Efficacy of *Chromolaena odarata*, *Curcuma longa* Extraction and Povidone-iodine on Surgical Wound Healing of Laboratory Rats

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Abstract

The Efficacy of *Chromolaena odarata*, *Curcuma longa* extraction and Povidone-iodine (PVI) were tested on surgical wound healing of adult female rats. Sixteen of adult female rats were divided into four groups. Group 1 is negative group, the rat don't treated with herbal extraction. Group 2, rats were treated with 10% of PVI. Group 3, rats were treated with 6% *C. longa* extraction and group 4, rats were treated with 6% *C. odorata* extraction by applied over the lesion. Wound healing was examined at day 3, 6 and 9 via wound reduction determination, wound area and histological evaluation. The results found 6% of *C. odorata* extraction showed great result of wound area and wound reduction, with better than that the result with 6% of *C. longa* and 10% of PVI treated. All rats were demonstrated decrease in wound size that were seen in all groups at all-time observe. At the final experiment, rats were treated with 6% of *C. odorata* had highest of wound reduction (0.43 cm), followed by treated with 6% of *C. longa* (0.35 cm) and treated with 10% of PVI (0.32 cm), the result of PVI is similar with negative group (0.33 cm). These results at day 6 and day 9 were presented different significant (P<0.05) between group of wound reduction. The result of wound area correlated with wound

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reduction, we demonstrate that the female rats were treated with 6% *C. odorata* extract had greatest result of wound area at all day treated. The finish experiment, rats were received with 6% of *C. odorata* can reduced surgical wound area from 1.00 cm to 0.57 cm at day 9. In addition result, histological evaluation, rat in all groups found similar cytology include collagen, connective tissues, lymphocyte and macrophage, while in group that treated with herbal extraction showed add of neutrophil and leukocytes.

***Keywords*:** Wound healing, *Chromolaena odarata*, *Curcuma longa*, Povidone-iodine

Introduction

Wounds are physical injury to the skin that have many forms including lacerated wounds, bruises and burns, etc. (Seyed et al., 2017). The injury is the skin continuous the tissues in both internal and external, causing traumatic effects (Sjahruddin et al., 2015). The physiological of wound healing, can be classified as term in open or closed wounds, and acute or chronic wounds. The acute and chronic wounds have similarity and difference in physiological characteristics. Acute wound has four phases include hemostasis, inflammation, proliferation and remodeling, however in chronic wound has the same phases, but it has a longer duration phase of inflammation, proliferation and remodeling caused lead of fibroblast tissues and leaving cicatrix (Sjahruddin et al., 2015). Normally, healing in the process that composed with three phases; inflammatory, proliferation and remodeling. Fist, inflammatory connected with vascular response by exudation reaction, blood coagulation and hemostasis. The process, would lesion was attracted by neutrophils cells that produced and secreted many of reactive oxygen species (ROS). This very important to protected body against developing infection. This action should occurred within a few days after the skin an injury. The proliferative phase, there is formation of epithelium to cover the wound surface, formation of granular tissue with proliferated of fibroblast, deposited of collagen and develop of new blood

vessel. Last, remodeling phase, found the tissue structural integration and functional competent (Shuid et al., 2018).

