

## Effect of Propolis Extract to Heal The Burns in New Zealand Rabbit

Maria Hilaria<sup>1</sup>, Elisma<sup>1</sup>, Adrianus L. Uhe<sup>1</sup>

<sup>1</sup>Study Program of Pharmacy Health Polytechnic of Kupang

### Abstract

Propolis contains arginine (amino acids), ferulic acid (a derivative compound cinnamic which promotes the formation of collagen), flavonoids (phenolic compounds), and other compounds that can prevent the growth of bacteria, virus, and fungi. The purpose of this study was to measure the most concentration of propolis extracts that effective to heal burns of New Zealand rabbit's. This was an experimental study with the post-test only control group design. As many as 15 male white rabbits were divided into 5 groups which treated by propolis extract with concentration of 50%, 75%, 100%, positive control by bioplacenta and negative control with propylene glycol. The macroscopic observation was done with detached necrosis tissue and the growth of collagen tissue as the parameter. Data were analyzed by using the one-way ANOVA and continued by the SNK test. The results of this study indicated that the propolis extracted have potency on healing burn wound with the significance of 0.00 ( $p \leq 0.05$ ). In the concentration of 100%, extract of propolis has potency on healing burn wound that proportionates with bioplacenta with the significance value of 1.000 ( $p \geq 0.05$ ). It is concluded that by increasing dose, the healing time of burn wound will be shortened.

**Keywords :** Propolis, healing burns, rabbit

### INTRODUCTION

Burn wounds have been a major challenge to deal with having high morbidity and mortality (Jalali, *et al.*, 2012). Burns caused by fire, exposure to high temperatures from the sun, electricity or chemicals. Patient with burns which require special treatment for burns is different from other injuries such as stab wounds because those burns are commonly followed with high pathogenesis of germs, there are a lot of dead systems, spend a lot of water and serum, open wounds for a long time, as well as the need to close the tissues (Nugroho, 2012).

The severity of a burn depends on tissues conditions and the intensity of trauma burning heat. Thick skin which contains a lot of pigments and sebaceous glands will be more resistant to heat trauma compared with thin and dry skin. The treatment for healing the burn aims to prevent infection and allow the regeneration of epithelial cells in wound surface. It is also necessary to find compounds that stimulate the formation of collagen, a structural protein that stimulates the wound healing process (Nugroho, 2012). One of the

products made from a bee that used for a long time to heal burn is propolis. Propolis contains arginine (amino acids), ferulic acid (a derivative compounds cinnamic which promotes the formation of collagen), flavonoids (phenolic compounds), and other compounds that can prevent the growth of bacteria, virus, and fungi from the burns that might leads to infection (Mahani, *et al.*, 2011).

Several scientific test to determine the efficacy of propolis for wound healing have been reported as the study conducted by Santoso entitled "Differences Between Speed Burn Healing Ointments Propolis 5% and Green Tea Concentration 6.4 g% On White Rat (*Rattus norvegicus*)", which concluded that propolis 5% of the most influential in the percentage of healing of burns compared with green tea 6.4 g% concentration. Based on this previous study, the use of propolis as a cure for burns has a good prospect for development in the province in terms of both cultivation and production of bee propolis itself.

\*Corresponding author e-mail : dmanehat@yahoo.co.id

## MATERIALS AND METHODS

### Materials

Raw propolis was obtained from the beehive of *Apis dorsata* honey that had taken from Oelpuah, Central Kupang.

### Phytochemical Screening

#### Phenol Compound

The classic way to detect simple phenolic compounds is to add a solution of iron (III) chloride 1% in water or ethanol to the solution of footage, which cause the color green, red, purple, blue or black solid (Harborne, 1996).

#### Arginine

Protein solution that has the amino acid arginine residues in its chemical structure when added to a solution of alpha-naphthol and sodium hypochlorite will form a red color. Amino acids arginine and the levels of 0.0004 mg/mL with this test still gives red color (Sumardjo, 2008).

