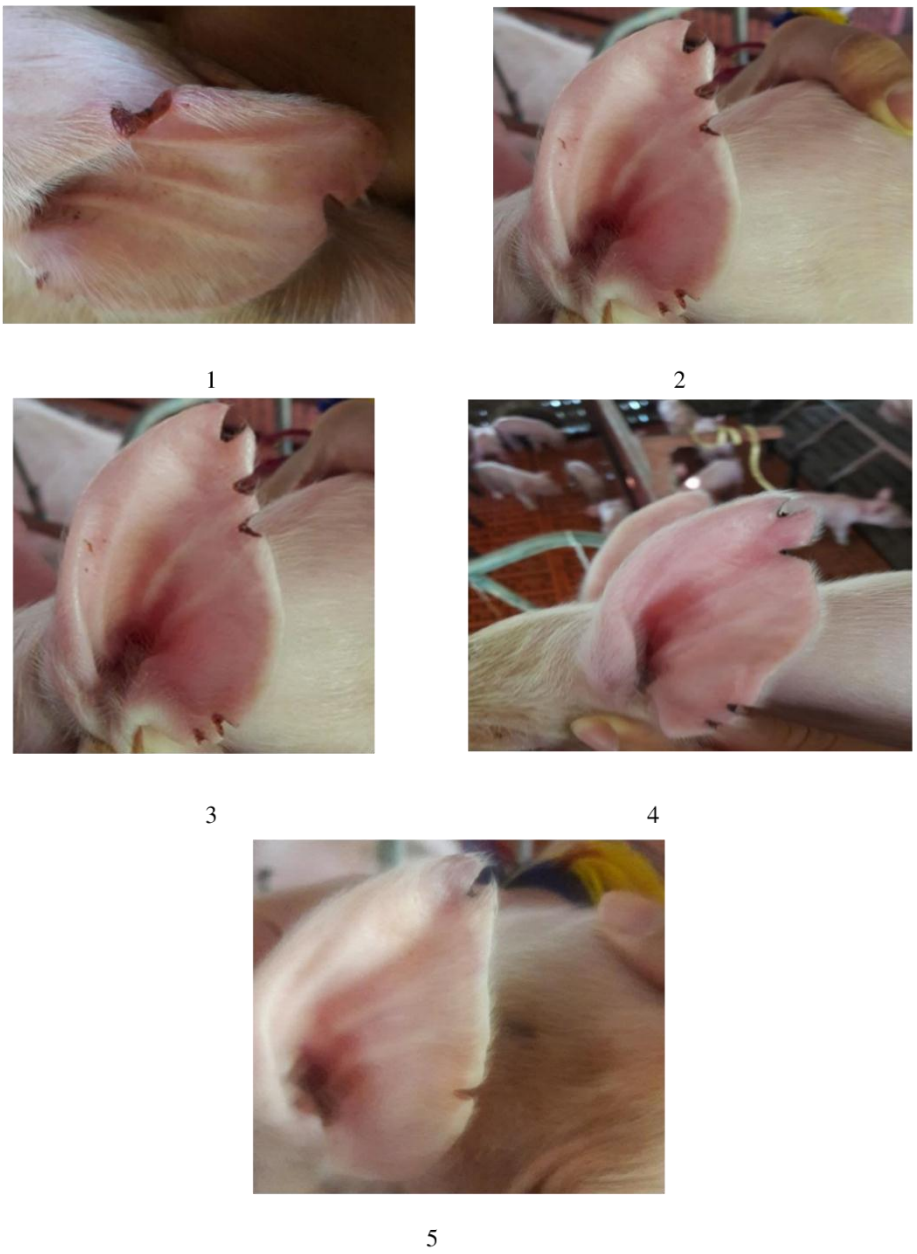

Figure 2 The characteristics of healing scores. Score one (1): the area around the wound is red; Score two (2): scabbed around the wound; Score three (3): scab wound with black colour; Score four (4): dried wound; and Score five (5): completely healed wound.

Results

Efficiency of herbals on blood-clotting time: From the experiment, the time of blood clotting after applying *J. podagrica* Hook. f. fresh exudate was 11.73 ± 21.19 s, which was statistically significantly (P <0.01) lower than the control group (35.45 ± 30.74 s). *C. odorata* (L.) 30% gel resulted in a bleeding time of 28.46 ± 9.22 s, while the control was 41.87 ± 16.67 s. Time of bleeding after applying *C. odorata* (L.) 40% gel was 43.62 ± 14.02 s (control 56.66 ± 16.11 s). When applying *C. asiatica* (L.) 50%, the time of bleeding was 15 ± 0.04 s (control 34 ± 0.05 s). The herbal gel of *C. odorata* (L.) at 30% and 40% and *C. asiatica* (L.) at 50% showed statistically significant reductions in the time of blood clotting from the control group (P < 0.01). The percentage reduction in blood-clotting time compared to control for each experiment indicated the highest percentage in *J. podagrica* Hook. f. (66.91%) followed by *C. asiatica* (L.) 50% gel (56%), *C. odorata* (L.) 30% gel (32%) *and C. odorata* (L.) 40% gel (23%) (Table 1).