The treatment process can be facilitated by medicinal and natural products. Variously researches, presented the great properties of natural products action in anti-inflammatory, antibacterial, antioxidant and good help to collagen synthesis. Many natural products have bioactive such as; alkaloids, essential oils, flavonoids, tannins, terpenoids and phenolic compounds (Thakur et al., 2011). Each bioactive agent had a great action in specific function on wound healing. The pro-collagen synthesis can be induced by saponins and for antibacterial and antiseptic of tissues could be help by tannins and flavonoids (Harbone, 1973). Many studies show the excellent action of natural products for selected to wound healing treatment. The great benefit of natural products including *Curcuma longa*, *Chromolaena odorata*, cucumber, vitamin E and honey. These products have been used in wound care management with excellent outcomes (Biswas & Mukherjee, 2003). This studies, we choose *Curcuma longa*, *Chromolaena odorata*, and povidone-iodine for treated wound healing in rats. Natural plant, *Curcuma longa* Linn. (Turmeric) is a plant in family Zingiberaceae, it has been used for many years in a traditional medicine. Turmeric has been recorded to treat in biliary, hepatic diseases, diabetic ulcers and rheumatic conditions in a rat (Emiroglu et al., 2017). *C. longa* is composed a bioactive substance called as curcuminoids (diferuloyl methane), low molecular weight, lipophilic molecule. Standard organize, U.S. Food and Drug Administration has classified the curcumin as general regarded as safe (Choudhary & Shivakumar, 2018). In addition, this substance is nonpolar drug, but soluble in organic solvents ethanol and acetone. The suggestion for used should intake 0.1-3.0 mg/kg body weight of daily dose. Many studies were applied curcumin to the animals with topical dressing or given orally by alone or incorporated into different formulations. A study of Bhaskar-Rao et al. (2015), tested the effect of curcuminoids (curcumin, tetrahydro curcumin (THC) and glucosyl-THC) on cutaneous excision wound in rat. The results found 2% of curcumin and

glucosyl-THC showed the best effect in wound healing with complete healing at 21 days after treatment. About of curcumin can be formulated into acid polymeric bandage (COP), resulting to increased wound reaction and enhanced cell proliferation (Mohanty et al., 2012). The COP can enables released of curcumin in solubilized form and sustained availability of curcumin metabolites with promote better healing at the wound side (Shuid et al., 2018). The application of curcumin, that used by combination with chemicals should possess excellent water absorption and fluid affinity; oleic acid, gelatin and hyaluronic acid thus promoted better wound healing. This report, similarity with El-Refaie et al. (2015) presented the wound healing efficacy of hyaluronic acid (HA) based on nanocarrier gel incorporating with curcumin in burn wound of rat. This HA had a better healing in wound more than treated with HA-curcumin or HA alone (Sharma et al., 2018). The study of Kant et al. (2014), showed curcumin combined with 25% of PF-127 hydrogel can increased enzyme activity and inflammatory cytokines, indicate enhance wound healing. This result, closely to Yen et al. (2018), reported the curcumin cooperate with hydrogel in male mice can increased inflammatory cytokines and good action on wound healing. Emiroglu et al. (2017) studied the effect of curcumin on nasal wound healing in 32 of Sprague-Dawley Albino rats' model by randomized into four groups as control, treated with 5 mg/mL, 10 mg/mL and treated with dimethyl sulfoxide (DMSO). They found, rats were treated with 10 mg/mL of curcumin can reduced the inflammatory response and significant accelerated wound healing. Mehrabani et al. (2014) administration of curcumin into decreased in size of burn wound and reduced inflammatory after 14 days in mice. They suggest that 2% of curcumin had great reduced in healing of burn wound. Opposite report from Sjahruddin et al. (2015), demonstrated the effectiveness of curcumin 0.5%, curcumin 5% and sodium chloride (NaCl) 0.9% on the acute wound healing of mice. They found no significant difference between applied of 5% of curcumin and 0.9% NaCl. After treated 7 days showed no significant all of three groups about the density of polymorpho nuclear (PMN)

and macrophage. From this result, concluded that the application topical curcumin is not better than NaCl 0.9% in acute wound healing in mice. In addition, curcumin showed decrease ROS production, but increase cellular proliferation, increase antioxidant enzymes; superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) (Kant et al., 2014).

