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Comparative Wound Healing Study of *Ocimum tenuiflorum* Leaves Extract and its Formulation on Sprague Dawley Rats

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This study has been designed to compare the leaves extract of *Ocimum tenuiflorum* with soy lecithin and the leaves extract of *Ocimum tenuiflorum* only and find out its effectiveness on wound healing properties. Drug release study was also evaluated to obtain a brief idea on the release of the drug after consumption of the formulation and extract. The wound healing effectiveness study suggest that the formulated extract has better wound healing activity in comparison to extract which is more similar to the standard drug which was used as positive control. It was also found that the drug release activity of the formulation shows sustained release of the marker compound in when compared to the extract. Thus, it can be concluded that the improved wound healing activity of the formulation could be due to the sustained released of the compounds which present that act accordingly to improve the activity. Further study on its mechanism of action need to done to understand it activity better.

Keywords: wound healing; *Ocimum tenuiflorum*; in vivo; soy lecithin

I. INTRODUCTION

Wound healing is a natural process that restore damaged wound tissue into its original state when injury occurred (Tan *et al.*, 2019). Prolonged inflammation condition can eventually affect the wound healing process (Maver *et al.*, 2018). This healing process need the association of several body physiological activities such as reversal of cytotoxicity, suppression of inflammation and stimulation of cellular viability and proliferation (Amin *et al.*, 2015). Hence, the aim of wound care is to ensure the products to promote tissue healing in shortest time possible, avoid secondary infections and reduce pain, discomfort and scarring by inducing tissue repair and regeneration (Builders *et al.*, 2013).

Herbal medicines have been used worldwide for several decades. The reason behind it was due to the presence of the active phytoconstituents (Jahan *et al.*, 2016). Thus, these plants can be used as an alternative and complementary herapeutics for improvement in wound healing process based on their multiple active and effective compounds (Hajialyani *et al.*, 2018). According to Mazumder *et al.* (2016), formulating plant extracts improve its effectiveness by resolving its physicochemical property issues; hence better biological and therapeutics activities can be seen.

Ocimum tenuiflorum is widely known for its medicinal properties. In Ayurvedic herb, it is called Tulsi, listed in Malaysian Herbal Monograph and Globinmed has proven lots of medicinal benefits including wound healing (Malaysian Herbal Monograph 2015, 2019). In this study, *O. tenuiflorum* leaves extract has been formulated using soy lecithin complex formulation.

Lecithin can be generally found in animal tissues and vegetables and its extensive amount can be obtained especially from soy bean (Pires *et al.*, 2017). Scientifically, lecithin is referring to phosphatidylcholine which was

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historically known as lipid molecules containing phosphate group that is isolated from brain or egg (Gnananath *et al.*, 2017). Amphiphilic properties of lecithin have shown increased in solubility and ease the permeation across the phospholipid bilayer of epithelium (Zhou *et al.*, 2017) which is beneficial because it enhances the bioavailability simultaneously (Lu *et al.*, 2018).

Similar to other flavonoids type compounds, apigenin also have been proposed to have antioxidant, anti-inflammatory, antimicrobial, anticancer and other medicinal properties (Erdogan *et al.*, 2017). It was reported that apigenin helps the epithelization of the wound and possess anti-inflammatory properties which is an essential factor in wound healing process (Manivannan, 2016). In this paper, apigenin was used as a marker compound to study the drug release activity. Apigenin was selected based on the liquid chromatography-mass spectrometry (LCMS) screening of the leaves extract. Many topical based product is available, hence this study has been designed to compare the leaves extract of Ocimum tenuiflorum with soy lecithin and the leaves extract of Ocimum tenuiflorum only and find out its effectiveness on wound healing properties especially to patients who do not like the peculiar taste/odour of snakehead fish and sea cucumber when taken orally.

