EFFEKTIVENESS OF ORALLY AND TOPICALLY GEL EXTRACT OF KELOR LEAF (MORINGA OLEIFERA) ON ACCELERATION OF CUTANEUOUS WOUND HEALING: AN EXPERIMENTAL STUDY IN MICE

Novi Lasmadasari[1], Mohammad Hakimi[2], Titih Huriah[3]
[1]Graduate School of Nursing Magister Program of Muhammadiyah University of Yogyakarta;
Institute of Nursing, Sapta Bakti Bengkulu.
[2]Profesor of Departement of Public Heath of Medical Science Gajah Mada University.
[3]Lecturer of Nursing Magister Program of Muhammadiyah University of Yogyakarta.

Abstract:

Hight nutrition on dry leaf of kelor (Moringa oleifera) and saponin, tannin, flavonoid, alkaloid substances that's can use on accelerating wound healing. The purpose of this study was to investigate the effectiveness of orally and topically gel extract kelor leaf on the accelerating of cutaneus wound healing. This study was true experiment post test design and data are expressed as mean of male mice were treath to produce circular (2 cm in diameter) full thickness skin wound on the dorsum were analyzed using one way anova and post hoc test were performed.They were randomly allocated to receive each 2 days gel CMC-Na topically (negative control), gel CMC-Na topically+orally extract of kelor leaf, gel extract topically+orally extract of kelor leaf, gel extract topically of kelor leaf, or transparant film (positive control). Macroscopic findings were observed on days each 2 days for wound measurement and using SWHT (Sussman Wound Healing Tools) weekly after wounding. Microscopic findings to observed capillary and collagen on day 14 after wounding were obtained. Wound contraction for oral intervention of extract kelor leaf groups were larger than those of negatif control group. Time of wound closure for oral intervention of extract kelor leaf groups rapidly closed until day 13 than negatif control group and its almost as effective as transparan film. Vascularisation and weight of collagen for oral intervention of extract kelor leaf group on day 14 more accelerating wound healing than negatif control group. The result indicate that orally of kelor leaf intervention can accelarated for wound healing.

Key words.

Extract of kelor leaf (Moringa oleifera Linn.), cutaneous wound

1.Introduction

Wound healing is a complex process and influenced by many factors. The overlapping process includes of homeostasis, inflammation, proliferation (granulation, contraction and epithelialization) and remodelling[1]. Nurses play a very important role in wound management both locally (cleansing, debridement, dressing) and systemic (nutrients). Velocity and delay of wound healing process can be affected by nursing intervention. Therefore, the nurses must be knowledgeable of the wound healing process and related wound management[2].

Local management of wound by making into moist wound environment, it can be created by selecting the type of closed bandage or dressing (occlusive dressings). Many modern dressings as an occlusive dressing, such as hydrocolloids, hydrogels, transparant film and foam that commonly have been used in clinicall setting as wound dressing but its still fairly expensive, so not all patient can use modern dressing.

Moringa oleifera is plant that can be grown in various countries can survive from dry and rain, has been known since ancient traditional medicine and this time with a variety of scientific studies reveal that this plant was benefit for treatment some of diseases, including wound. The benefit of Moringa leaves is the high nutrient content, especially class of proteins, minerals, fatty acids and vitamins that making it ideal as a nutritional supplement found in dried leaves or powder form[3]. So that Moringa leaves can be used as medicine and diet serves as a curative and therapeutic therapy[4]. Nutrient content of Moringa leaves and phytokimia proved effect as antioxsidant[5], antimicrobial and wound healing[6].

Wound healing activity of aqueous extract of Moringa leaves or Methanolic extract showed significant results as antibacterial in inhibiting the growth of organisms of wound orthopedics (orthopedic wound)[6], administration of oral doses 300mg/kgBB of extract significant increase in the average of wound closure, skin breaking strength, granuloma breaking strengt and decrease scar on excision wounds in mice[7]. Moringa leaves contain a number of minerals such as iron, magnesium, sodium, potassium, calcium and zinc as well as substances phitokimian largest saponins in the leaves

of Moringa rest flavonoids, tannins, phenols, and alkaloid[6,9]. It also contains a number of vitamins such as vitamin C, E, K, A in the leaves of Moringa leaves [8,10].

