International Journal of Research in Pharmacy and Pharmaceutical Sciences
ISSN: 2455-698X; Impact Factor: RJIF 5.22
www.pharmacyjournal.in
Volume 1; Issue 4; September 2016; Page No. 07-12

Burn wound healing activity of the combination of *Centella Asiatica* extract and papaya latex on male white mice

Delladari Mayefis

Institute of Health Science, Mitra Bunda Persada Batam Jl Seraya No.1 Kota Batam, Kepulauan Riau, Indonesia

Abstract
Research on the combined effect of *centella asiatica* extract and papaya latex. The healing of burns on white male mice. Examination burn healing done by measuring the diameter of the burn until the wound completely healed. Dose combination *centella asiatica* extract and papaya latex given row is (1%: 1%); (1.5%: 0.5%); (0.5%: 1.5%) on a daily basis. Measuring the diameter of burns conducted up to the 10th day. The results showed that the dosing combination *centella asiatica* extract and papaya latex can accelerate the healing of burns significantly (P <0.05). Giving combination *centella asiatica* extract and papaya latex showed a faster recovery time and better than the singular respectively. Healing time of burns optimal dosing is achieved by a combination of *centella asiatica* extract 1% and 1% papaya latex. Measurement of the percentage of healing from the first day until the 6th day, showed a significant effect (P <0.05).

Keywords: *centella asiatica* extract, papaya latex, burn wound healing, male white mice

Introduction
Burns is a form of tissue damage of the skin caused by contact with a heat source such as a fire, hot water, chemicals, electrical and radiation (Chandrosama and Taylor, 1998; Mansjoer, 2001; Syamsuhidayat and Jong, 1997) [5, 30, 42].

In connection with efforts to burn therapy, the treatment in the healing of burns include preventing infection, promotes the formation of collagen and to ensure that the remnants of epithelial cells can develop so as to cover the surface of the wound (Syamsuhidayat and Jong, 1997) [42].

The compound that plays a role in healing wounds, especially burns include latexonins and papain (Hariana, 2004) [20]. Plants that contain latexonin is *Centella asiatica* (L.) Urban, while papain from papaya latex produced or Carica papaya (L). Both of these plants contain a compound that was instrumental in the process of healing wounds, especially burns (Mackay and Miller, 2003) [27].

Centella asiatica contains a group of compounds terpenoids, flavonoids, polyphenolic compounds, and compounds poliasetilena. The most important compounds that have been examined have the effect of healing open wounds and burns are the class of compounds triterpene latexonin and latexogenin, namely asiatat acid, acid madekasat, and asiatikosida (Duke *et al*., 2002; Shukla *et al*., 1999) [15, 46].

While the latex of papaya contains papain, kimopapain, papaya peptidase, pectin D - galaktase and L - arabinose (Starley *et al*., 1999; Azarkan *et al*., 2003) [43, 2]. Papain from papaya latex has been shown to be used as an antibacterial (Starley *et al*., 1999) [43], inflammatory (Gupta *et al*., 2000) [17], meat tenderizers (Koswara, 2007) [26], and accelerate the healing of wounds, especially burns (Ayob, 2003; Martin, 1996; and Salmah Mahmood, 2005; Hewitt *et al*., 2002) [1, 31, 28, 24].

Based on the description in the above, *centella asiatica* and papaya latex each been proven to heal burns. But research on the combination of both as burn treatment has not been done. *Centella asiatica* contains triterpene that can stimulate the formation of collagen as the early stages of tissue repair, while the latex of papaya contains papain that can clean up the dead cells on the skin as well as the control of inflammation (Mackay and Miller, 2003) [27].

Both compounds are contained in two of these plants was instrumental in healing burns. We perform research on the combination of herbal extracts and papaya latex *centella asiatica* for the treatment of burns studied in animal experiments, so expect a mixture of extracts can be used as an alternative medicine is efficacious as the healing of burns.

The study was conducted experimentally using 35 white male mice were divided into 7 groups. The methods used in this study is the topical method with the parameters measured were diameter burns. The data were processed using ANOVA test followed by Duncan test.

Materials and methods
Plant material
Centella asiatica extract and papaya latex for the proposed study were collected from localities of Padang, Indonesia. The collected plants were carefully examined and authenticated in Departement of Biology, University of Andalas, Padang, Indonesia.