II. MATERIALS AND METHOD

A. Plant Material Collection and Extraction

O. tenuiflorum plants were collected from the botanical garden, Perak only to resolve the issue about standardization of apigenin as marker compound. The plant was identified and authenticated from School of Biological Sciences, Universiti Sains Malaysia (USM) (Herbarium number is 11400).

A 1 kg of the leaves was washed with running tap water. The washed leaves were oven dried at 60 °C for 24 hours. Then the weight of the dried sample was approximately 100 g, and the leaves were ground to fine powder mesh 60. The weight of the powdered leaves was approximately 90 g and stored in air-tight container in freezer -20 °C.

Aqueous extraction method was chosen based on the effectiveness of the plant in wound healing study done by Shetty *et al.* (2008) while the extraction steps according to (Rabeta & Lai, 2013) with some modification. Approximately 10 g (modification step) of leaves was mixed with 100 ml of distilled water in a conical flask. The mixture was then shaken in an orbital shaker at 160 rpm at 27 °C for 24 hours followed by centrifugation at 3500 rpm (modification step) for 30 minutes to separate the sediment and supernatant. The supernatant was transferred to a beaker and evaporated using the oven at 60 °C until thin layer dry extract was obtained. The obtained extract was stored in an airtight container in freezer -20 °C until use.

B. Formulation Preparation

The formulation was prepared according to Wang *et al.* (2013). Soy lecithin and extract were dissolved separately using 50% ethanol until complete dissolution and mix together. The solutions beaker was closed and allowed to mix until equilibrium was reached for about 3 hours with agitation at 90 rpm in orbital shaker. During solvent evaporation, the beakers were opened and placed in oven at 45 °C until dried solids were obtained.

C. In vivo Wound Healing Study

Comparative wound healing study was conducted according Shetty *et al.* (2008) with slight modifications. Modification done was the dissolving agent used for diluting extract and number groups tested. Comparative wound healing study was conducted according Shetty *et al.* (2008) with slight modifications. Modification done was the dissolving agent used for diluting extract and number groups tested.

Total of sixteen male rats were used in this study. Adult female Sprague-dawley (SD) rats age 8-10 weeks old, weighing around 180-200 g were used in this experiment. Rats were acclimatized under 12 h light and dark cycle and allowed for free access to food and water. The experiment was carried out in accordance to Universiti Sains Malaysia (USM) animal ethics committee guidelines and approval. The ethical approval number was USM/IACUC/2018/ (111) (915).

All the rats were individually caged and divided in four groups which are the negative control, positive control, formulation and extract. Negative control group received 200 mg/kg BW of plant lecithin solution, positive control group received 222 mg/kg BW of Channa striatus solution while 400 mg/kg BW and 200 mg/kg BW of formulation and leaves

extract were given to formulation and extract group, respectively. Religious and vegetarian lifestyle choices may prohibit certain consumer groups from taking animal-based wound healing products (Boyer, 2013). Effective wound care from plant-based is needed because some people do not like the peculiar taste of Channa striatus and sea cucumber when taken orally, which are the popular animal-based products with wound healing properties. In our in vitro study showed that, at dosage of 400mg/kg body weight the wound heals faster than the extract treated group (Rohini *et al.,* 2019). The dose treatment for extract group was obtained from (Shetty *et al.,* 2008) as baseline dose and equivalent dose of formulation was chosen for the formulation group treatment. All the treatments were done once daily via 50 µl oral administration using oral gavage needle for 15 days until the treatment group animals wound closed. Positive control dose was calculated by converting human dose to rat dose based on animal equivalent dose table (Nair & Jacob, 2016).