#### Cinnamic Compounds and Derivatives

Cinnamic derivative compounds found widely in nature, particularly hydroxycinnamate derivatives, such as p-coumarate, caffeate, ferulic and sinapate. These compounds are usually found in the form of esters. These compounds are easily detected because it taints on filter paper (or paper chromatogram) gives a blue or green fluorescence under ultraviolet light. The color intensity can be increased when treated with ammonia vapors.

### Propolis Extract

#### Extract of Propolis

Extraction is done by maceration with 70% ethanol. A total of approximately 200 grams of propolis obtained from the honeycomb soaked with 650 mL of 70% ethanol, closed and kept in a dark room and beaten repeatedly for one week. After that, the filtrate was taken and macerated residue back with 300 mL of 70% ethanol. Furthermore, filtrate taken every day for one week. After seven days, or after the resulting clear color, maceration terminated (Ning, 2009).

#### Preparation of Propolis Extract Concentration of 100%, 75%, 50%

In this study, the concentration of propolis extracts from the village of Oelpuah District of Central Kupang Kupang regency used was 100%, 75%, and 50%. Results of propolis extract evaporated using a rotary evaporator and a water bath is referred to as extract of

propolis 100% from the village of Oelpuah District of Kupang Tengah Kabupaten Kupang and then to manufacture the concentration of 75% and 50% use dilution method using propylene glycol.

#### Burn Healing Effects Testing

The hair on the back of the rabbit was shaved clean. Rabbit locally anesthetized using ethyl chloride sprayed on the back skin of rabbits. Created burns on the backs of rabbits using heat inducers (metal, diameter 2 cm) were heated on a fire-free for 1 minute 30 seconds and then placed on the backs of rabbits for 30 seconds. Each burn is given treatment by applying drug use by 5 drops (0.25 mL) for each treatment the positive control, negative control and test materials. Basting is done every day 1 time a day for 21 days of testing. Do the observation of the effects of the healing of burns every day until the burns healed (diameter zero), record observations (Ning, et al., 2006).

### Data Analysis Techniques

Calculation of percentage of healing performed by the formula:

$$Px = \frac{d1^2 - dx^2}{d1^2} \times 100\%$$

Description:

Px = the percentage of healing day x  
d1 = diameter of the wound first day  
dx = diameter wound to the day-x  
(Ning, et al., 2006).

Data analysis with ANOVA and SNK test (Sudrajat, et al., 2005)

## RESULTS AND DISCUSSION

### Preparation of Propolis Extract

The extraction of 200 grams of crude drugs (raw propolis) from the village of Oelpuah District of Central Kupang by using ethanol 70% as much as 650 mL conducted maceration with 300 mL ethanol 70% obtained liquid extract as much as 740 mL of dark brown, after evaporated with rotary evaporator, followed by evaporation of the water bath obtained viscous extract as much as 44.5860 grams (22.29%) blackish brown with a distinctive odor extracts.

### Test Free Ethanol

This test aims to determine whether the District Oelpuah propolis extract from the

village of Central Kupang district who have gone through the process of evaporation no longer containing 70% ethanol. From the test results, data showed that there was no smell typical of the ester of ethanol extract of propolis extracts from the village so Oelpuah District of Central Kupang declares that free of ethanol.

## Screening Phytochemicals

### Test of phenol

This test aims to identify the presence or absence of phenolic compounds contained in extracts of propolis from the village of Oelpuah District of Central Kupang. Testing is done by adding a solution of iron (III) chloride 1% in water or ethanol to the solution of footage, which of the test results obtained from the data that these tests produce colored footage strong black color so that the extract contains phenolic compounds tested positive.

### Test compounds and derivatives of cinnamic

The test is performed to identify the presence or absence of cinnamic and derivatives of compounds contained in extracts of propolis from the village of Oelpuah District of Central Kupang. Testing is done by observing the stains footage propolis extracts from the village of Oelpuah District of Central Kupang on the filter paper which in this test data showed that footage on filter paper under observation of ultraviolet rays fluoresce green that propolis extracts from the village of Oelpuah Subdistrict Central Kupang declare

that cinnamic containing compounds and derivatives.