The purpose of this study is to determine the effectiveness of the leaf extract of Moringa (Moringa oleifera) orally and topically in enhancing wound contraction, accelerates wound closure time, vascularization and collagen density activity in wound healing of full-thickness excision wounds in comparson with occlusive dressings transparant film and formulating gel of CMC-Na.

The benefits of this research for the nursing profession is the basis of knowledge to understand the use of the leaves of Moringa (Moringa oleifera) both systemic and local wound management and can add to their repertoire in wound care with complementary therapies. While the benefits to the community to be the basis for utilizing Moringa leaves (Moringa oleifera) in support of wound healing.

2.Methode

Animal. Twenty-five 2-3 months old male mice, weighing 18.0-23.0 g were placed in a cage and given food and drink experimentation on animal treatment standards of Biomedical Laboratory of the Medical School, University of Muhammadiyah Yogyakarta. This study has been approved and permission of the Research Ethics Committee of the Faculty of Medicine and Health Sciences, University of Muhammadiyah Yogyakarta.

Extract. Moringa leaves are choiced that old leaves, solid green, not attacked by pests or diseases and bully free. Moringa leaves are extracted using maceration with ethanol according to existing procedures in the Laboratory of Pharmacology Unit III Faculty of Medicine, University of Gajah Mada. Moringa leaf powder 500 g of solvent extracted with ethanol (1:9) for 2x24 hours. This extract is processed back through maceration method becomes viscous extract which is then used as dried extracts that ready for use or re-processed for gel preparation.

Gel. CMC-Na gel made of CMC-Na powder, glyserin and water content of more than 90%. While the Moringa leaf extract gel 10%, gelling formula CMC-Na was added powdered moringa leaf extract about 10 g and water up to 100%.

Wounding. Mice were general anesthized with inhaled chloroform that put in a large glass jar, covered for 20-30 seconds. Dorsal of mice cleaned with 70 % alcohol and skin on the backs of mice sterile manner excision of 2 cm diameter circular shape and depth of deep cutaneous layers on each animal. The wound was cleaned immediately with 0.9 % NaCl, performed debridement for tissue excision and giving of the wound dressings appropriate intervention group intervention group topical gel that CMC - Na (negative control), topical gel oral CMC - Na + Moringa leaf extract, topical gel extract + oral moringa leaf extract, primary dressings transpsran films (positive control), and topical gel CMC - Na. All wounds received the same treatment in one group. Wound dressings were given CMC - Na topical gel or topical gel extract of moringa leaves were covered with melolin gauze as a secondary dressing to prevent the out of a topical gel of wound and to absorb exudate and to minimize trauma of the wound surface when dressing change. Local wound care and systemic conducted once every two days. Measurements done manually wound with clock methods for once every two days starting at day 3 to day 13 assessment and qualitative development of the wound with SWHT (Sussman Wound Healing Tool) were performed per week.

Histological prosedure. The mice were euthanized by chloroform inhalation performed on day 14 after injury. Excision performed on the wound and surrounding skin tissue ± 0.3 cm from the edge of the wound, stapled on to thin cardboard to prevent excessive contraction of the sample and fixation with 10% formaldehyde for ± 24 hours. Sample dehydrated with alcohol series, clearing in pure toluene and embedded in paraffin to prepare serial 6 μm sections.

Alternate sections of the wound center were stained with hematoxylin-eosin (H&E) or mollary. Stage of staning, put the glass object on xylol for 15 minutes x 3, 96 % alcohol for 15 minutes x 3, then washed with running water for 15 minutes, then put on a glass object Hematoxylin dye for 15 minutes and washed with running water for 15 minutes . Object glass put on Lithium carbonate for 20 seconds and washed with running water for 15 minutes. Further object inserted in glass eosin dye for 15 min , 96 % alcohol for 15 minutes x 3 and xylol for 15 minutes x 3. The last stage is the preparation using covered with a deck glass Entellan .

