

THE EFFECT OF 40% RED BETEL LEAF SOLUTION ON THE NUMBER OF BACTERIA COLONIES, AND EXUDATE IN CHRONIC WOUNDS: A CASE SERIES

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ABSTRAK

Luka kronis adalah luka yang membutuhkan waktu lebih lama dari proses normal penyembuhan luka, disebabkan adanya gangguan selama proses penyembuhan luka. Pelayanan keperawatan yang diberikan secara menyeluruh salah satunya adalah perawatan luka yang harus dilaksanakan sesuai dengan prosedur tetap. Prosedur perawatan luka ini bertujuan agar mempercepat proses penyembuhan dan bebas dari infeksi. Metode perawatan luka secara herbal diantaranya dengan menggunakan larutan daun sirih merah. Studi ini bertujuan untuk mengetahui pengaruh larutan daun sirih merah 40% terhadap jumlah koloni dan eksudat pada luka kronis pasien *home care* di wilayah Banyumas. Studi ini merupakan case series yang mengikutsertakan 6 responden dengan luka kronis dengan perawatan luka di rumah. Perawatan luka dengan larutan daun sirih merah 40% sebagai bahan pencuci luka dilakukan sebanyak 3 kali dalam 1 minggu. Koloni bakteri dan jumlah eksudat diambil sebelum dilakukan perawatan luka 1 (pre-test) dan setelah perawatan luka ke 2. Sampel dianalisis di laboratorium Farmasi UMP. Analisis data yang digunakan dalam penelitian ini menggunakan Uji T- test. Hasil penelitian menunjukkan bahwa usia responden dengan rata-rata 66,53 tahun dengan jenis kelamin yang paling dominan perempuan (66,7%). Penghitungan jumlah koloni bakteri sebelum diberikan terapi dengan larutan daun sirih merah 40% adalah 1266.67 ± 152.75 . Sedangkan setelah diberi larutan daun sirih merah dengan 1x pengukuran adalah 1040.00 ± 495.17 (p value=0.325). Mengikuti sedikit penurunan koloni bakteri, jumlah eksudat juga berkurang. Meskipun terjadi penurunan jumlah koloni bakteri, namun secara statistik jumlahnya tidak signifikan. Namun larutan tersebut masih dapat digunakan sebagai alternatif larutan berbahan dasar herbal untuk membersihkan luka kronis yang terinfeksi dalam pengobatan luka.

Kata Kunci : Larutan Daun Sirih Merah 40%, Perawatan Luka, Luka Kronis

ABSTRACT

Chronic wounds are long-standing or chronic wounds with longer healing due to disturbances during the wound healing process. One of the nursing services provided as a whole is wound care which must be carried out according to standard procedures. This wound care procedure aims to speed up the healing process and be free from infection. Herbal wound care methods include using red betel leaf solution. The aim of this study was to determine the effect of 40% red betel leaf solution on the number of colonies in chronic wounds of home care patients in the Banyumas Region. This study was a case series study. 6 patients with infected chronic wound were selected using convenience sampling method. Wound care with 40% red betel leaf solution as a wound cleansing was carried out 3 times in 1 week. Bacterial colonies and the amount of exudate were taken before the 1st wound treatment (pre-test) and after the 2nd wound treatment. The samples were analysed at the UMP Pharmacy laboratory. Data analysis used in this research was T-test analysis. The research results show that the age of the respondents with an average of 66.53 years with the most dominant gender is female (66.7%). Measurement 1x before being given a red leaf solution was 1266.67 ± 152.75 obtained an average number of colonies. Whereas after being given red betel leaf solution with 1x measurement was 1040.00 ± 495.17 (p value=0.325). Following the slight decrease of the bacterial colonies, the amount of exudate also reduced. Although there was a decrease in the number of bacterial colonies, the number was not statistically significant. However, the solution still can be used as an alternative solution herbal based for cleansing an infected chronic wound in the wound treatment.