### Testing Results Burn Healing Effects on Rabbit

Results of testing the effect of the concentration of propolis extract on the healing effects of second-degree burns on rabbits are characterized by superficial skin damage from the superficial dermis where the common bullae wound, the wound is red or white and are often located higher above the normal skin. Changes in average diameter measured burn until the wound healed declared for each treatment. Data change burns diameter is obtained by calculating the average change in the diameter of burns with each result measurement time interval measurements can be seen in Fig. 1.

The results showed that the propolis accelerates healing of burns compared with control rabbits were healed within 25 days. The healing of burns is also those through a series of phases in which the wound healing process in burn healing process consists of three phases, namely the inflammatory phase, the proliferative phase, and maturation phase.

In the treatment extract 100%, the phase of the healing process takes place within 19-20 days. Inflammatory phase lasts from day 1 until day 2 where the inflammatory phase is characterized by swelling and formation of bullae. Proliferative phase occurs on day 3 until day 8 where the proliferative phase is characterized by the outbreak bullae which then dries resulting in the formation of exudate and

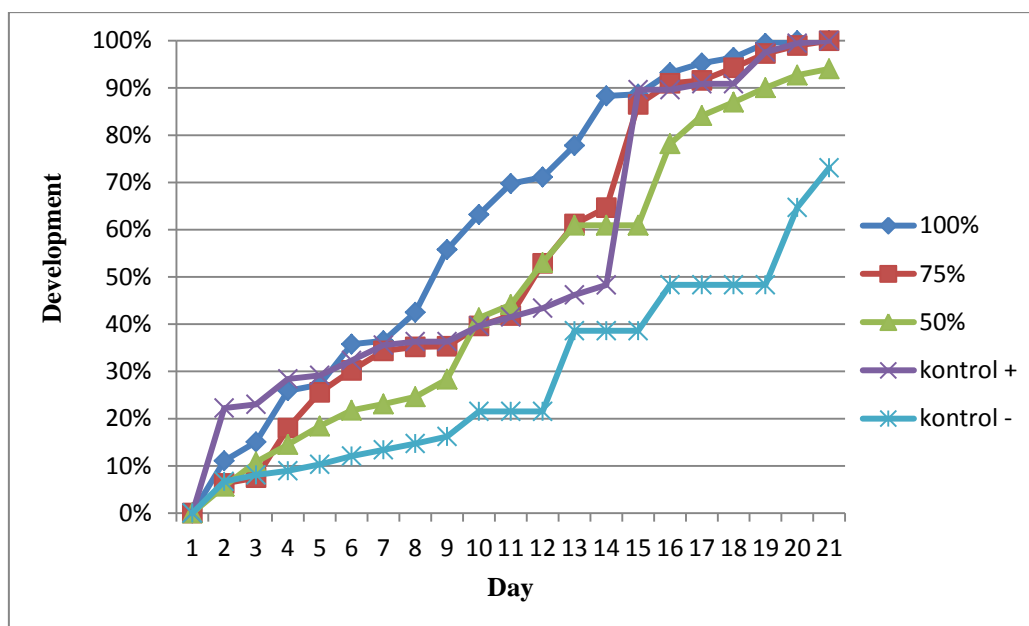


Figure 1. Time interval measurements

fibroblasts that looks like a crust on top of the wound and on the 9th day to 18<sup>th</sup> day injuries suffered maturation process where end phase is characterized by the formation of new tissue which means that the wound had shrunk or recovered, then on days 19 and 20 wounds had healed.

In the treatment extract 75%, the phase of the healing process takes place within 19-21 days. Inflammatory phase lasts from day 1 until day 3 in which the inflammatory phase is characterized by swelling and formation of bullae. Proliferative phase occurs on day 4 until day 14 where the proliferative phase is characterized by the outbreak bullae which then dries resulting in the formation of exudate and fibroblasts that look like a crust on top of the wound and on the day 15 until the day 18 injuries suffered maturation process where maturation phase is characterized by the formation of new tissue which means that the wound had shrunk or recovered, then on day 21, the wound had healed.