Animals
Experimental animals used were healthy male white mice aged approximately 3 months with a weight of approximately 20-30 grams total of 35 birds. Before the study was conducted, mice in acclimatization for 7 days. Pets are healthy if the difference in weight before and after the adaptation of not more than 10 % and visually showed normal behavior.

Chemicals
Fenol 50 %, HPMC, propilenglikol, nipagin, Bioplacenton® jelly.

Methods

Preparation of Papain Course

Samples were taken from Sinta papaya fruit varieties are aged between 2.5 and 3 months. Latex carried out early morning at 6:00 to 08:00 pm. Papaya fruit is cleaned of impurities exist, then scratched the needle from the base to the tip of the fruit as deep as 1-2 mm. Latex dripping out accommodated by a stainless steel container. Furthermore, the latex is collected and dried using a vacuum oven to obtain dry papain. Papain is pulverized and sifted.

Preparation of *Centella Asiatica* Extract

Manufacture of extracts made with weigh one kilogram of powdered herb gotu kola, then put into maserator electrical stirrer, plus 5 liters of 70% ethanol, stirred for 30 minutes, allowed termaserasi for 24 hours. Once filtered with filter paper, the filtrate obtained is evaporated at the evaporator at reduced pressure until thick but can still be poured. Evaporation followed on stainless steel saucepan over a water bath until extracts obtained viscous to be weighed, counted rendemennya and analyzed further.

Setup Preparations

Dose Planning

The concentration of the active substance in the gel formulation is a single *centella asiatica* herb extract 2%, 2% single papaya latex, and combinations of *centella asiatica* herb extract and papaya latex ratio of 2% to 3 doses are:

- The combination of herbal extracts of *Centella asiatica*: papaya latex (1%: 1%).
- The combination of herbal extracts of *Centella asiatica*: papaya latex (1.5%: 0.5%)
- The combination of herbal extracts of *Centella asiatica*: papaya latex (0.5%: 1.5%)

Making Preparations Test

Centella asiatica extract and papaya latex formulated in a topical dosage forms which gel, using gel basis as follows:
Formula gel base (Fransiska, 2008; Putri, 2008) [16, 37]:
Every 100 grams of gel base contains:

HPMC	3.5
Propilenglikol	50
Nipagin	0.1
Aquadest	ad 100

Table 1: Formula gel *centella asiatica* extract and papaya latex

Nama zat (gram)	F 1	F 2	F 3	F 4	F 5
Centella asiatica extract	2%	-	1%	1,5%	0,5%
Papaya latex	-	2%	1%	0,5%	1,5%
Base ad	10	10	10	10	10

Preparation of medicated gel base

All the ingredients are weighed, HPMC (3.5 grams) was dispersed with most propilenglikol (28 grams) in a mortar (mass I). Nipagin propilenglikol diluted with the remainder (22 grams) (mass II). Massa II mixed into the first mass (mass III). At first, the mixture was stirred strong to prevent the precipitation, then the mass of III is added water and stirred gently to prevent the formation of air bubbles until the preparation is quite thick and not too sticky to be poured.

Making gel *centella asiatica* herb extract and papaya latex

Centella asiatica extract and papaya latex dissolved in propylene glycol (Massa IV). Base added gradually into the mass IV while stirring until homogenous.

Treatment of Animal Experiments

Experiments using 35 mice and were divided into 7 groups where each group consisted of 5 mice. As shown in Table II.

Table 2: Grouping mice by the treatment given

Kelompok	Perlakuan	Dose 2%	
		Centella extract	Papaya latex
1	without drugs (negative control)	-	-
2	Bioplacenton® jelly (positive control)	-	-
3	*Centella asiatica* extract	2	-
4	Papaya latex	-	2
5	Combination 1	1	1
6	Combination 2	1,5	0,5
7	Combination 3	0,5	1,5

Test Effect of Herbal Extract *Centella asiatica* and Papaya Latex Burn Wound Healing against Animal Experiments