Capillary and collagen observation. The number of capillaries in the granulation tissue was caunted by observation of the four slides from five differences mice tissue of one group through a microscope with 10x objectif and software raster image on one field of view. The collagen density were observed with a field of view at 40x.

Contraction and wound closure time. Wound contraction is defined as a broad new epithelialization (mm^2) divided by the area of the wound preview of each sample from day 5 to day 13. Wound closure time (day) is caunted result of extensive prior treatment divided by the initial wound epithelialization area and multiplied by the long of the treatment.

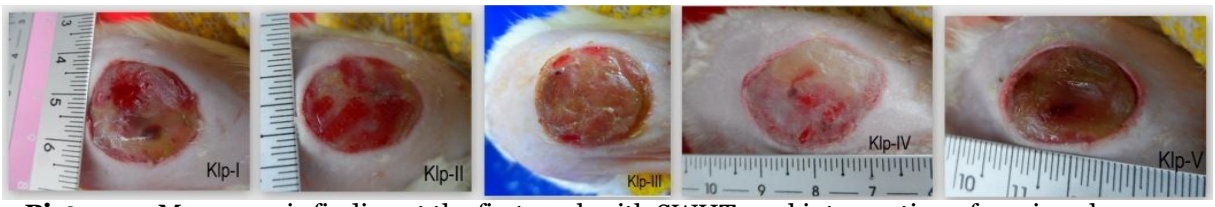
Evaluation of wound care intervention. The progress of intervention in wound care management both locally and systemically in the wound assessed by SWHT (Sussman Wound

Healing Tool) every week, the progress of wound healing characterized by signs of 'good for healing' which consists of wound edges fused at the base of the wound, granulation, contraction perform, which can be measured further contraction, and epithelialization. And the delay of the wound healing characterized by signs of 'not good for healing' which consists of erythema, necrosis tissue, hemorrhage, undermining, maceration.

Statistical analysis. Data are expresed as mean±SD and were analyzed using one-way ANOVA and Bonferroni multiple comparison test were performed. The differences were consedered significant at p <0.05.

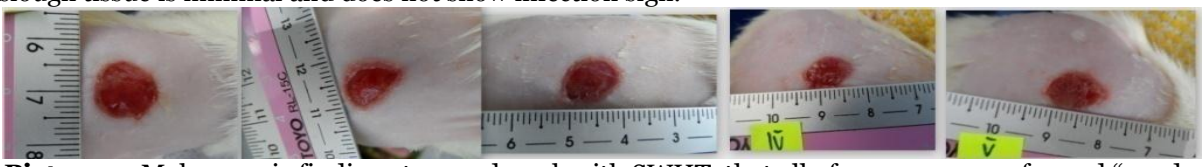
3.Results

Macroscopic observation of wound healing with SWHT. On the first week at day 5 after wounding, the skin around the wound in the oral intervention with moringa leaf extract visible redness, bleeding sign of the previous day is only found in one sample, the wound edges are form and intact on the wound base, presence of granulation that filled the wound bed and visible contraction. The almost same between group of the negative control and intervention of Moringa leaf extract topical gel which are average still bleeding marker at the previous day, although the edge of the wounds have also been fused at the wound base.



Picture 1. Macroscopic finding at the first week with SWHT, oral intervention of moringa leaves extract (pic 2 and 3 of the left) performed “good for healing” edge intact of the wound base, wound surface were filled with granulation tissue, sign of “not good for healing” consist of erythema and necrotic tissue. Negative control (pic 1 of the left) perrformed of “not good for healing” sign were hemorrhage, erytema, and necrosis tissue.

Observation on the second week at 11 day after wounding that all of Moringa leaf extract intervention group and the control group were performed the signs of "good for healing", there were continuous contraction and epithelialization that reduces the wound size, the wound surface was covered with slough tissue is minimal and does not show infection sign.



Picture 2. Makroscopic finding at second week with SWHT, that all of group were performed “good for healing” sign, there were edge intact on the wound base, granulation filled in the wound surface, continue contraction were performed and epitelization.

