

Potential Use of Clove Oil Diluted in VCO On Contaminated Wound Healing

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Abstract

Essential oils, including clove oil, has been used broadly through human civilization due to its multiple benefits, including its utilization as natural antiseptics. In spite of its benefits, clove oil can cause irritation if applied neat to the skin, thus a carrier oil is needed for topical application of clove oil. Clove oil together with virgin coconut oil as a carrier, has potential synergic benefit if applied on contaminated wounds due to its enhanced antiseptic and anti-inflammatory properties. The aim of this study is to investigate the effect of topical application of clove (*Syzygium aromaticum*) oil diluted in Virgin Coconut Oil on contaminated wound. This study was a quasi-experimental research with post-test only control group design. Clove oil that had been diluted in VCO was applied on contaminated excisional wound in white male rat wistar strain once daily. The wound was inflicted using unsterilized scalpel. The wound was then left exposed to the environment without any dressing and the healing process was then evaluated by measuring the wound area percentage once every four days for sixteen days. Friedman analysis used in this study showed a significant result ($p < 0,001$) and Concentration of 5% clove oil diluted in VCO was found to be the most effective concentration based on the measurement of wound area percentage on the last day of measurement. This study concludes that the topical application of clove oil diluted in VCO showed a significant difference in wound healing process.

Keywords: Clove Oil, Wound Healing, VCO

INTRODUCTION

Essential oil is a volatile material isolated from one species of plant through physical method of extraction¹ It can be extracted from various parts of plants. Essential oil plays the role of giving plants its distinct aroma and as chemical signal that is used by the plant to regulate its surrounding². The oil itself is a secondary metabolite constituted of complex combination of terpenic hydrocarbons primarily monoterpene and sesquiterpene with oxygenated derivatives such as aldehyde, ketone, epoxy, alcohol, and ester³. It has been known to be used traditionally as natural remedies due to its broad antimicrobial properties capable of acting as antifungal, antibacterial, and antiviral agent⁴. Aside from its antimicrobial property, essential oil with its terpenoid constituents also have the potential of acting as antioxidant agent⁵.

Out of many essential oils that has been used through human civilization, clove oil is the oil that has been used in various facets of human life such as

industrialization, farming, culinary, and medicine⁶. Clove oil medicinal use can be observed in many cultures across the world, it has been used as natural antiseptics and as remedy for numerous symptoms both in their topical or inhalant form⁷. Its antiseptic property, along with anti-inflammatory potency, is due to its high eugenol content⁸.

One potential use of clove oil is to aid in wound healing process⁹. The loss of skin tissue integrity risks infection to occur and cause the worsening of inflammation that will disturb physiological process of wound healing¹⁰. This is one of the conditions that can benefit from clove oil's antimicrobial and anti-inflammatory properties. This usage has been demonstrated in previous research by Alam (2017) that found clove oil significantly influence the process of wound healing¹¹.

Despite their numerous benefits, clove oil does have its adverse effect. Clove oil, like other essential oil, has irritative effect if used neat or in high concentration

to the skin. Because of this, the oil needs to be diluted first before usage⁷. one of the carrier oils that can be used to dilute clove oil while also have synergic effect to its benefit is coconut oil.

Coconut oil is believed to make the clove oil less toxic to the skin, slows down its evaporation rate, and increase its absorption¹². Beside the potential combined effect of clove and coconut oil, coconut oil in itself could be beneficial to the skin due to its protective effect by enhancing skin barrier function and had been known to be used as skin moisturizer traditionally by the people in the tropical regions¹³.

Disturbance in wound healing process such as infection has the potential of becoming a problem to the patient and the healthcare system due to the increase of time and cost needed to treat the wound. Finding alternatives in wound care that are safe, effective, and easily accessible is important and relevant. This research aims to investigate the effectivity of clove oil diluted in virgin coconut oil (VCO) on contaminated excisional wound.

MATERIALS AND METHOD

The clove oil used on this research was first analyzed using gas chromatography-mass spectrometry method to investigate the oil's constituent. While the VCO used on this research is a BPOM (National Food and Drug Agency)-approved commercially available VCO.