D. Wound Creation

Rats were anaesthetized using ketamine and xylazine intraperitoneally 50 mg/kg and 5 mg/kg body weight (BW) respectively prior to wounding. Excision wound was created by cut off a circular piece of skin, thickness ~ 500 mm2 at the dorsal interscapular region of the rat. The wound size was traced using a plastic sheet on the day of wounding and consequently every three days until healing was complete. The wound contraction was calculated using the formula below (Shetty *et al.,* 2008):

Wound contraction = (healed wound area/ total wound area) × 100

E. In vitro Drug Release Assay

Equivalent amount of extract and formulation samples were weighed and dissolved in 4 ml of water. The samples were sonicated at 45 °C at 60 kHz for 15 min until the samples were completely dissolved. The samples were transferred to 4-inch long dialysis tube (cellulose membrane molecular weight cut off range 12,000- 14,000) separately and secured with clips at both ends. The tube was incubated in a beaker containing 80 ml of PBS, pH 7.4 in beaker at 37 °C under mild shaking in incubator shaker. About 1 ml of aliquot was withdrawn from the incubation medium of both samples at specific intervals of 0, 15, 30 min and every 1 h for 12 h. Each time an aliquot was withdrawn, equal volume of PBS was added to the solution. The amount of drug release into the incubation medium was analyzed using UV- spectrophotometer at wavelength of 340 nm using apigenin (API) as reference (Telange *et al.,* 2017).

G. Statistical Analysis

Results were tabulated with SPSS software version 20 and displayed as mean +SEM. The SEM quantifies how precisely you know the true mean of the samples. Data were statistically analyzed using one-way ANOVA and Tukey post host descriptive test where $p<0.05$ were considered statistically significant.

III. RESULT AND DISCUSSION

A. In vivo Wound Healing Study

The comparison was made based on the treatment. Figure 1 shows the wound healing activity of four different groups. On 9th day, it was observed a significant reduction ($p<0.05$) in wound size for the formulation group compared to the extract group. Besides, it was also observed that the wound healing activity of the formulation treated group was equivalent to the positive control group. In overall, it can be observed that the formulation treated wound closes faster ($p<0.05$) when compared to extract treated group which is within fifteen days of the study. In Figure 2, the macroscopic view of the rat wounds before and after treatment of the test samples support that the wound is completely closed within the study period for formulation treated rats when compared to the extract treated rats.

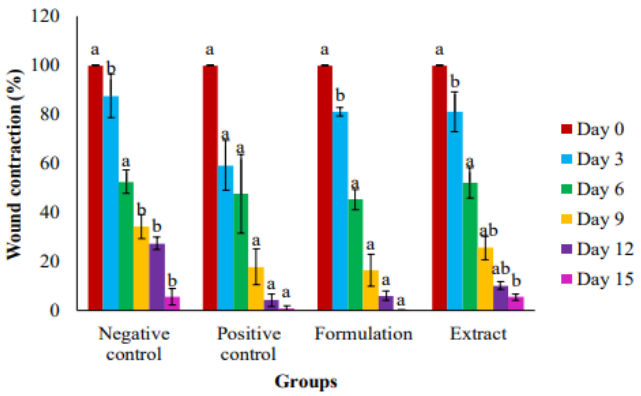

Figure 1. The wound healing activity of different treatment given to rats

*Value represented as mean ±SEM (n=4) and different alphabets were considered statistically significant when ($p<0.05$).

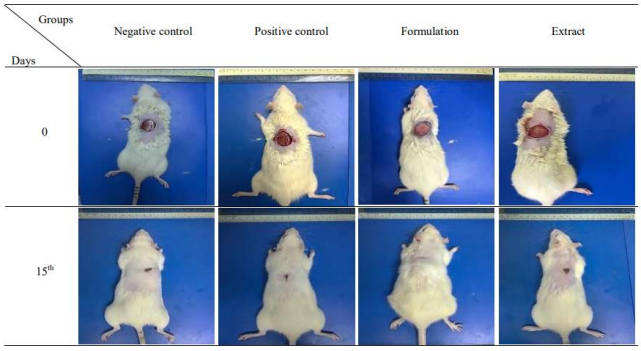

Figure 2. Macroscopic view of wound healing process in rats treated with formulated and non-formulated aqueous extract of *Ocimum tenuiflorum* leaves

It was also of interest to determine whether extract formulated, could enhance the wound healing activity. Based on the obtained results, it showed that the formulation treated rats wound healed faster compared to the extract treated rats. This suggest that the formulation do enhance the wound healing activity of the extract when it is formulated rather than its original state. This improvement in the wound healing activity could be related with the complex action formed between the lecithin.