Keywords : 40% red betel leaf solution, wound care, chronic wounds

BACKGROUND

Healing of chronic infected wounds, such as diabetic foot wounds and cancerous wounds, requires a long process and complex wound treatment. One of the factors that determine the success of healing chronic infected wounds is wound care. Wound care that supports healing includes wound care techniques and materials used (Han & Ceilley, 2017)(Kusumastuty & Dewi, 2020). Research states that the challenge in healing chronically infected wounds is to regulate and maintain these factors. In patients with chronic wounds who are hospitalized, treatment techniques and wound care materials have been adapted to standard operating procedures (SOPs) that can support wound healing ((Mardiah et al., 2016)Mardiah, 2016; Wijaya, 2018). However, treating chronically infected wounds until they heal cannot be done forever in a hospital because it will take too long to treat (Frykberg & Banks, 2015). The patient will be discharged or referred back to continue wound care at home. However, there are many challenges in treating these types of wounds at home that can lead to delayed wound healing (Smith et al., 2016).

The most common cause of delayed healing in chronic wounds is infection. Microbial contamination of wounds can progress to colonization, localized infection, systemic infection, sepsis, multi-organ dysfunction, and subsequently life- and limb-threatening infections (Han & Ceilley, 2017)(Sy, 2017). Determining true infection can be complicated by the presence of biofilms, not on the surface but deep in the wound tissue. The presence of biofilms also contributes extensively to keeping the wound in a state of prolonged inflammation by stimulation of nitric oxide, inflammatory cytokines, and free radicals (Boulton & Bus, 2017)(Sy, 2017). As a result, one of the best ways to get a wound back on track is an effective wound bed preparation starting with debridement and topical antimicrobial application. The large number of bacteria and due to bacterial activity in the wound will cause the wound to produce a pungent odour and a lot of exudates (Han & Ceilley, 2017)(Smith et al., 2016) (Mardiah et al., 2016).

In modern wound care techniques, several uses of advanced wound care materials have been shown to reduce the number of bacteria, odour and exudate (Akhmetova et al., 2016). However, this is often an obstacle for patients who need it in the long term because the prices of the ingredients are quite expensive. Therefore, it is necessary to have a wound care material solution that is proven to be a disinfectant and antimicrobial at a more economical price. The use of 40% red betel leaf (*Piper Crocatum*) solution in the treatment of chronic wounds has been shown to reduce the number of bacteria in wounds (Fadlilah, 2015) (Rahmasari & Fitriani, 2021) (Yuliarta, 2013). The objective of this study was to measure the effect of a 40% red betel solution on bacterial colonization and exudate (pus). This study aimed to measure the effect of 40% red betel leaf solution on the number of colonies in chronic wounds of home care patients in the Banyumas Region

METHODS

This study was a case series study involving 6 patients with chronic illness using convenience sampling method. The bacterial colonization and exudate were measured before and after cleaning the wound with 40% red betel leaf solution. During July 2022, six patients with chronic infected wounds, aged >18 years in Banyumas Regency were enrolled in this study. The patients who had been discharged from hospital for a home care were taken care by the researchers and a nurse who regularly visit. The treatment was carried out once in two days.

The researcher prepared 40% red betel leaf solution at the UMP Pharmacy Laboratory assisted by a laboratory assistant. Initially the 40% red betel solution was prepared as follows: fresh betel leaves are washed and finely sliced, then dried in the shade, followed by drying in

a drying cupboard for three days. Materials that have been dried, blended into powder form. The fine powder was weighed using a blender for 40gr. It then mixed with 400ml aquades and boiled it with 110⁰C. The solution was rested for 10-15 minutes, then filtered with a clean flannel cloth. Extract with a concentration of 40% is put into a syringe as much as 20 ml.

For the 1st wound treatment, the sample for a bacterial count and exudate were taken. The wound was then irrigated with aqueous extract at a dose of 20 ml using a 20 ml syringe with an 18-gauge needle. In the 3rd wound treatment, the samples were again taken for a post test. The bacterial counting was conducted in the UMP integrated laboratory (Microbiology Lab). The preparation of bacterial media was as follows: weigh 0.1 gram of peptone and 3.4 gram of PCA, then dissolved using distilled water and heat it to 250 degrees Celsius using a hotplate and magnetic stirrers. Pour 12 ml of peptone and PCA into each test tube.

The normality test using Shapiro-Wilk yielded value sig.> 0.05, thus Paired t-test was used to determine the effect of treatment with 40% red betel leaf solution on wound healing.