Wound contraction. Contraction has been able to count on day-5, its means that the wound size at day 5 were smaller than at day-3 before. The highest Percentage of contraction at day 5 shown in the intervention group with transparent films, followed by oral intervention group Moringa leaf extract and then the intervention group Moringa leaf extract topical gel. Until at 9 day, the percentage of contraction of transparant film was the highest. Ot day 11 and day 13 percentage contraction of oral intervention group Moringa leaf extract more highest than transparant film and negative control group.

On day 13, were counted of contraction (mm^2) in each group, the average contraction of both groups oral interventions moringa leaf extract greater (0.85 ± 0.85 ± 0:07 & 0:03) than the negative control group (0.76 ± 0:06) and group Moringa leaf extract topical intervention (0.77 ± 0:08). There is no significant difference between the negative control group and the intervention group of oral moringa leaf extract (p = 0.388 & p = 0277). While the transparant film shown the greater contraction than both oral intervention group with moringa leaf extract, but there was no significant (p = 0767 & p = 1.000).

Table 1. mean and SD of contaction (mm^2), wound closure time (mm^2/day), new capillary and collagen

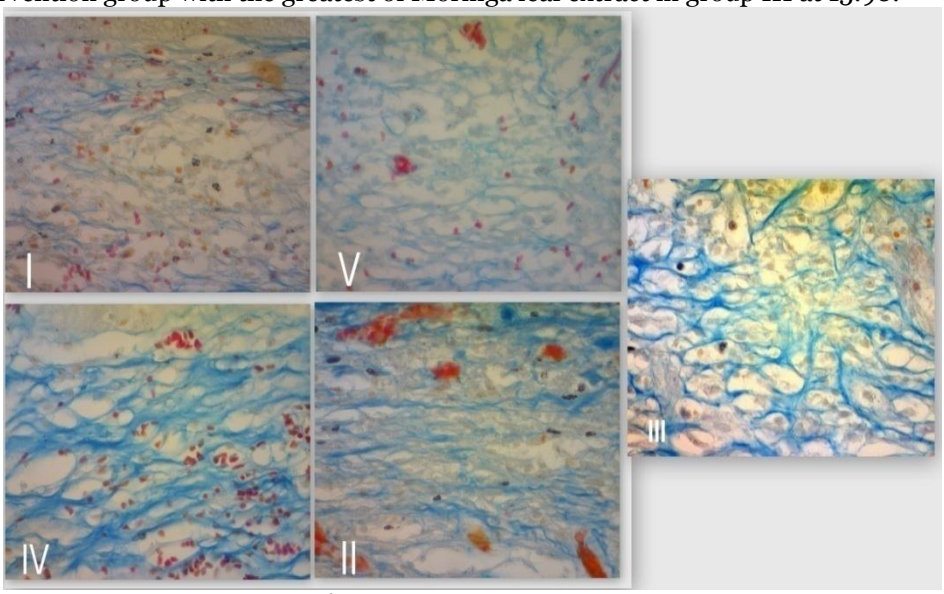
Group	Contraction	Closure time	capillary	collagen
Gel CMC-Na (I)	0.76±0.06	17.17±1.49	30.50±5.08	4.00
Gel CMC-Na + oral ekstrak (II)	0.85±0.07	15.30±1.42	35.40±6.19	14.20
Gel ekstrak + oral ekstrak (III)	0.85±0.03	15.24±0.62	37.60±2.70	15.90*
Transparan film (IV)	0.92±0.03*	14.18±0.61*	44.60±4.28*	19.30*
Gel ekstrak (V)	0.77±0.08	16.96±0.87	34.00±6.43	7.40

Value are expressed as mean, ANOVA, Bonferroni. * $p<0.05$

Wound closure time. Both of oral intervention group with moringa leaf extract took time for wound closure (15:30 ± 1:42 and 15:24 ± 0.62) in day are almost similar with the intervention group dressings transparant film with that took time for 14:18 (day) and its only difference about ± 0.61 (day). The intervention groups Moringa leaf extract orally and transparant films goup were p = 1.000. While topical intervention group moringa leaf extract (16.96 ± 0.87) had almost the same time of wound closure with the control group topical gel CMC-Na (17:17 ± 1:49) with p = 1.000.

