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Effect of Turmeric Ethanol Extract Ointment (Curcuma Longa) on the Healing of Grade II Burns in Wistar Rats (Rattus Norvegicus)

Ji Junfeng[1], Florenly[2], Johannes Bastira Ginting[3], Fioni[4]
[1,2,3,4]*Master of Clinical Medicine, Faculty of Medicine, Universitas Prima Indonesia*
ly@unprimdn.ac.id

Abstract: *Burns is still a choir for clinical nurses as severe burns have led to high postburn morbidity. In Indonesia, the death rate from burns is still high at about 40%, mainly caused by severe burns. Curcuma longa herbal products have several such antioxidants, anti-inflammatory, anti-cancer. The purpose of this study was to find out the phytochemical content and effect of turmeric Ethanol Extract Ointment (Curcuma longa Linn) in curing grade II burns in Wistar rats (Rattus norvegicus). This study is an experimental study with a pre and post-test control group design approach. This research was conducted at Riwandi Pet Shop and Animal House and Laboratoirum Taxonomy of Plants, Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), the University of North Sumatra in February-March 2021. The study sampled 20 turmeric rhizomes and male Wistar rats (Rattus norvegicus) divided into 4 treatment groups. Independent variables of topical formulation (base ointment, nebacetin ointment®, Turmeric Ethanol Extract Ointment (Curcuma longa) 5% and Turmeric Ethanol Extract Ointment (Curcuma longa) 10%, as well as independent variables that include wound contraction and epithelial period. The results of phytochemical screening in turmeric found phytochemicals in the form of flavonoids, alkaloids, and tannins. The results of the study obtained significant differences in the epithelial period of the standard group, turmeric ointment 5% and 10% against the control group. However, in the turmeric ointment group and the standard group, there was no difference in epithelialization periods. This is evident from the value P < 0.05 (Value P = 0.028). Where the wound contraction rate of turmeric ointment is 10% better than nebacetin ointment as standard. But in both turmeric ointments, both 5% and 10% and nebacetin ointment as standard did not show significant differences in the parameters of the epithelial period.*
Keywords: *curcuma longa; burns; ointments*

I. Introduction

The prevalence of injuries increases every year, burns rank sixth cause of unintentional injury (unintentional injury) after a fall, motorcycle, and others (Badan Penelitian Dan Pengembangan Kesehatan Kementerian Kesehatan RI 2013). In 2008, more than 410,000 burns occurred in the United States, with about 40000 requiring hospital treatment. In India, more than 1 million people suffer burns each year (Fitria, Saputra, and Revilla 2014). Until now, burns are still a chor for clinical nurses as severe burns have led to high postburn morbidity. In Indonesia, the death rate from burns is still high at around 40%, mainly caused by severe burns (Mutia 2015).

A combustion is an injury (injury) as a result of direct contact or exposure to sources of heat (thermal), electricity, chemicals, or radiation (Tutik Rahayuningsih 2012). Based on depth, burns are divided into 4 types: superficial (degree 1), deep partial thickness (degree 2), and full thickness (level 3), and level 4 (Hakim 2020). Burns can usually be prevented, and different treatments are applied based on the severity of the burn.

Sometimes, ointments, creams, biological and nonbiological dressings, and antibiotics are recommended levels 2, 3, and 4 burn, while misuse of such drugs can increase the risk of antibiotic resistance and fungal infections, even slowing wound healing and increasing the depth of burns (Avni et al. 2010).

Herbal products have been used since long ago in the medical world, one of which is curcuma longa root. The main compound of turmeric is curcumin (Nabofa et al. 2018). Many researchers are interested in studying the benefits of turmeric and its level of safety. Some of the benefits of turmeric from the results of research are turmeric is antioxidant (Wanninger et al. 2015); (Razavi 2021); (Razavi 2021), anti-inflammatory (Manarin et al. 2019); (Setiadi, Khumaida, and Wahyuning Ardie 2017); (Kocaadam and Şanlier 2017), anti-cancer (Hartati 2013); (Razavi 2021); (Razavi 2021). The purpose of this study is to find out the phytochemical content and effect of turmeric Ethanol Extract Ointment (Curcuma longa Linn) in curing grade II burns in Wistar rats (Rattus norvegicus).

II. Review of Literature

Turmeric with the scientific name Curcuma longa linn is one of the spice plants and is also a medicinal plant (Ariani 2017); (Sabale, Modi, and Sabale 2013). The main compound of turmeric is curcumin (Nabofa et al. 2018). Curcumin is the most important element among the natural curcuminoids found in the turmeric plant (curcuma longa). Curcumin derivatives have been evaluated for bioactivity and structure-activity relationships (SAR=Structure-activity relationships) (Itokawa et al. 2008). The efficacy and interesting properties of chemical physics of curcumin compounds, making this plant used as a lead compound for the development of new drug compounds (Wanninger et al. 2015). Curcumin, its main active constituent, is very powerful and antioxidants such as vitamins C, E, and Beta-Carotene, making the use of turmeric the consumer's choice for cancer prevention, liver protection, kidney protection, anti-aging, anti-inflammatory activity, anti-spasmodic and analgetic function (Ahmad et al. 2010). The National Cancer Institute has clarified that turmeric plants are non-toxic, even at high doses, so they are recognized as safe ingredients (GRAS=Generally recognized as safe) (Itokawa et al. 2008). The skin is part of the integumental system there are three main layers of skin: epidermis, dermis, and hypodermis (subcutaneous fat). The focus of this topic is on the skin layer of the epidermis and skin. Skin appendages such as sweat glands, hair follicles, and sebaceous glands are in-depth reviewed elsewhere (Kalangi 2014).