This test uses male white mice consisting of 7 groups gel formula, each of which consists of 5 mice. First white male mice were shaved on the back, then peel blistered with 50% phenol solution. Blister skin by dipping a filter paper with a diameter of 2 cm into a solution of phenol 50%, then the filter paper taped to the skin of mice that have been sheared for 30 seconds. With a time of 30 seconds, 50% phenol solution has been able to create mouse skin blister characterized by bleaching the skin of mice. Carried out observations of shapes, colors and types of burns that can be determined the degree of the burn.
Injuries that occur wound measured diameter and mean diameter calculated by the following formula:

$$dx = \frac{dx_1 + dx_2 + dx_3 + dx_4}{4}$$

Where;
dx = diameter wound to the day-x

Then the test preparation + 0.1 gram applied to the skin of mice that had blistered once a day for 10 days and observe the changes that occur. Measure the diameter of the wound until the wound completely healed. Wound diameter equal to zero when the wound is covered by the new network.
Calculation of percentage of healing of burns can be done by the following formula (Wannarat *et al*., 2009) [52]:

$$Px = \frac{d_1{}^2 - dx^2}{d_1{}^2} \times 100\ \%$$

Where;
Px = Percentage of healing day-to-x
d1 = diameter of the wound first day
dx = diameter wound to the day-x

Healing burns monitored from the burns until the mice were cured. Complete healing can be seen with the formation of new skin layer with terkelupasnya the wound.

Data analysis

Data were analyzed statistically using the method of analysis of variance (ANOVA) in both directions. The data analysis followed by Duncan multiple range test (Duncan's Multiple Range Test).

Result

After doing research on the effect of the combination of *centella asiatica* herb extract and papaya latex to the healing of burns on white male mice, the results are as follows:

1. The percentage of healing of burns an average of the negative control group was given no treatment at day 10 was 75.34%
2. The percentage of healing of burns an average positive control group given the drug dosage burns on the market that is Bioplacenton® jelly and a group given a combination of herbal extracts of *Centella asiatica*: papaya latex (1%: 1%) on the 6th day already showed 100%
3. The percentage of healing of burns an average group given a combination of herbal extracts of *Centella asiatica*: papaya latex (1.5%: 0.5%) and the group given a combination of herbal extracts of *Centella asiatica*: papaya latex (0.5%: 1.5 %) on the 7th day already showed 100%
4. The percentage of healing of burns an average group were given doses of *centella asiatica* herb extract 2% and the group papaya latex dose of 2% on the 8th day already showed 100%

Table 3: Data on average the percentage of healing of burns from a combination of total triterpenoids *Centella Asiatica* herb extracts and papaya latex on white male mice after treatment

Groups	The percentage of healing mean + SD during the day-										Total percentage of the average healing + SD
	1	2	3	4	5	6	7	8	9	10	
Negative control + SD	0,00 + 0,000	11,52 + 20,514	20,82 + 17,874	20,66 + 17,770	24,51 + 18,770	32,4 + 21,745	62,57 + 30,406	65,92 + 26,969	69,49 + 26,351	75,34 + 20,709	38,321 + 32,6489
Positive control + SD	0,00 + 0,000	48,07 + 20,144	59,33 + 17,150	78,59 + 17,368	91,20 + 9,6462	100 + 0,000					77,7210 + 33,193
Centella extract 2%	0,00 + 0,000	32,07 + 27,050	49,36 + 21,838	20,46 + 93,420	62,41 + 26,711	81,70 + 21,259	98,66 + 1,8844	100 + 0,000			64,4692 + 46,852
Papaya latex 2%	0,00 + 0,000	4,638 + 29,104	30,2 + 26,202	30,2 + 26,202	59,8 + 17,636	79,56 + 13,086	91,34+ 4,9364	100+ 0,000			55,5198 + 46,761
Combination Centella extract: papaya latex 1%:1%	0,00 + 0,000	7,832 + 36,044	32,6 + 47,447	81,19 + 10,566	98,42 + 3,5329	100 + 0,000					72,0046 + 43,443
Combination Centella extract: papaya latex 1,5%:0,5%	0,00 + 0,000	15,92 + 9,2456	49,46 + 14,481	80,69 + 11,024	93,04 + 4,3119	98,5 + 2,0567	100 + 0,000				73,7612 + 37,134
Combination Centella extract: papaya latex 0,5%:1,5%	0,00 + 0,000	11,68 + 42,392	29,22 + 39,899	66,46 + 21,536	91,54 + 6,6628	99,18 + 1,8335	100 + 0,000				69,8080 + 42,871

Treated groups

Fig 1: Burns first days after administration of the combination *Centella asiatica* extracts: papaya latex (1 % : 1 %)

Fig 2: The healing of burns on the 7th day of the administration of the combination *Centella asiatica*: papaya latex (1% : 1%)