In the treatment extract 50%, the phase of the healing process takes place within 21-23 days. Inflammatory phase from day 1 until day 8 wherein the inflammatory phase is characterized by swelling and formation of bullae. Proliferative phase occurs on day 9 until day 20 where the proliferative phase is characterized by the outbreak bullae which then dries resulting in the formation of exudate and fibroblasts that looks like a crust on top of the wound and on day 21 the wound undergo a process maturation where end phase is characterized by the formation of new tissue which means that the wound had shrunk or recovered, then on day 21 to day 24, the wound had healed.

In this test was also conducted on rabbits treated using propolis on the market as a positive control and propylene glycol as a negative control where each treatment shows the time and the healing process is different.

On the positive control, the healing process takes place within 21 days, in which the inflammatory phase lasts only 1 day later on day 2 had undergone a process of proliferation. The apparent differences between positive control with propolis extract is in the healing process of burns make positive control inflammatory phase only characterized by swelling and the bullae formed not filled with liquid so that the proliferative phase there is a

process of the breakup of bullae that cause injury wet and bleeding yet this did not happen and instantly dries on day 2 which meant cuts through a phase of rapid proliferation. The process of proliferation without going through the process of the breakup of bullae lasted until the 19th and then the 20th day the wound undergo a process maturation characterized by the formation of new tissue which means that the wound had shrunk or recovered and on day 21 the wound had cured.

In the negative control, the healing process takes longer than 25 days. The healing process starts from the inflammatory phase from day 1 until day 11 in which the inflammatory phase is characterized by swelling and formation of bullae. Proliferative phase occurs on day 12 and at day 20 where the proliferative phase is characterized by the outbreak bullae which then dries resulting in the formation of exudate and fibroblasts that looks like a crust on top of the wound and on day 21 until day 24 injuries suffered maturation process where end phase is characterized by the formation of new tissue which means that the wound had shrunk or recovered, then on day 25, the wound had healed.

### Results of Data Analysis In Statistics

Data analysis method used was ANOVA (analysis of variance). Analisa conducted on the results of changes in the diameter of burns from 0 days up to 21 days after the formation of burns. Analysis of variance to change the diameter of the burn is used to see whether there is any difference in the effect of the concentration of propolis extract on the healing effect of skin burns backs of rabbits. To see which treatment group who have similar effects or different and the effects of the smallest to the greatest effect between each other in order to obtain the composition of the different groups SNK test. ANOVA can be seen in table F counted value of  $44.953 > F$  table 2.46 and the probability of  $0.000 < 0.05$  so it can be concluded that the five treatments were significantly different effect (significant). Because there is a real difference, it is necessary to further examine the differences lie with further test SNK (Student-Newman-Keuls). In the SNK test data obtained between the extract 50%, 75%, positive control has no significant difference. In the table of data that existing real or significant differences are a

negative control treatment group and 100% in which the group has a significant difference to the treatment of real or extract 50%, 75%, positive control. So that the obtained sequence of SNK's most effective concentration to which have the smallest effect is the concentration of 100%, positive control, concentration 75%, the concentration of 50%, a negative control.

## CONCLUSIONS

### Conclusions

The test results burn healing effects of propolis extracts from the village of Oelpuah District of Central Kupang shows the effect as burn treatment which saw the healing process characterized by a reduced diameter burns faster than the diameter of burns on the negative control treatment.

In the statistical test using ANOVA two-way be deduced that there are effects of the concentration on the healing effects of burns on the back skin of rabbits New Zealand and through testing subset of the statistics using SNK data showed that of the five treatment has the effect of healing of burns are the most effective propolis extract 100% from the village Oelpuah District of Central Kupang Kupang district and the smallest effect is propylene glycol as a negative control.

### Suggestions

1. It is advisable to conduct further research on the efficacy of propolis from East Nusa Tenggara as antioxidants given the content of propolis is rich in bioflavonoids.
2. The test should be conducted to determine the toxicity and pharmacological safety Oelpuah propolis extracts from the village of East Nusa Tenggara Kupang district before applied to humans.