The plant, *Chromolaena odorata* Linn. (Siam weed) is in the family Asteraceae with about 165 species that are distributed in tropical and subtropical (Jiraungkoorskul & Sirinthipaporn, 2017). It has been used variety of aliments in many tropical countries. Many studies had demonstrated that Siam weed extract stimulate hemostasis and wound healing management (Pandith et al., 2013). The phytochemical substances in leaf of *C. odorata* were used for antibacterial, antifungal, anti-inflammatory, anticancer, antidiabetic, antidiarrheal and help hepatoprotective activities. These advantages are from many reviews literature of *C. odorata* dried leaf. The dried leaf contained 31% of carbohydrate, 18% of crude protein, 15% of mouisture, 15% of fiber, 11% of crude fat and 11% of ash. The active substances are found flavonoid aglycones substance, terpenes, terpenoids, essential oil, alkaloids, saponins, tannins, phenolic acid and found chromomeric acid (Jiraungkoorskul & Sirinthipaporn, 2017). Same research with Ukwueze et al. (2013) showed the ointments containing in leave extract of *C. odorata* that compound with alkaloids, flavonoids, tannins, terpenoids and carbohydrates were the major constitutes present. Addition result, the leaf was extracted with acetone fraction had highest percentage of wound contraction and shortest epithelialization. The benefits for wound healing are enhances the fibroblast and keratinocyte proliferation and the aqueous extract from leaf has been treated the soft tissues burn or skin infections (Phan et al., 2001). Several studies were applied *C. odorata* to wound healing process, such as the study of Mahmood et al. (2005) reported that the honey combined with 10% of *C. odorata* aqueous extract can decrease scar formation and period of epithelialization in adult male Sprague-Dawley rats. Pandurangan et al. (2015) investigated the wound healing activity of *C. odorata* leaves extract ointments for 14 days in rat. These activities were

tested at 2.5%, 7.5% and 10% of leaves extract. They found that varying concentration of this herb was produced cutaneous wound dressing activity by inducing wound concentration and closure time. Pandith et al. (2013) studied the effect of *C. odorata* leaf extract on hemostatic and has been used to stop bleeding in wound healing activities. This extract was promoted the Balb/c 3T3 fibroblast cell migration, proliferation and found the oxygenase-1 enzyme that expedite wound healing. Seyed et al. (2017) investigated the efficacy of *C. odorata* leaf extracts for the healing of rat excision wounds. The result presented 5% (w/w) of leaf extract treated the rat group that exhibited a faster reduction in wound area that compared to control and group treated with Betadine solution. In addition, the group that applied with this leaf extract can increased collagen synthesis by increase hydroxyproline and hexosamine level and expression of collagen.

For the effect of Povidone-iodine (PVI) in wound healing, it has been used for several broadcast activity; antimicrobial effect, penetrated biofilm and lack of acquired resistance (Bigliardi et al., 2017). It reduced wound infection after gridiron appendectomy (Gilmore et al., 1977). Iodine is a combination between of molecular iodine and polyvinylpyrrolidone surfactant, and has been used as an antiseptic in the treatment of wound. The polyvinyl pyrrole iodine caused microorganism cell death. This act directly to the cell surface of microorganism by the free iodine penetrates into cell wall and target protein, fatty acid and nucleotide. Wang et al. (2017) studied the effect of Povidone-idodine to transforming growth factor β (TGF β) that play role in enhancing wound healing. The result found 0.5% of PVI on acute skin wound can attenuate congestion, edema and pain induced by pressure sores. In addition result, PVI treated rat skin can enhance wound healing via promote the expression of TGF β, α SMA, neovascularization and epithelialization. However, some reports showed the PVI have risen concern over allergy and toxic effect on host cell (Bigliardi et al., 2017). All reviews can summarized that the some herbals plant and PVI can good act in wound healing. So, in this study, we focus on the objective to the effect of some herbals to keep surgical wound

compared with the general medicine.

Research Objective

To determine the effect of *C. odarata*, *C. longa* extraction and Povidone-iodine on surgical wound healing of adult female rats.

Research Methodology

1. Animal and animal's preparation

We obtained 16 adult female rats, 4-5 months of age and each rat was weighted 250±5 g. All rats were received from the Medical Faculty, Khon Kaen University. After acclimation, the animals were divided into four groups include group 1 was negative control (rats no treated with herbal or PVI), group 2 was positive control (rats treated with 10% of PVI), group 3, rats were treated with 6% of *C. longa* extract (w/w) and group 4, rats were treated with 6% of *C. odorata* extract. All groups were carried out under aseptic conditions in laboratory. The animals were housed one per cage, maintained under controlled the environments 25±2°C, 65-70% HR and balanced diet with free access to food and water ad libitum. Before treatment, all rats were knocked with ether. Then, shaving and surgical wound were performed on the area of 1 cm long x 1 cm wide, that located in the back region of the body of rats. Each rat was received the 0.1 ml of penicillin antiseptic medical for reduced pain. The animals were just treated the herbal extraction and PVI immediately.