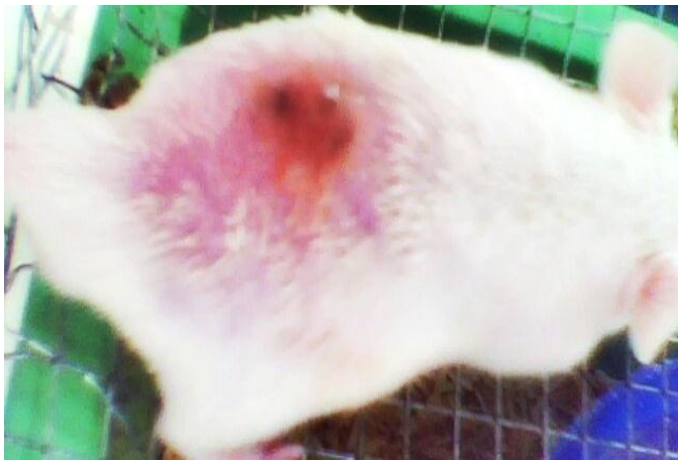

Fig 3: Burns first days after administration of the combination *Centella Asiatica* extracts: papaya latex (1.5 % : 0.5%)

Fig 4: The healing of burns on the 7th day of the administration of the combination *Centella Asiatica* extracts: papaya latex (1.5 % : 0.5%)

Fig 5: Burns first days after administration of the combination *Centella asiatica* extracts: papaya latex (0.5 % : 1.5%)

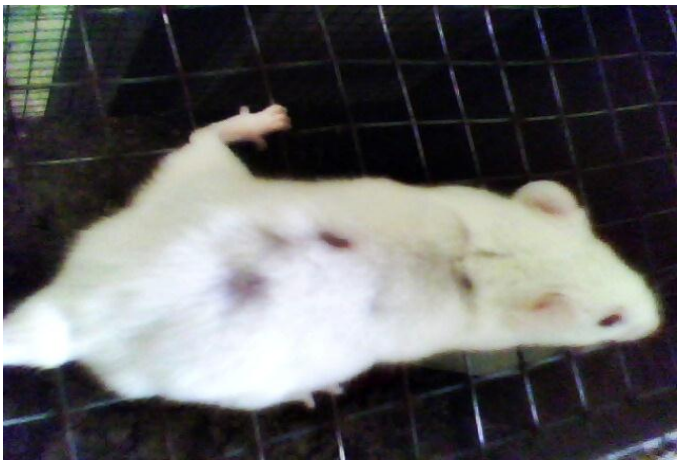

Fig 6: The healing of burns on the 7th day of the administration of the combination *Centella asiatica* extracts: papaya latex (0.5 % : 1.5%)

Discussion

In this study used samples of *Centella Asiatica* herb extract and papaya latex. Basic selection of the two types of this substance is the potential of both in accelerating the healing of burns both traditional and based on the research that has gone before. *Centella Asiatica* contains triterpenoids that can stimulate the formation of collagen as the early stages of tissue repair, while the latex of papaya contains papain that can clean up the dead cells on the skin as well as the control of inflammation (Mackay and Miller, 2003) [27]. Combining both are expected to be effective in accelerating the healing of burns and the effects of the treatment given in addition to optimal for healing burns are also able to remove scars and stains of the burn.

Caucasian male white mice blistered with phenol 50% for 30 seconds. With a time of 30 seconds, 50% phenol solution has been able to create mouse skin blister characterized by bleaching the skin of mice. The use of phenol in inducing burns due to phenol can simultaneously burn blister the skin and mucous membranes. This is because phenol containing not less than 99.0% and not more than 100.5% C_6H_6O and is corrosive to skin tissue (Depkes, 1995).

These combined administration of *centella asiatica* herb extract and papaya latex are topically in a gel dosage form. Selection gel as the dosage form based on several considerations, including the raw materials used one form of gum, gelling easier and the composition is quite simple and has an interesting shape, creates a feeling of cool soothing and easily washed after treatment and water content can reduce the high mechanical on the skin, mucous membranes and the treatment of wounded and burned skin tissue (Swarbick *et al*., 1992) [44].