Efficiency of herbals on wound healing: The result of wound healing by applying *J. podagrica* Hook. f. fresh exudate was observed from day one to day three. The results showed that on day one the notched wound became red. Then a scab appeared around the wound on day two. A black scab was found with a dry wound at day three. The completed healed wound had to be pinked and stretched or puckered.

However, the experimental group showed better scores on wound healing than the control group (Table 2). The result of herbal gel on wound healing showed that *C. asiatica* (L.) 50% and *C. odorata* (L.) 50% could increase wound healing score. Using *C. asiatica* (L.)

50%, the wound-healing score from day 2 to day 5 was statistically significantly better than the control group ($P < 0.01$). The healing was complete on day 6, similar to the control group. Using *C. odorata* (L.) 50%, the wound-healing score showed better results from day 1 to day 4; there was a statistically significant higher score than the control group. The wounds were completely healed by day 5, which was faster than the control group (Table 3).

Table 1 Time of blood clotting in the control and experimental groups for each herbal application

Herb	Control (second)	Experiment (second)	P-value	Percentage blood clotting reduction
J. podagrica Hook. f.	35.45 ± 30.74	11.73 ± 21.19	<0.01	66.91
C. asiatica (L.) *10%*	32 ± 0.07	34.21 ± 45.90	NS	-
C. asiatica (L.) *20%*	27 ± 0.08	26.42 ± 19.25	NS	-
C. asiatica (L.) *30%*	38 ± 0.20	29 ± 0.10	NS	-
C. asiatica (L.) *40%*	28 ± 0.05	32 ± 0.12	NS	-
C. asiatica (L.) *50%*	34 ± 0.05	15 ± 0.04	0.01	56
C. odorata (L.) *10%*	33.44 ± 7.87	31.72 ± 8.32	NS	-
C. odorata (L.) *20%*	25.96 ± 5.27	24.37 ± 6.99	NS	-
C. odorata (L.) *30%*	41.87 ± 16.67	28.46 ± 9.22	<0.01	32
C. odorata (L.) *40%*	56.66 ± 16.11	43.62 ± 14.02	<0.01	-
C. odorata (L.) *50%*	32.30 ± 13.47	31.90 ± 10.59	NS	23

Table 2 Result of *J. podagrica* Hook. f. fresh exudates on wound healing in notched piglets

Day	Wound-healing score		P-value
	Control group	*J. podagrica* Hook. f.	
1	1.53 ± 0.51[a]	2.07 ± 0.45[b]	<0.01
2	2.34 ± 0.6[a]	3.17 ± 0.53[b]	<0.01
3	3.06 ± 0.76[a]	3.97 ± 0.18[b]	<0.01
4	4.00 ± 0.00	4.00 ± 0.00	NS
5	5.00 ± 0.00	5.00 ± 0.00	NS
6	5.00 ± 0.00	5.00 ± 0.00	NS

Table 3 Result of *C. asiatica* (L.) and *C. odorata* (L.) gels on wound healing in notched piglets

Wound-healing score						
Day	Control	*C. asiatica* (L.)				
		10%	20%	30%	40%	50%
1	1.20 ± 0.32	1.50 ± 0.53	1.30 ± 0.50	1.00 ± 0.0	1.40 ± 0.52	1.60 ± 0.53
2	1.86 ± 0.7	2.00 ± 1.00	1.80 ± 0.42	1.60 ± 0.52	2.00 ± 0.0	2.60 ± 0.53*
3	2.70 ± 0.53	2.80 ± 0.63	2.90 ± 0.60	2.50 ± 0.53	3.00 ± 0.0	3.44 ± 0.53*
4	3.60 ± 0.52	3.60 ± 0.52	3.60 ± 0.52	3.70 ± 0.50	3.90 ± 0.40	4.33 ± 0.70*
5	4.60 ± 0.50	4.90 ± 0.32	4.40 ± 0.52	4.60 ± 0.52	4.70 ± 0	4.90 ± 0.01*
6	5.00 ± 0	5.00 ± 0	5.00 ± 0	5.00 ± 0	5.00 ± 0	5.00 ± 0
Day	Control	*C. odorata* (L.)				
		10%	20%	30%	40%	50%
1	1.28 ± 0.53	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	2.00 ± 0.00**
2	1.88 ± 0.82	1.80 ± 0.42	1.80 ± 0.42	1.60 ± 0.52	1.60 ± 0.52	2.80 ± 0.42**
3	2.84 ± 0.63	2.70 ± 0.48	2.70 ± 0.48	2.40 ± 0.52	2.50 ± 0.53	3.60 ± 0.52**
4	3.74 ± 0.52	3.90 ± 0.32	3.90 ± 0.32	3.60 ± 0.52	3.40 ± 0.52	4.00 ± 0.00**
5	4.80 ± 0.32	4.90 ± 0.32	4.90 ± 0.32	4.30 ± 0.48	4.50 ± 0.53	5.00 ± 0.00
6	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00

* *P*-value <0.01 ** *P*-value <0.05

Discussion

Ear notching is widely used in the pig industry to identify a pig's litter and to give each pig a unique identity number. Ear notching allows for easy identification of pigs for breeding and health and performance records. It takes about one week for complete healing. During that time stress and secondary infection may be caused in the piglets. Some studies have indicated that piglets who were ear-notched displayed more grunting vocalisations, head shaking, squeals, escape attempts, ear scratching and shivering (Noonan *et al*., 1994; Marchant-Forde *et al*.,2009; Leslie *et al*., 2010). The reduction in blood-clotting time and wound healing from notching may reduce acute pain and the chance of secondary infection.