2. Plants material collection and plant extraction.

The herbal plants, Turmeric and Siam weed were collected from Kalasin province, Thailand. The rhizomes of turmeric and the leaf of Siam weed were chopped and dried in hot air oven at 60°C for 24 h. The dried samples were ground into moderate powder. Then, 50 g of powder of each plant was extracted with 250 ml of 70% ethanol at ratio 1:5 w/v by shaking at 25°C, 120 rpm for 24 h. The mixer was filtrated through the Whatman filter paper no. 1, and concentrated by rotary evaporator at 40°C until constant 1/6 (v/v) of the mixer. The bioactive from each plant was kept in a closed flask at 4°C until used.

3. Wound healing determination

The method of wound healing measurement was applied from Pandith et al. (2013) and Mehrabani et al. (2014). Sixteen of adult female rats were divided into four groups, four healthy rats per group. After shaving and surgical wound, and the rats were injected the penicillin for reduced pain. Treatments group were applied the herbal extraction over the lesion. Wounds were examined at day 3, 6 and 9 after treatment. This wounds was recorded for any changes in appearance and wound healing by measured the length of wound area and reduction of wound in the time range course. The histological evaluation was prepared after finish complete test, the section from the back tissues region was preserved in 10% of formalin. This tissues was stained and microtome section by Faculty of Medicine, Khon Kaen University. The slides were viewed and described under the light microscope. Picture of all groups were taken by digital camera before and after finish tested. The histological diagnosis under the light microscope.

4. Statistical analysis

All data were analyzed by using the IBM Statistical Package for the Social Science ver. 19.0. The average of length and reduction of wound were used One-Way ANOVA of SPSS program. Difference between groups were compared analyzed by least significant difference (LSD). The statistical results were showed as Mean±S.D. and group effects were reveal and Duncan Multiple Range Test that employed significant at $P<0.05$.

Results

The experiment presented the wound on female rats in all groups were seen in the fig 1. However, our results demonstrated improve wound healing within 9 days in povidone-iodine, *C. longa* and *C. odorata* treated female rats. The experiment found the *C. odorata* extraction showed great result to reduced wound healing and wound reduction more than the result of *C. longa* and povidone-iodine. All rats were found that a decrease in wound size at day 3, 6 and 9 were presented in all groups, there are significantly difference among group within the finish experiment (Table 1).

Wound reduction at day 3, the negative control group showed highest of wound reduction at 0.21 cm and rats were treated with 10% of povidone-iodine (0.14 cm) had least of wound reduction, but all groups were shown not significant difference. However, the results at day 6 and day 9 were presented different significant ($P<0.05$) of wound reduction at the same time. These results at day 6 found the female rats were treated with 6% of *C. odorata* had highest of wound reduction (0.46 cm), followed by group treated with 6% of turmeric (0.37 cm), rats treated with 10% of povidone-iodine (0.34 cm) and negative group (0.31 cm). The wound reduction at day 9 demonstrated similarity with the result at day 6. The female rats were treated with 6% of *C. odorata* had highest of wound reduction (0.64 cm), followed by treated with 6% of *C. longa* (0.52 cm) and treated with 10% of povidone-iodine (0.49 cm), but, rats in negative group showed least of wound reduction (0.47 cm). From all results, we can conclude that the *C. odorata* extraction can be reduced lesion of the wound with highest the average of wound reduction at 0.43 cm. The result of wound area of wound healing in all groups demonstrated similar with the result of wound reduction. The adult female rats that treated with *C. odorata* extract had greatest result of wound area at all day treated. The finish experiment at day 9, rats were 6% of *C. odorata* can reduced wound area from 1.00 cm to 0.57 cm. In addition result, rats were treated with 6% of *C. longa* extract showed the wound area reduced to 0.65 cm, followed by rats in negative group fond wound area at 0.67 cm and rat treated with 10% of PVI showed wound area at 0.68 cm (Fig. 2).

Figure 1. The adult female rats were injured on the back position with 1 cm x 1cm diameter. G1 is negative control, G2 treated with 10% of povidone-iodine, G3 treated with 6% of *C. longa* extract and G4 treated with 6% of *C. odorata* extract.