New capillary. In the group treated with leaf extracts of Moringa, the mean of new blood vessels was variated and 37.60 in the third group, when compared to Group I (negative control), the three groups with of Moringa leaf were have better conditions vascularization in the scar tissue.

Collagen. On day 14, the identification of collagen density contained in the scar tissue in each group. The analysis showed there were differences in the average density of collagen in each group is significant with p = 0.002 (p <0.05) with the lowest mean rank 4.00 in the group with topical gel CMC-Na and the highest mean rank 19.30 on the group by giving transparant films. Collagen density in the intervention group with the greatest of Moringa leaf extract in group III at 15.90.



Picture 3. Collagen at day 14

4.Disccussion

Indonesia is a tropical country overgrown with various types of plants that can be used as herbal plants to treath health problems. Moringa plant (Moringa oleifera) thrives scattered in various regions in Indonesia. Most researchers outside of Indonesia has conducted various research on the content of Moringa leaves as antimicrobials, antioxidants and nutrients that can be used to support tissue repairing included in wound healing. Researcher hopes that the Indonesian Moringa leaves can be used for wound care. Today, the effectiveness of moringa leaves in indonesia for wound contraction, wound closure time and vascularity and collagen density conditions of wound have not been observed histologically. Therefore, authors invetigated effectiveness of oral and topical administration of Moringa leaf extract gel in wound healing in mice. As far as, outhors' knowledge, this study is the first to compare the Indonesian moringa leaf extract to modern dressings transparant film in mice.

The results of macroscopic observation with SWHT on the first week at day 5 showed progress by intervention group with Moringa leaf extract especially oral administration is better than the negative control group. The observation of the percentage of wound contraction was greater in the intervention group with Moringa leaf extract compared to negative control group on day 5 to day 13. That is because the moringa leaf extract can inhibit inflammatory processes through the effects of antimicrobial and antioxidant content of Moringa leaves. The inflammatory stage is clearing phase of microorganisms of wound that beginning at the first day until day 7 after wounding. At this stage

needed activity of compounds that can help get rid of and kill microorganisms on the wound. Moringa leaves contain saponins, flavonoids, tannins, phenolic which are bactericidal or bacteriostatic by damaging the cell wall and sitoplasm membrane of bacterial cell and damaging the protein denaturation of bacteria[6,7,11]. Anseptic properties of the compounds may have an important role in the inflammatory phase of the wound healing process. Whereas the negative control group is a topical gel CMC - Na did not have bactericidal and bacteriostatic effect, but only moisturize the wound even it may be have the potential for bacterial growth if the secondary bandage opened..

All of three groups of intervention with Moringa leaf extract showed the presence of wound granulation tissue and new epithelium that facilitates the wound closure. In previous research also show the results of intervention with moringa leaves wos no signs of infection, the presence of epithelialization and wound contraction[7], which is consistent with the results in this study. The macroscopic observation that contraction of the wound with intervention group of Moringa leaves were faster than the negative control group and it is supported by the results of the time for wound closure measurements rapidly on the intervention group with moringa leaves until the 13 day. These results are supported by previous studies in combination with oral and topikal administration of fruit and leaves extracts of Moringa for the wound within 14 days can accelerated wound closure.

The results of microscopic observation at day 14 were performed the collagen density and the number of new capillary blood vessels in the intervention group with Moringa leaf extract was found more support accelerated wound healing compared to negative control group topical gel CMC - Na. Significant difference to the spread of collagen density occurred in the intervention group topical gel + oral extract of Moringa leaf extract and negative control group. This is caused by moisture in addition to relatively balanced oxygen on the surface of the wound due to the use of CMC-Na as a gel preparation in topical gel extract moringa leaves, but the Moringa leaves also contain with nutrients such as Fe , Ca, protein, Mg ,zinc and some vitamin[6,8.9,10] orally and topical may work synergistically so that will affect the increase of immunity. An integral role in the immune system as a defense of the body, and the immune system secreted the cytokin, lymphokin and growth factor[13] which very important for the inflammatory phase beginning of the wound healing.