RESULT

Respondent Characteristics

Table 1 shows that the respondents' age ranging from 57 to 72 years old and had suffered from chronic wounds.

Table 1. Respondent Characteristics (n=6)

Characteristics	n	%
Age (Years)		
58	1	16,66
69	1	16,66
72	1	16,66
61	2	33,33
57	1	16,66
Gender		
Male	3	50
Female	3	50
Wound diagnosis		
Ganggren	3	50
Decubitus	1	16,66
Combustio	1	16,66
Vena Leg Ulcer (VLU)	1	16,66

Table 2 shows that there was a decrease in the number of bacterial colonies after the treatment. However, the decline after cleansing the wounds for 2x using the solution was not significant (*p value* > 0.05).

Table 2. The Difference of Bacterial Count Before and After the Intervention Using 40% Red betel Leaf Solution

Bacterial count	n	Mean Bacterial (SD)	<i>p value</i>
Before	6	1266.67 (152.75)	
After	6	1040.00 (495.17)	0.325

Source: primary data, 2022

The amount of exudate in the chronic wounds shows a slow decrease during the treatment (Table 3).

Table 3. The Amount of Exudate During the Wound Care

Patient	The Amount of Exudate per Day		
	I	II	III
Combustio	< 1cc	< 1cc	< 1cc
VLU	< 1cc	< 1cc	< 1cc
Decubitus	< 1cc	< 1cc	< 1cc
Gangrene 1	1,5cc	1,4cc	1,2cc
Gangrene 2	0,9cc	0,8cc	0,7cc
Gangrene 3	1,3cc	1,1cc	1cc

DISCUSSION

Infected chronic wound takes complex factors to heal. It may vary from systemic to local factors. Patient's overall health, age, gender, and diabetes are example of factors which can hinders wound healing (Raziyeva...). Whereas the presence of bacteria which then lead to infection, environmental exposure, therapeutic modalities and low level of oxygen in the blood stream (hypoxia) are some of the local factors preventing successful wound healing (Beyene, Raziyeva, Visha). Aging would cause cells and organ deterioration and slow down wound healing (Beyene). Respondents in this study were categorized as elderly. The results showed an average age of 66.53 years. Currently the largest age population according to WHO (2019) is over 60 years old. In this age population there is a high risk of delayed wound healing. In healthy elderly, the effect of age causes a delay in wound healing, but does not affect the quality of healing. The wound healing response is influenced by activities that stimulate an anti-inflammatory response in the wound (Beyene & Barbul, 2020). Thus, in the treatment of wounds over the age of 60 years, it is necessary to pay more attention to matters that affect activities that stimulate an anti-inflammatory response in wounds so that wound care becomes more effective (Boulton & Bus, 2017)(Krzyszczuk et al., 2018).

Female respondents in this study might experience longer wound healing process compared to their counter-part. A report from a systemic perspective showed that two components; age and gender may affect this condition. In terms of gender, one important hormone plays a key role in cell granulation, that is oestrogen (Ashcroft et al., 2003)(Horng et al., 2017). Sex hormones greatly affect the length of time wound healing takes. Several previous studies reported factor, what needs to be underlined in this study is that elderly women might experience a slowdown in wound healing because the low level of oestrogen in their body. Oestrogen supports wound healing by regulating, regenerating, inhibiting proteases, epidermal function, and inflammation-related genes (Ashcroft et al., 2003)(Horng et al., 2017)(Mohtadi et al., 2021). Aging and gender are two non-modified factors that could influence wound healing, there are other aspects that could be managed for instance patient's nutrition, wound exudate, necrotic tissue in the wound, hypoxia and wound care technique (Akhmetova et al., 2016)(Horng et al., 2017)(Raziyeva et al., 2021).