Doses of *centella asiatica* herb extract and papaya latex used is 2%. Doses of *centella asiatica* herb extract equated with a dose of papaya latex to be able to see a comparison of the potential effects of both at the same dosage. Dose combination *centella asiatica* herb extract and papaya latex is also equated with the use of a single dose of 2%, so that a large number of doses of variation combinations are the same as single use (1%: 1%) (1.5%: 0.5%) and (0.5%: 1.5%) (Total both 2%), with dose reduction principles either of these substances would give effect to the healing of burns. Old topical administration of both substances is 10 days to see the effect of duration of the percentage of healing of burns on male white mice.

In this study showed that administration of doses of *centella asiatica* herb extract 2%, 2% dose papaya latex, and combinations thereof (the dose (1%: 1%) (1.5%: 0.5%) (0.5%: 1.5%) shows the speed the healing of burns on white male mice. After testing the analysis of variance (ANOVA) bidirectional showed very significant influence ($P < 0.05$) between treatment factors, the healing and the interaction

with the healing of the percentage of healing of burns. This is presumably due to the combined effect of synergism granting work of each of the test preparation that has a different working mechanism in accelerating the healing of burns.

Based on data from the comparison of the percentage of healing of burns on *centella asiatica* extract and papaya latex either a single dose or a combination of both, showed a significant correlation between the percentage of time healing with the healing of burns (Appendix 7 Figure 23). This graph clearly visible on the comparison between the healing time with the percentage of healing of burns on *centella asiatica* extract and papaya latex either a single dose or dose combination. This is because both of these plants contain compounds with different mechanisms in the healing of burns.

On observation it was found that the percentage of healing of burns by *centella asiatica* herb extract triterpenoids total single dose provides the same healing time with a single dose of papaya latex. But still lower than the positive control group. In granting *centella asiatica* herb extract triterpenoids total single dose of 2% indicates the percentage of healing higher than the day to day administration of a single dose of papaya latex 2%, even though the treatment is the same. In total triterpenoids extract herb *centella asiatica* is a single dose on day 2 began to show marked the early healing wound began to dry up. While the administration of a single dose of papaya latex initial healing is shown on the 3rd day with a marked wound began to dry up. This is thought to be caused by the physiological condition of each animal and the enzyme papain in the cleaning of necrotic tissue and controlling inflammation that affects the comprehensive process and drying of the wound.

In the treatment dose combination of either (1%: 1%); (1.5%: 0.5%) and (0.5%: 1.5%) speeds healing better than the single group of triterpenoids total herb *centella asiatica* and papaya latex dose of 2%. This is expected because the provision of a combination of *centella asiatica* herb extract and papaya latex causing speed healing of burns from two sides, namely of increasing the synthesis of collagen and acid mucopolysaccharides, as well as by inhibiting the inflammatory phase and also helps cleansing of necrotic tissue or dissolve dead cells attached to the skin difficult physically separated so as to accelerate healing of burns.

Based on the results obtained can be assumed that the combination of *centella asiatica* herb extract and papaya latex can be used as an alternative medicine healing of burns.

Conclusion

From the research that has been done can be concluded as follows:

- Provision of a single dose of *centella asiatica* herb extract, papaya latex single dose and combination of herbal extracts of *Centella asiatica*: papaya latex can accelerate time to healing of burns significantly ($P < 0.05$).
- Providing a combination of *centella asiatica* herb extract and papaya latex showed a faster recovery time and better than the singular respectively.
- Providing a combination of herbal extracts of *Centella asiatica*: papaya latex 1%: 1% give the same healing time with positive control and faster than the combination of *centella asiatica* herb extract 1.5%, 0.5% and 0.5%: 1.5%.
- The first treatment day until day 6 showed a significant effect on the percentage of healing of burns. While the 7th day up to day 10 showed no significant effect on the percentage of healing of burns.

Suggestion

Advised on the next researcher to conduct further research on the histopathological study the combined effect of *centella asiatica* herb extract and papaya latex on wound healing burns on white male mice.