The histological evaluation, rats in negative group exhibited collagen, connective tissues, lymphocyte and macrophage. This cytological similar with the tissue of rats were treated with 10% of PVI found collagen, found connective tissues, lymphocyte, macrophage and neutrophil. However, rats were received 6% of *C. longa* and *C. odorata* extract showed similar diagnosis as collagen, connective tissues, lymphocyte, macrophage, neutrophil and leukocytes.

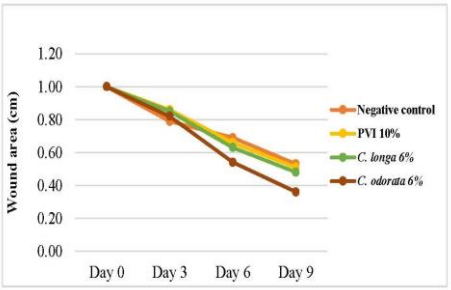

Figure 2. An effect of povidone-iodine, *C. longa* and *C. odorata* extracted on wound area contraction (cm2) of skin wound healing in adult female rats at Day 0, 3, 6 and day 9. The orange line is group 1, yellow line is group 2, green line is group 3 and red line is group 4.

Table 1.

The effect of povidone-iodine, *C. longa* and *C. odorata* extracted on wound reduction (Mean±S.D.) of adult female rats at day 3, 6 and 9 of the experiment.

day	Negative control (cm)	Povidone-iodine (cm)	Turmeric (*C. longa*) (cm)	Siam weed (*C. odorata*) (cm)
Day 3	0.21±0.06 a	0.14±0.08 a	0.15±0.04 a	0.18±0.06 a
Day 6	0.31±0.04 a	0.34±0.06 ab	0.37±0.09 ab	0.46±0.07 b
Day 9	0.47±0.03 a	0.49±0.08 a	0.52±0.02 a	0.64±0.06 b
Average	0.33	0.32	0.35	0.43

The results are as mean of wound reduction at day 3, 6 and 9. The value of a, b in each row at the same time followed by a different letter indicate significant differences ($P<0.05$) according to Duncan test.

Discussions

Successful wound healing management is important for the quality of life of the patients in human and animals. This studies was demonstrated Thai herbals as *C. longa* and *C. odorata* extraction that can be distributed in local in Kalasin province or general in Thailand. This we were compared the herbals with general medical antibiotic as PVI and negative control rats. All resulting concluded that Thai herbal as Siam weed or *C. odorata* had greatest result in wound reduction and wound area, these mean the wound healing action. The result had great action in wound healing that similar with the study of Ukwueze et al. (2013) they reported that 5% of *C. odorata* was extracted by methanol show good wound healing in rat at day 9 as 23.33 cm of wound area. This plant was attributed to presence the phytoconstitutents like tannins, flavonoids and terpenoids. All phytochemicals had reported and confirmed to reduce wound area, antimicrobial and anti-flammatory properties might equally to wound healing effect. In addition study from Seyed et al. (2017) concluded that 7.5% of *C. odorata* aqueous leaf extract can reduced wound area in Albino rats from 1.80 cm to 1.21 cm, but 5% of aqueous leaf extract showed great faster reduction of wound area at 0.51 cm at day 16 the finish experiment. The majority phytochemical analysis of aqueous leaf extracts of *C. odorata* found conclude saponins, tannins, phenol and alkaloids. The rats were treated with 5% of *C. odorata* leaf extract showed increase the level of hydroxypoline, hexosamine and total protein. However, this study investigated that *C. odorata* of varying concentration can also exert cutaneous wound healing activity. *C. odorata* exhibited its wound healing property using multiple mechanisms. Theses mechanisms can be summarized as follows: these plant extract contains the many compounds that enhance wound healing. It can be reduced the bleeding and clotting time may be the first of action in the physiology of wound healing (Jiraungkooskul & Sirinthipaporn, 2017). *C. odorata* can protected the cells from destruction by inhibiting the imflammatory mediators. The important action can antibacterial activity by against both positive and negative grams of bacteria. Wound repair

consists of a decade of events that re-establish the injured of damage tissues. The repair damage is categorized into haemostasis, inflammatory, proliferation and remodeling phases. The healing require the collaborative efforts of numerous tissues and cell lineages (Seyed et al., 2017). This action may involve platelet aggregation, blood clotting, fibrin formation, angiogenesis and re-epithelial. The healing process is not complete until the disrupted surfaces are firmly closed together collagen (Owoyele et al., 2008; Seyed et al., 2017).