In the process of wound healing, the vitamin A is required for inflammatory responses that may accelerate inflammatory to the proliferative phase, increasing the formation of collagen and improve epithelization of the wound[17]. The vitamin E is an antioxidant protected the white blood cells so can improves immune function, vitamin C also increases the activation of leukocytes and macrophages in wounding on the inflammatory phase, and increasing the synthesis of collagen by fibroblasts. While the iron is important for the transport of oxygen and essential zinc as a cofactor and for collagen synthesis protein[14, 15,16].

The intervention group with extract Moringa leaves both at local wound and administered orally are also compared to the intervention group with modern wound dressings transparant film. All of intervention group Moringa leaf extract administered topical gel-based CMC-Na. CMC-Na is one of the substances contained in the hydrocolloid dressing that absorbs exudate and accommodate excessive, stimulate the formation of granulation tissue and reducing pain. The CMC-Na gel that binds water is expected to provide a moist environment in the wound. while the transparant film dressing is applied as a primary occlusive dressings is a good arbsobent and atraumatic thus making an optimal environment for the growth of new blood vessels that will affect the growth of granulation, the synthesis of collagen by fibroblasts and the occurrence of wound contraction which will facilitated wound closure by new skin[18].

The results in this study also showed that between the wound intervention with transparant films faster compared to the intervention group with Moringa leaf extract. This suggests that although the intervention group wound care with moringa leaf extracts using gel as a primary wound dressings were covered with gauze melolin, it contains antibacterial, antioxidant and nutritional support in a number of tissue repair is not enough to compete with the effect of a transparant film used as primary dressings on the wound. So the gel base used is not sufficient to provide moist or humid conditions on the surface of the wound such as that provided by a transparant film.

Comparisons between intervention with Moringa leaf extract and transparant film for wound contraction and time to wound closure and supported by microscopic observation form capillary blood vessels and collagen are not significant to the intervention group orally extracts of Moringa leaves. The significant value were performed on the intervention group with topical gel compared to transparant films on observations contraction, wound closure time, vascularity and collagen density.

Based on this study showed the intervention orally with extract Moringa leaf more effective to accelerate wound healing than topical administration. The results are consistent with some studies the effect of oral consumption of Moringa leaf to improved nutrition and it work as herbal medicine for some diseases[8,19,20] and wound healing properties in several states such as India, Africa, philipine and some countries in Asia[7]. Previous study used Moringa leaf extract topically combined with extract of Moringa fruit orally as antimicrobial for orthopaedic wound[12] can be justified because in this study with topical intervention does not prolong inflammation sign and overall elongated slightly further accelerate wound healing compared to intervention only with topical gel CMC-Na.

5.Conclusion

The rate of wound healing effectiveness of oral administration of Moringa leaf extract and transparant film were have similar for contraction and wound closure time. wound contraction of oral intervention of Moringa leaf extracts more slowly and increased gradually, while the transparant film increased more rapidly during the inflammatory phase. Scar tissue on the wound surface that treath with transparant film was minimum comapred to group CMC-Na topical gel, or topical gel Moringa leaf extract. Oral administration can be used alternative in wound care management.