References

1. Ayob I. Khazanah herba dalam koleksi perubatan tradisional yang hebat dan petua orang tua. Hlm.86. Golden Book Centre, 2003.
2. Azarkan M, Moussaoui AE, van Wuytswinkel D, Dehon G, Looze Y. Fractionation and purification of the enzymes stored in the latex of Carica papaya. J Chromatogr B, 2003; 790:229-38.
3. Baga Kalie. Bertanam papaya. Jakarta: Penebar Swadaya, 1996.
4. British Pharmacopoeia. Centella. Incorporating the requirements of the 3rd edition of eropaea pharmacopoeia 1997 as amended by supplement, 2001.
5. Chandrasoma R, Taylor CR. Healing and repair in concise pathology. 3rd Ed.London. Prentice Hall International Inc., 1998.
6. Cheng CL, Guo JS, Luk J. The healing effects of Centella extract and asiaticoside on acetic acid induced gastric ulcers in rats. Life Sci. 2004; 74(18):2237-2249.
7. Departemen Kesehatan Republik Indonesia. Acuan sediaan herbal. Jakarta. Hal, 2000, 121-125.
8. Departemen Kesehatan Republik Indonesia. Extra Pharmacopeia Indonesia. Penerbit Lembaga Farmasi Nasional. Jakarta, 1974.
9. Departemen Kesehatan Republik Indonesia. Farmakope Indonesia (Edisi III). Jakarta, 1979.
10. Departemen Kesehatan Republik Indonesia. Farmakope Indonesia (Edisi IV). Jakarta, 1995.
11. Departemen Kesehatan Republik Indonesia. Materia Medika Indonesia. Jilid I. 1977, 34-39.
12. Departemen Kesehatan Republik Indonesia. Materia Medika Indonesia. Jilid VI, 1995.
13. De Sanctis MT, Belcaro G, Incandela L. Treatment of edema and increased capillary filtration in venous hypertension with total triterpenic fraction of *Centella asiatica* a clinical, prospective, placebo controlled, randomized, dose-ranging trial. Angiology, 2001; 52:55-59.
14. Djatmiko Hertami. Pepaya. Jakarta: CV Yasaguna, 1985.
15. Duke JA, MJ Bogenschutz-Godwin J. DU Cellier, Duke PAK. Handbook of medicinal herbs. Second edition. CRC Press London-New York, 2002, pp. 344-346.
16. Fransiska A. Formulasi gel minyak atsiri daun sirih (Piper bettle Linn) menggunakan HPMC sebagai basis gel untuk anti kandidosis kutis. (skripsi). Padang: Universitas Andalas, 2008.
17. Gupta OP, Sing S, Bani S, Sharma N, Malhotra S. Anti-inflammatory and anti-arthritic activities of silymarinacting through inhibition of lipoxygenease. Phytomedicine. 2000; 7:21.