This research was a quasi-experiment with post-test only control group design. After gaining the ethical clearance from the Faculty of Medicine Universitas Muhammadiyah Purwokerto health research ethics committee (research ethics number KEPK/FK/VIII/164/2019), twenty-five male wistar strain rats (*Rattus norvegicus*) that weighs 150-200 gram and aged 6-7 weeks were used in this research. The inclusion criteria are as follow: the animal is actively moving, no visible anatomical abnormalities, and not showing any skin-related diseases. Any animal that

shown to be sick are excluded from the research. Any animal found dead during the duration of the research are included in the drop out criteria and therefore eliminated from the research. Before the any treatments were induced, the animals underwent acclimatization period for seven days.

One full-thickness excisional wound with the depth of +/- 2 mm were induced on the interscapular region of all animals in this study using unsterilized scalpel. The wounds were then left exposed to the environment without any dressing before the animals were put into five different treatment group. Group I was the negative control treated with only VCO and 0 % Clove oil, group II was treated with 2,5% clove oil diluted in VCO, group III was treated with 5% clove oil diluted in VCO, group IV was treated with 10% clove oil diluted in VCO, and group V was the positive control treated with povidone iodine. The treatment consists of applying the oil mixtures and control once every day during the duration of the research.

Wound healing evaluation on this research is done with a similar method to Nagar (2016) by measuring the wound area once every four days using millimetres block paper and tracing paper. The data then presented using percentage of wound area on the day of measurement per original wound area induced. Once all data had been obtained, the animals used on this research were then terminated using cervical dislocation method.

The results of measurement were then analyzed using Shapiro-Wilk normality test followed by Friedman test.

RESULT

The gas chromatography-mass spectrometry analysis showed that the detectable constituents of the clove oil used in this research are as follow: eugenol (72%), caryophyllene (22,8%), humulene (3,24%), caryophyllene oxide (1,03%), and copaene (0,80%).

The number of data and the mean of each treatment group is presented in table 1. The percentage value is obtained by dividing the wound area on the day

Table 1 Wound area mean for each group

Treatment Group	Sample number	Day 0	Day 4	Day 8	Day 12	Day 16
Negative control	5	100%	83%	59%	26%	12%
Clove oil 2,5 %	4	100%	84%	43%	8%	6%
Clove oil 5 %	4	100%	80%	48%	15%	5%
Clove oil 10 %	5	100%	78%	46%	12%	5%
Positive control	4	100%	62%	34%	10%	2%

of the measurement with the original wound area. From the table, it can be obtained that the groups have varying number of sample size. This is due to some animals were included into the drop out criteria during the period of the research.

Normality test was carried to determine the data distribution. By having less than fifty samples, the method most suitable for this research was the Shapiro-Wilk test. From table 2, It was found that not every group has the p value more than 0,05 thus concludes that the data distribution was not normal.

Table 2 Normality Test Results

Variable	P	Note
Wound healing on day 0	-	-
Wound healing on day 4	0.206	Normal
Wound healing on day 8	0.749	Normal
Wound healing on day 12	0.001	Not normal
Wound healing on day 16	0.003	Not normal

The hypothesis of this research was there was a significant effect on the topical application of clove oil diluted in VCO on contaminated wound in male wistar strain rats. This hypothesis was then analyzed using comparative Friedman test. Table 3 show that the analysis results has the significance value of $< 0,001$ meaning that the results was significant.

Table 3. Hypothesis Test results

Measurement days	P	note
Wound healing day 0		
Wound healing day 4		
Wound healing day 8	< 0.001	significant
Wound healing day 12		
Wound healing day 16		

DISCUSSION

The gas chromatography-mass spectrometry analysis of clove oil used in this research revealed eugenol as it's constituent with the largest percentage. This is similar with previous research done by Uddin (2017) that also found eugenol to be clove oil main constituent⁸.