Referensi

1. Diegelmann RF, Evans MC. Wound healing: an overview of acute, fibrotic and delayed healing. *Front Biosci*. 2004;9:283–289.
2. Bryant RA. (2007). *Acute and chronic wounds: Current management consept* (3th ed). St Louis: Mosby Elsivier
3. Moyo B, Patrick J. Masika, Hugo A, and Muchenje V. (2011). Nutritional characterization of Moringa (*Moringa oleifera*) leaves. *African Journal of Biotechnology*., Vol. 10(60), pp. 12925-12933 diakses 15 Juni 2013 dari http://www.academicjournals.org/AJB
4. Fahey JW. (2005). Moringa oleifera: A review of the Medical evidence for its nutritional, Therapeutic and prophylactic properties. *Trees for Life Journal Part 1*., diakses 15 Juni 2013 dari http://www.TFLjournal.org/article.php/20051201
5. Shahriar M, Hossain I, Nizam Md Bahar AN, Akhter S, Md. Haque A and Bhuiyan MA. (2012). Preliminary Phytochemical Screening, *In-Vitro* Antioxidant and Cytotoxic Activity of Five Different Extracts of *Moringa Oleifera* Leaf. *Journal of Applied Pharmaceutical Science* 02 (05); 65-68 diakses 10 Februari 2012 dari www.jabsonline.com
6. Oluduro AO. (2012). Evaluation of Antimicrobial properties and nutritional potentials of Moringa oleifera Lam. leaf in South-Western Nigeria. *Malaysian Journal of microbiology* 8(2): 59-67 diakses 12 Juli 2012 dari http://web.usm.my/mjm/issues/vol8no2/Research%201.pdf
7. Rathi, B.S., S.L. Bodhankar and A.M. Baheti. (2006). Evaluation of aqueous leaves extract of *Moringa oleifera* for wound healing in albino rats. *Indian Journal of Experimental Biology*, 44: 898-901 diakses 21 November 2012 dari http://www.IJEB 44(11) 898-901.pdf
8. Fuglie, L.J. (2005). The Moringa Tree: a local solution to malnutrition? Church World Service in Senegal. Diakses 16 Juni 2013 dari *www.moringanews.org/documents/Nutrition.pdf*
9. Zakaria, Abdullah Tamrin A, Sirajuddin, Hartono R. (2012). Penambahan tepung daun kelor pada menu makanan sehari-hari dalam Upaya penanggulangan gizi kurang pada anak balita. *Media Gizi Pangan, Vol.XIII, Edisi 1, 2012*
10. Luthfiyah, fifi. (2012). Potensi gizi daun kelor (moringa oleifera) nusa tenggara barat. *Media Bina Ilmiah* Volume 6, No. 2 pp. 42-50 diakses 16 Juni 2013 dari http://www.lpsdimataram.com
11. Rahman, M, Islam.M, Sheikh, S. Sharmin and Alam, M. (2009). Antibacterial Aktivity of leaf Juice and Extrack of *Moringan oleifera* Lam.again some hman Phatogenik Bacteria. *CMU Journal* 8(2): 219-228.
12. Hukkeri VI, Nagathan CV, Karadi RV, Patil BS. (2006). Antipyretic and wound healing activities of moringa oleifera lam. in rats. *Indian Journal of Phaceutical Sciences* Vol. 68: 124-126 diakses 15 Juni 2013 dari http://www.ijpsonline.com/text.asp?2006/68/1/124/22985
13. Park, JE and Barbul, A. (2004). Understanding the role of immune regulation in wound healing. *The American Journal of Surgery* 187:11–16 diakses 9 July 2013 dari www.sciencedirect.com
14. Baronoski S & Ayello EA. (2012). *Wound care essentials: Practice principles* (3th ed). New York: Lippincott Williams and Wilkins.
15. Potter and Perry. (2009). *Fundamental of nursing* Fundamental Keperawatan (7th ed) . St Louis: Mosby Elsivier Jakarta: Salemba Medika
16. Sussman C & Bates-Jensen B. (2012). *Wound care*: *A collaborative practice manual for health professionals essentials* (4th ed). New York: Lippincott Williams and Wilkins
17. Jeffcoate WJ, Price P, dan Harding KG. 2004. Wound healing and treatments for people with diabetic foot ulcers. *Diabet Metab Res Rev* 20(1): S78-S89
18. Lippert H. The use of wound dressing. Compendium Wound and Wound Management. 1th ed. 1999. HARTMANN medical edition. Heidenheim : 88-101
19. Anwar, F et al. (2006). Review Article *Moringa oleifera*: A Food Plant with Multiple Medicinal Uses. *Wiley InterScience* 21, 17–25 diakses 27 Juni 2013 dari www.interscience.wiley.com
20. Biswas S, Chowdhury A, Das J, Roy A and Hosen S.M. (2012). Pharmacological potentials of moringa oleifera lam.: a review. *International Journal of Pharmaceutical Sciences and research*., Vol. 3(2): 305-310 diakses 12 Juli 2012 dari www.ijpsr.com