18. Guo JS, Cheng CL, Koo MW. Inhibitory effects of *Centella asiatica* water extract and asiaticoside on inducible nitric oxide synthase during gastric ulcer healing in rats. Planta Med. 2004; 70(12):1150-1154.
19. Harborne JB. Metode fitokimia: Penuntun cara menganalisis tumbuhan. terbitan ke-2. Diterjemahkan oleh K. Padmawita dan I. Soediro. Penerbit ITB. Bandung, 1987.
20. Hariana HA. Tumbuhan obat dan khasiatnya. Seri 1. Penebar Swadaya. Jakarta, 2004.
21. Harmely F, Salman. Studi aktifitas papain dalam krim. Artikel Dosen Muda (BBI) Dikti. Padang, 2007.
22. Heimbach D. Burn patient then and now. Burns. 1999; 25:1-2.
23. Herlich K, AOAC. Official methods of analysis. Vol II. Ed 15. United State of America, 1990.
24. Hewitt H, Wint Y, Tolobere L, Lopez S, Bailey E, Parshad O, *et al*. The use of papaya on pressure ulcers. Am. Jour. Nutr. 2002; 102(12):73-77.
25. Jayashree G, Kurup M, Sudarsial S, Jacob VB. Anti-oxidant activity of *Centella asiatica* on lymphoma-bearing mice. Fitoterapi. 2003; 74(5):431-434.
26. Koswara Sutrisno. Tepung getah papaya: Pengempuk daging, 2007. Ebook Pangan.com.pdf. 20 Juni 2010.
27. Mackay D, Miller AL. Nutritional support for wound healing. Alternative medicine review. 2003; 8(4):359-377.
28. Mahmood AA, Sidik & Salmah I. Wound healing activity of Carica papaya L. aqueous leaf extract in rat. Int J Moleculer Med Ad Sci. 2005; 1:398-401.
29. Mann J. Chemical aspect of biosynthesis. 1st ed., Oxford University Press, New York, 1994.
30. Mansjoer Arif. Kapita selekta kedokteran. Jilid II. Edisi III. Media Aesculapius. Jakarta, 2001.
31. Martin A. The use of antioxidant in healing. Dermatol. Surg. 1996; 22:156-160.
32. Menard R, Khouri HE, Plouffe C, Dupras R, Ripoll D, Vernet T. A protein engineering study of the role of aspartate 158 in the catalytic mechanism of papain. Biochemistry. 1990; 29:28.
33. Muhidin Dudung. Agroindustri papain dan pektin. Jakarta: Penebar Swadaya, 2001.
34. Nazrun A, Mohd Syukri, Ahmad AY. The Effects of Carica papaya Linn. latex on the healing of burn wound in rats. Jurnal Sains Kesehatan Malaysia. 2005; 3(2):39-47.
35. Oyedeji OA, Afolayan AJ. Chemical composition and antibacterial activity of the essential oil of *Centella asiatica* growing in South Africa. Pharm Biol. 2005; 43(3):249-252.
36. Parfitt K, (Ed). Martindalle the Extra Pharmacopedia. 32nd Ed. The Pharmaceutical Press, London, 1999.
37. Putri WE. Formulasi gel dari ekstrak etanol buah belimbing wuluh (Averhoa bilimbi Linn) sebagai Antijerawat. (Skripsi). Padang: Universitas Andalas, 2008.
38. Rao SB, Chetana M, Uma Devi P. *Centella asiatica* treatment during postnatal period enhances learning memory in mice. Physiol Behav. 2005; 86(4):449-457.
39. Sabiston David C. Buku ajar ilmu bedah (Essentials of surgery). Penerbit Buku Kedokteran. EGC, 1995.
40. Sebayang Firman. Imobilisasi enzim papain dari getah pepaya dengan alginat. Jurnal Komunikasi Penelitian. 2006; 18(2):34-38.
41. Setianingsih Dwi, Wahyudi Budi. Kesetimbangan papain dalam getah papaya padat dan air pada ekstraksi papain: variasi kadar NaHSO3 dalam air. Disajikan dalam Seminar Nasional Teknik Undip. Semarang, 2004.
42. Sjamsuhidayat R, Wim De Jong. Buku ajar ilmu bedah. Penerbit Buku Kedokteran. EGC, 2007.
43. Starley IF, Mohammed P, Schneider G, Bicckler SW. The treatment of paediatric burns using topical papaya. Burns, 1999; 25(7):633-639.
44. Swarbrick James, James C Boylan. Encyclopedia of pharmaceutic technology. Marcel Dekker INC. New York. 1992; Vol. 6.
45. Stewart PJ. Prediction of drug stability. Aust Hosp. Pharm. 1994; 14(4):165-171.
46. Shukla A, Rasik AM, Jain GK. In vitro and in vivo wound healing activity of asiaticoside isolated from *Centella asiatica*. J Ethnopharmacol. 1999; 65:1-11.
47. Suguna L, Sivakumar P, Chandrakasan G. Effects of *Centella asiatica* extract on dermal wound healing in rats. Indian J Exp Biol. 1996; 34:1208-1211.
48. Teixeira da silva JA, Zinia R, Duong Tan Nhut, Dharini, S. Papaya (Carica papaya L.) biology and biotechnology. Tree and forestry science and biotechnology. 2007; 1(1):47-73.
49. Thompson EB. Drug bioscreening fundamental of drug evaluation techniques in pharmacology. New York: Graceway Publishing Company, Inc., 1990.
50. Tjitrosoepomo G. Taksonomi tumbuhan obat. Gadjah Mada University Press. Yogyakarta. 1994, 295-297.
51. Van Steenis CGGJ. Flora malesiana, Vol 4, Noordhof N.V. Batavia, 1984.
52. Wannarat K, Mayuree H, Boonyong T. Wound healing effects of a standardized extract of *Centella asiatica* ECa 233 on burn wound in rats. Thai J Pharmacol. 2009; 31(1):120-123.
53. Wasitaatmadja Syarif M. Ilmu penyakit kulit dan kelamin. Fakultas Kedokteran UI. Jakarta. 1999, 3-8.
54. WHO. WHO monograph on selected medicinal plants. Vol 1. Geneva, 1999.
55. WHO Regional Publications. Medicinal plants in the south pacific. Westren Pacific Series. 1998; 19:35-36.
56. Winarto WP, dan Subakti. Khasiat dan manfaat pegagan: Tanaman penambah daya ingat. Jakarta. Agromedia Pustaka, 2003, p. 64.