One of the nursing services provided as a whole is wound care which must be carried out according to standard procedures. This wound care procedure aims to speed up the healing process and be free from infection, indicators of infection due to poor wound care, one of which is the occurrence of nosocomial infections which are infections that are acquired or that arise when the patient is hospitalized (Olsson et al., 2019)(Kusumastuty & Dewi, 2020) (Frykberg & Banks, 2015). The results showed that the mean 1x measurement of bacterial colony before being given red betel leaves solution was 1266.67 ± 152.75 , whereas after being treated with the solution, the number of bacteria decreased to 1040.00 ± 495.17 . The condition happened might due to the anti-bacterial effect that the solution has. Red betel leaf has flavonoid, a substance which functions forming complex compounds against extracellular protein that could damage bacterial membrane (Fadlilah, 2015)(Purwaningsih, 2016).

Not only flavonoids, the antibacterial effect of red betel leaves is due to the presence of several compounds such as phenols. Phenols work to change the properties of bacterial cell proteins so that the permeability of the bacterial cell wall increases and the bacteria become lysed (Sukmana & Nopriyanto, 2020). Furthermore, flavonoids interfere with the integrity of the bacterial cell membrane, and alkaloids interfere with the peptidoglycan component in bacterial cells. In addition, the inhibitory effect is due to the presence of active substances contained in red betel leaf extract, namely catechins, phenols, tannins, flavonoids, and others which have bactericidal activity (Fadlilah, 2015)(Sukmana & Nopriyanto, 2020).

Red betel leaf has a high phenol content. Phenol compounds have several properties, including; easily soluble in water, easily forms complexes with proteins and is very sensitive to enzyme oxidation. Phenol works to damage the protein bonds that make up the bacterial cell wall and then enters and inactivates the enzymes that play a role in the metabolic process of the bacterial cell so that the biological activity of the bacteria stops. Phenol compounds can also damage the hydrophobic bonds of cell membrane components (such as proteins and phospholipids) and dissolve other cell components that bind hydrophobically so that the permeability of the cell membrane increases. This will cause lysis of the bacterial cell (Akhmetova et al., 2016)(Fadlilah, 2015)(Sukmana & Nopriyanto, 2020).

This study proves that red betel leaf infusion can inhibit the growth of *Staphylococcus aureus*. The higher the concentration of red betel leaf infusion, the smaller the inhibition zone formed and the weaker the inhibition response. This is due to the reduced diffusion power of the infusion into the media. The decrease in diffusion power is caused by the higher the concentration of red betel leaf infusion, the lower the solubility (Sukmana & Nopriyanto, 2020).

The results of this study were not in line with research conducted by Pashar (2018) which showed that topical administration of red betel leaf infusion (solution) with concentrations of 10%, 20%, and 40% had a wound healing effect (Pashar et al., 2018). Red betel leaf infusion concentration of 40% had a better effect on increasing the percentage of wound healing than red betel leaf infusion concentrations of 10% and 20%. Some previous studies showed that irrigation with 40% red betel leaf infusion affected wound healing in alloxan-induced white rats (Purwanti & Astuti, 2021)(Riami et al., 2019).

The result of this study showed that although there was a decrease in the number of bacterial colonies after treatment with red betel leaf solution, statistically the decrease did not yield a significant result statistically (p value > 0.05). This could be due to the small number of samples used in this study. The number of patients with chronic wound who were treated at home were not many. Many patients preferred to get to a hospital polyclinic for treatment. In addition, not all patients who got homecare treatment would involuntarily enrolled in this study. Three more patients who involved in this study were counted as Drop Out (DO) because they were admitted to a hospital during the wound treatment because of their comorbid disease complications. In the hospital, the patients' wounds would be treated using conventional wound care treatment or based on the hospital SOP for treating chronic wound. Some procedure of wound care would be carried out using physiological fluid antiseptics (NaCl or RL). If it is necessary or if the wound had necrosis part, a debridement would be addressed. According to our pilot study, drugs such as cloramphenicol, tetracycline HCL, silver sulfadiazine 1%, basitracin, bioplacenton, mafenide acetate and gentamicin sulfate are the list of antibiotics which are commonly used in the hospital.

The limitation in this study is that the study still involves a small number of samples so that for further research it can be tested the effectiveness of red betel leaf solution in washing wounds against a greater number and variety of chronic wounds.

CONCLUSION

During one week of infected chronic wound treatment, 40% red betel leaf solution showed the effect to reduce bacterial colony and the amount of exudate. Although the decrease was small or not statistically significant, this solution could still be used as an alternative yet economical solution to cleanse chronic wound.

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