This study showed the efficiency of *J. podagrica* Hook. f., *C. asiatica* (L.) and C. *odorata* (L.), which are readily found in Thailand, in reducing the time of blood clotting and wound healing in ear-notched pigs. Fresh exudate of *J. podagrica* Hook. f. applied directly to the cut area resulted in reductions in the bleeding and healing time of the wound in piglets compared to the control group. A review of the activity of the genus Jatropha (Euphorbiaceae) on wound healing has been reported, using ointment in a dose-related manner as compared to blank ointment (Abdelgadir and Van Staden, 2013). Herbal ointment containing 50% w/w *J. curcas* leaf extract showed 91.3% wound healing on the 16th day in an excision wound model in female rats, while a standard drug povidone iodine (Betadine®) showed 82.1% wound healing (Esimone *et al*., 2009). Moreover, several compounds of the root of *J. podagrica* Hook. f. such as japodagrin (1), japodagrone (2), macrocylic diterpenoids possessing lathyrane and jatrophane skeletons and four diterpenoids (3–6) displayed antibacterial activity against some gram-positive bacteria (Aiyelaagbe *et al*., 2007).

The experimental group that used high concentrations of the extract gel reduced the time of blood clotting and promoted wound healing faster than the normal drug (povidone iodine). *C. asiatica* (L.) gel (50% v/v) showed efficiency in stopping bleeding by reducing the time of blood clotting (15 ± 0.04 s) compared to the control group (34 ± 0.05 s). It significantly promoted wound healing from day 2 to day 5. This is similar to a previous study which showed the important health benefits of *C. asiatica* (L.) on wound-healing, with antidiabetic, antimicrobial, memory-enhancing, antioxidant and neuroprotective activities (Chandrika *et al*., 2015). An alcoholic extract of *C. asiatica* (L.) significantly increased wound breaking strength in an incision wound model by inducing epithelisation as well as being able to overcome the wound-healing suppressing action of steroids (Shetty *et al*., 2006). *C. asiatica* appeared to be effective in the treatment of wound-healing disturbances (Brinkhaus *et al*., 2000).

The efficiency of *C. odorata* (L.) on blood clotting showed the best significant reduction at 30% v/v, followed by 40% v/v, while it promoted wound healing at a high percentage (50%) from day 1 to day 4. The lyophilised aqueous leaf extract and alcoholic extracts (50, 70 and 95% ethanol) from the fresh and dried leaves of C. *odorata* (L.) displayed a significantly reduced bleeding time (<2.5 min) in rats but did not induce platelet aggregation or blood clotting in the in vitro study (Pandith *et al*., 2012). *C. odorata* (L.) is reported to have antibacterial, anti-inflammatory, antioxidant, anthelminthic, antifungal, cytotoxic, anticonvulsant, antiprotozoal, antispasmodic, antipyretic and analgesic properties (Omokhua *et al*., 2016). The efficiency in wound healing comes from the antioxidant properties, which enhance conservation of the fibroblast and keratinocyte proliferation on wounds (Vaisakh and Pandey, 2012). The ethanoic extract could, to a certain extent, inhibit the growth of various human pathogens, such as Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, and Escherichia coli (Vijayaraghavan *et al*., 2018).

Comparisons of the efficiency of blood-clotting time reduction indicated the best to be by applying fresh exudates of *J. podagrica* Hook. f. followed by *C. asiatica* (L.) 50% gel, *C. odorata* (L.) 30% gel and *C. odorata* (L.) 40% gel. The herb that significantly showed better results in wound healing than the control group was *J. podagrica* Hook. f., with a higher score from day 1 to day 3 and complete healing within 3–5 days, while *C. odorata* (L.) 50% gel showed high efficiency in promoting wound healing from day 1 to day 4 and complete healing by day 5. *C. asiatica* (L.) 50% gel could the improve wound-healing score compared to the control group from day 2 to day 5 and complete healing within 6 days.

As the result, this study implies that those three herbals can be used as a herbal gels for applying to ear-notched piglets in order to reduce bleeding time and promote wound healing faster (5 days) than normal (7 days). The study suggests that the first choice might be *J. podagrica* Hook. f. followed by a high percentage of *C. odorata* (L.) This will be an alternative more humane way by which to improve healing as quickly as possible in order to avoid attachment from other piglets. Reduction in bleeding time may prevent secondary infection as a result of antibacterial activity. However, preparing the extract for ease of use should be considered.

Conflict of interest: The authors declare that there are no conflicts of interest.

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