## REFERENCES

- Ansel, H. and Prince, S., 2004, *Pharmaceutical Calculation: The Pharmacist's Handbook*, In: Aisyah, C. and Elviani E., translator, 2006, *Kalkulasi Farmasetik: Panduan Untuk Apoteker*, Jakarta: EGC Publishers.
- Darmono, S., 2011, *Buku Ajar: Farmakologi Eksperimental*, Jakarta: Universitas Indonesia Press.
- Departemen Kesehatan RI, 1977, *Materia Medika Indonesia Jilid I*, Jakarta: Departemen Kesehatan RI.
- Departemen Kesehatan RI, 1979, *Farmakope Indonesia Edisi III*, Jakarta: Departemen Kesehatan RI.
- Departemen Kesehatan RI, 1986, *Sediaan Galenika*, Jakarta: Departemen Kesehatan RI.
- Gunawan, D. and Mulyani, S., 2004, *Ilmu Obat Alam (Farmakognosi) Jilid I*, Jakarta: Penebar Swadaya.
- Jalali, F.S.S., Tajik, H. and Hadian, M., 2012, Efficacy of Topical Application of Alcoholic Extract of Yarrow in the Healing Process of Experimental Burn Wounds in Rabbit, *Comp. Clin. Path.*, 21(2), 177–181.
- Moenadjat, Y., 2001, *Luka Bakar*, 2nd Edition, Jakarta: Universitas Indonesia Press.
- Mahani, 2011, *Keajaiban Propolis Trigona*, Jakarta: Pustaka Bunda.
- Ningsih, D. and Pamuji, G., 2009, *Potensi Propolis Trigona Spp Pandeglang Sebagai pemacu Pertumbuhan Pada Sapi Peranakan Ongele*, Essay, Sekolah Pascasarjana Institut Pertanian Bogor, Bogor.
- Ningsih, D. and Pamuji, G., 2006, *Petunjuk Praktikum Farmakologi*, Surakarta: Universitas Setia Budi.
- Nugroho, T., 2012, *Mengungkap Tentang Luka Bakar dan Artritis Reumatoid*, Yogyakarta: Nuh Medika.
- Priyatna, N., 2011, *Beternak dan Bisnis Kelinci Pedaging*, Jakarta: AgroMedia Pustaka.
- Pujilestari, R., 2007, *Efek Penyembuhan Luka Bakar Krim Ekstrak Etanolik Daun Nanas (Ananas Comosus Merr) Pada Kulit Punggung Kelinci Jantan Newzealand*, Essay, Universitas Muhammadiyah Surakarta, Surakarta.
- Santoso, T., 2010, *Perbedaan Kecepatan Kesembuhan Luka Bakar Antara Olesan Propolis 5% dan Teh Hijau Konsentrasi 6,4gr% Pada Tikus Putih (Rattus Norvegicus)*, Essay, Universitas Muhammadiyah Yogyakarta, Yogyakarta.
- Sihombing, 1997, *Ilmu Ternak Madu*, Yogyakarta: Gadjah Mada University Press.
- Sudrajat and Subana, M., 2005, *Dasar-Dasar Penelitian Ilmiah*, Bandung: Penerbit Pustaka Setia Bandung.

- Sumardjo, D., 2008, *Pengantar Kimia: Buku panduan Kuliah Mahasiswa Kedokteran dan Program Strata I Fakultas Bioeksakta*, Jakarta: EGC Publisher.
- Suranto, A., 2010, *Dahsyatnya Propolis Untuk Menggempur Penyakit*, Jakarta: PT. Agromedia Pustaka.
- Voigt, R., 1994, *Buku Pelajaran Teknologi Farmasi Edisi ke-5*, Yogyakarta: Gadjah Mada University Press.
- Weller, B., 2005, *Kamus Saku Perawat*, In: Harton, A., *Kamus Saku Perawat*, 22nd Edition, Jakarta: EGC Publishers.