Religious and vegetarian lifestyle choices may prohibit certain consumer groups from taking animal-based wound healing products (Boyer, 2013). Effective wound care from plant-based is needed because some people do not like the peculiar taste of snakehead fish and sea cucumber when taken orally, which are the popular animal-based products with wound healing properties. People with seafood allergies should avoid the use of sea cucumber for wound healing purpose (Wan Zanariah *et al.*, 2016). Plant-based wound healing may cause minimal adverse effects (Ekor, 2013).

B. In vitro Drug Release Assay

Figure 3 shows that the amount of API released to the environment (PBS solution) was significantly increased ($p<0.05$) up to 7th hours of study in both extract and formulation. However, the API release in extract had slight fluctuation during the 8th hour and increases slightly up to 10th hour. In contrast, the API release in formulation was significantly increased ($p<0.05$) up to 12th h of study when compared to extract whereby the release reduces significantly ($p<0.05$) after 10th h.

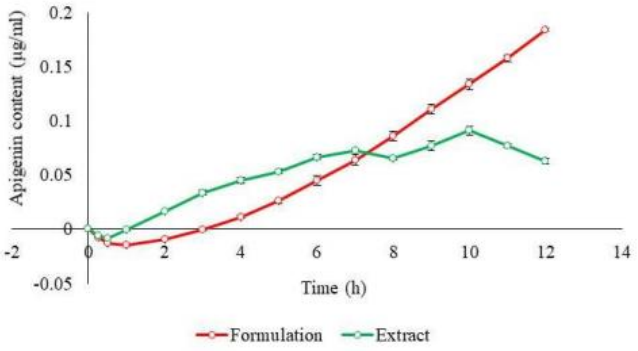

Figure 3. *In vitro* drug release of extract and formulation

In drug release study, the release of target marker compound API was observed to be increased up to 12 h when compared to extract which has fluctuated release. This result indicates that the complex action with lecithin has control the drug release whereby a sustained release of the drug can be observed. The sustained release activity may be due to the gradual drug dissociation from the complex and later diffuse to the medium through the membrane (Freag *et al.*, 2018). Hence, it can be said that the wound healing activity of the formulation was better and improved in formulation treated group was because of the slow release of the compounds. The release of compounds was controlled by the complex formed with lecithin which help the compounds stay longer in the body to give a better effect rather administering extract which shows fluctuated release after few hours.

Besides that, it was believed that by formulating the extract with lecithin, which is the type of phospholipid having both hydrophilic and hydrophobic site increases the bioavailability by wetting and dispersion action when the solubility was

enhance (Semalty *et al.,* 2010). Therefore, this can be said that the phospholipid complex of extract may have improve the absorption of active compounds to the lipid barriers in gastrointestinal tract after oral administration (Semalty *et al.*, 2012). This could be another reason why improvement in wound healing activity was observed in formulation treated group compared to extract treated group. Similarly, literature studies also reported that drugs or compounds that have been formulated with phospholipid have improved its effectiveness by improving its absorption to target site when compared to the application of crude compounds or drugs alone (Kumar *et al.*, 2014).

IV. CONCLUSION

The comparative study between the extract and formulation suggest that, formulating the extract with phospholipid has improved its wound healing effectiveness by sustained release of the compounds to the target site which can be seen through the drug release study. Hence, formulating the extract to improved it biological activity has been a good attempt on *Ocimum tenuiflorum* leaves. Further study on its mechanism of action need to done to understand it activity better.

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