The success of transdermal therapy depends by the ability of the therapeutic substance to penetrate the stratum corneum of the epidermis through its intercellular lipid structure. One way to assist the penetration of the therapeutic substance through this least permeable layer of the skin is by using a vehicle that is absorbable by the skin. This type of vehicle are the ones that contains fatty acid, one example being the virgin coconut oil. The fatty acid can increase the skin permeability by disordering the alkyl chain within the intercellular space causing lipid fluidization of the

stratum corneum. This action will increase the permeability of the therapeutic agent applied together with the carrier oil¹³. This was the reason why the research combined clove oil with virgin coconut oil.

VCO with its constituents plays role in skin protection by keeping the balance between water-ion in skin cells through increasing the expression of aquaporin, a transmembrane canal that facilitates water transport through epidermal cell's membrane¹⁴. Furthermore, VCO also significantly increases filaggrin expression, a protein needed in the development of epidermal corneocytes which intracellular metabolism aids in skin hydration¹⁵.

The negative control group which was only given VCO topical application shown to have larger percentage of wound area on the last day of measurement compared to other groups. This could be attributed to the fact that the VCO alone mainly only act as skin emollient with minor effect on inflammation and did not contain significant antimicrobial or anti-inflammatory properties like clove oil does to aid with the wound healing process¹⁶.

Treatment groups that combined clove oil with VCO were found to have faster wound healing process judged by the wounds area percentage shrinkage on the five consecutive measurement days. This could be attributed to the properties possessed by the clove oil and the synergic benefit the VCO brings. The use of VCO as carrier oil could slows down evaporation rate of the clove oil making it having longer absorption rate while diluting it so that the cytotoxicity effect of the clove oil can be minimalized¹².

On the fourth day of the observation period, groups treated with clove oil and VCO mixture with varying degree of concentrations were already having smaller wound area percentage compared to the negative control. This could happen due to the eugenol effect on wound healing process that had entered its inflammation phase. During this phase, the number of macrophages would reach its peak¹⁷. These macrophages would produce large number of inflammatory mediators to eliminate pathogenic bacteria that might colonize the wound. The casualty of this action was the tissue surrounding the wound could be also damaged along with the bacteria. This was due to the high number of protease and ROS released by the macrophage. This is when clove oil's eugenol played its role. Eugenol had the ability to lower macrophage's migration rate to wound site thus minimized the excessive inflammatory mediators and ROS¹⁸.

As the wound healing progresses, on measurement day 8 and 12, the wound area percentage shrinkage was consistent with that of the fourth day. Groups treated with clove oil and VCO mixture had smaller wound area percentage compared to the negative

control. During this period, the wound healing process entered its proliferative phase¹⁹. In proliferative phase, the body tried to replace the damaged tissue by forming granulation tissue, angiogenesis, collagen deposition, epithelialization, and wound retraction. These processes all happened on the same time¹⁷.

One of the mediators that contributed to this phase was matrix metalloproteinase (MMP). MMP functions to regulate extracellular matrix degradation and wound re-epithelialization. Although its role was important, uncontrollable expression of MMP could potentially disrupt wound healing process by inducing uncontrolled epithelial migration, degradation of newly-formed matrix, and increasing keratinocyte cell deaths on wound edges²⁰. There are multiple factors that can contribute to MMP upregulation, one of which is the pathologic state of inflammation that associated with increased proteolytic activity and defective macrophage function²¹.

Previous research by Nam & Kim (2013) reported that eugenol can inhibit the expression of MMP²². This is consistent with another research by Tsai (2017) that demonstrated MMP expression inhibition property of eugenol extracted from cloves²³. This ability of eugenol might contribute to the faster wound healing process observed on day 8 and 12 in this research.

Between the varying degrees of concentration of clove oil used in this research, one concentration with the highest effectivity is the 5% of clove oil in VCO. This is because compared to the wound area percentage on the last day of the measurement, both 5% and 10% group has the same percentage. Thus, the group with least concentration while bring the most benefit was chosen.

This research concludes that there is a significant correlation between the topical application of clove oil diluted in VCO and contaminated wound healing process in male wistar strain rats (*Rattus norvegicus*).

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CONFLICT OF INTEREST

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