For the great action with 6% of curcumin or *C. longa* extraction, this plant has many research could confirmed the benefit of it's used in rats model. This study we can summarized this concentration showed high of the wound reduction and reduced wound area from 1.00 cm to 0.65 cm at day 9. This similar result with previous study of Sjahruddin et al. (2015), they tested the rats with 5% of *C. longa* extraction. The study showed at day 7 of experiment, this concentration had reduced wound diameter from 0.5 cm to 0.28 cm. The percentage of density of polymorpho nuclear (MPN) and macrophage presented at 32.4% and the thickness of fibroblasts play at 9 μm. Curcumin (difeurlolyl-methane) is the most majority action in anti-inflammatory, anti-infectious, antiapoptotic that action in wound healing (Shuid et al., 2018). If the concentration of curcumin less than the above suggested that it could be action for long time. Example study, Bhaskar-Rao et al. (2015) reported 2% of curcumin with complete healing at 21 days. These reason were accorded with the study by Emiroglu et al. (2017), they demonstrated that rats were treated with 10 mg/mL of curcumin at day 15 had significantly reduced edema, cellular hyperplasia and leukocytic infiltration to the normal level and accelerated wound healing. This curcumin exerts anti-inflammatory, anti-infectious and antioxidant effect. Wound healing and tissues repaired in burn injuries are considered as a complex process as inflammation, granulation and remodeling of the tissues (Mehrabani et al., 2014). Curcumin substance excellent in inhibited the production of two cytokines (IL-1 and TNF-α) that active the monocytes and macrophage. This macrophage play role in regulate the imflammatory response (Emiroglu et al.,

2017). Curcumin prolonged the imflammatory phase only minimally and accelerated the proliferation phase, which commences 4 to 21 days after injury (Lima et al., 2011). Beside this, it should be able to induced cell proliferation for re-establishment of cell damage. In addition reason, should be an antioxidant since free radicals are the main cause of imflammatory in wound repair (Choudhary and Shivakumar, 2018). These can be concluded that the curcumin exhibits powerful modulating effects on healing by acting on various phase of skin wound healing.

The medical PVI, is widely and general concentration used at 0.5%-10% of PVI in health care. The use of PVI as an effective and antiseptic to prevent the bioburden in wound healing, that supported by many in vitro and in vivo testes (Bigliardi et al., 2017). This medical has broad spectrum of activity, the ability to penetrated biofilm and antibacterial. Some studies reported the application of 0.5% of PVI can promoted acute cutaneous wound healing and that TGF-β has play role action in this process (Wang et al., 2017). However, some studies fond about the allergy and cytotoxicity could often raise *in vitro* cellular and *in vivo* animal studies. Seyed et al. (2017) they indicated that the concentration more than 10% w/w of Betadine or PVI and herbal extract of *C. odorata* could be lethal for animals.

In addition good reason, the histological that exhibited of lymphocyte and macrophage. This result showed that rats were act in good response to skin injury. More result, the cytological of the tissue found collagen and connective tissues can diagnosis as the rats were trended to complete the composition of skin tissue. All evidences of histological are very important to support the effect of herbal extraction act and apply used in wound healing.

Conclusions

In summary, our study found 6% of *C. odorata* leaf extract had greatest result in wound healing in female rats. This is evident from the substantial increased wound reduction and decreased wound area, which are necessary of wound healing. In addition, the rats that applied with leaf extract can increased collagen synthesis by increase

hydroxyproline and hexosamine level and expression of collagen. Consist of increase the connective tissues, enhance the fibroblast and keratinocyte proliferation. Hence, based on the present study we conclude that *C. odorata* is a promising wound healing agent and this study has attempted to confirm the use of *C. odorata* to be the medicinal plant, to be the one choices in the wound treatment.

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