III. Research Methods

This study is an experimental study with a pre and post-test control group design approach. This research was conducted at Riwandi Pet Shop and Animal House and Laboratoirum Taxonomy of Plants, Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), the University of North Sumatra in February-March 2021. In this study sample of turmeric rhizomes and male Wistar rats (Rattus norvegicus) as many as 20 are divided into 4 treatment groups so that each group consists of 5 rats (Muthia Milasari 2019). The variables in this study are independent in the form of giving several topical formulations (base ointment, nebacetin ointment®, Turmeric Ethanol Extract Ointment (Curcuma longa) 5% and Turmeric Ethanol Extract Ointment (Curcuma longa) 10%, as well as independent variables that include wound contraction and epithelialization period. pellets.

3.1 Tool

Maceration vessel, knife, rotary evaporator, water handler, gel container, stirrer rod, plate measuring 2 x 2 cm.

3.2 Material

Turmeric, aquades, lanolin, solid paraffin, Cetostearyl alcochol, white vaseline, gauze, oil paper, filter paper, 1mm2-sized paper, oil paper, nebacetin ointment®.

3.3 Phytochemical Test

Turmeric rhizomes identified several groups of compounds such as flavonoids, tannins, alkaloids, phenols, steroids/triterpenoids, terpenoids and saponins (Rahmawati, Febrina, and Tjitraresmi 2016).

a. Identify Flavonoids

As much as 1 ml of test solution is each put into 3 test tubes. Tube 1 as a control, tube 2 coupled with 1 mL of 5% FeCl3 solution, flavonoid positive if there is a dark green/blue discoloration. Tube 3 coupled with a few drops of NaOH 10% formed yellow color if it contains flavonoids.

b. Identification of Tannins

Tannins As much as 2 mL of test solution is inserted into the test tube added with a few drops of 1% FeCl3 solution, a positive sign of tannin if the color formed is dark green/ blue.

c. Identification of Alkaloids

For alkaloid tests, as much as 2 mL of test solution is evaporated on a porcelain cup until residue is obtained. The residue is then dissolved with 5 mL HCl 2N. Once cool, the solution is filtered. The solution obtained is divided into 3 test tubes. The first tube serves as a control. The 2nd tube is added 3 drops of the dragendroff reagent and the third tube is added 3 drops of mayer reagent (through the tube wall). The formation of orange deposits in the second tube and yellow deposits in the third tube indicates the presence of alkaloids.

d. Identification of Phenols

Tests are carried out in drip plates, the test solution is added FeCl3 (1% dalaim water / ethanol), if there is a change in color green / red / purple / blue / black shows the presence of phenol content.

e. Identification of Terpenoids

The modified Salkowaski test. For 2 mL of water plant extract, added 2 mL of chloroform and this is followed by the addition of a few drops of concentrated sulfuric acid. The solution is well shaken. The formation of a yellow lower layer indicates the presence of terpenoids.

f. Identification of Saponins

Saponins 4 mL test solution is added with 5 mL aquadest, shake, see the presence of a stable foam. A little extract is added 5 mL of water, shake in a test tube, form a stable foam (foam as high as 1 cm and stable for 30 minutes). 4 mL of test solution is inserted into the test tube as a control (Muthia Milasari 2019).

3.4 Manufacture of Ointment Preparations

Table 1. Topical Preparation Formulations of Each Ointment

Material Name	**Ointment**	**Turmeric Ointment *(Curcuma longa)* 5%**	**Turmeric Ointment *(Curcuma longa)* 10%**
Turmeric Ethanol Extract	-	0.5 ml	1 ml
Lanolin	2.5g	2.5g	2.5g
Solid paraffin	2.5g	2.5g	2.5g
Cetostearyl alcochol	2.5g	2.5g	2.5g
White vaseline	42.5g	42.5g	42.5g

3.5 Testing on Animal Trials

All animals try in the form of wistar rats are done need by using electrical solder that has been motivated with a round-shaped tip then found on the dorsal part of the rat for 10 seconds, before doing the need for the rats dianastesi using ketamine (50 mg / kg i.m) that has previously been satisfied. Before continuing with the sampling of extract gels and controls, different tests are carried out to assess the degree and extent of the grade II burn (Thakur et al. 2011). As for the treatment given to 24 wistar rats as tried animals that are divided into groups as in the table below:

Burns evaluation is carried out every 2-4 days, with aspects evaluated from the healing activities of the burn including wound contration and epithelialization periods (Thakur et al. 2011). Wound contration is measured by displacing the diameter of the wound using a ruler, then wound contraction is calculated by the following formula (Thakur et al. 2011):

$$\text{Wound Contraction (\%)} = \frac{(\text{size of the initial wound - size of the wound on a specific day}) \text{X } 100\%}{\text{Wound Size on Specific Day}}$$

The epithelialization period is measured by calculating the length of time eschar is removed to escape, during which the epithelial period is calculated in the day (Thakur et al. 2011); (Ghazali et al. 2016). The static analysis used in the study was Anova's One-Way test, followed by a post-hoc test. Before another test is done descriptive analysis of wound contraction and epithelial period. If the data in this study is distributed abnormally, then there will be a transformation of the data so that the data is distributed normally.

Table 2. Treatment Group in Rats

Group	**Treatment**
Control	In this group only given the base of ointment
Standard	In this group is used Nebacetin Ointment which is generally used in the treatment of burns.
Turmeric ethanol extract ointment (Curcuma Longa) 5%	In this group used Turmeric Ethanol Extract Ointment (Curcuma Longa) 5%
Turmeric ethanol extract ointment (Curcuma Longa) 10%	In this group used Turmeric Ethanol Extract Ointment (Curcuma Longa) 10%

IV. Discussion

4.1 Phytochemical Screening of Turmeric Extract

From the results of phytochemical screening in fresh samples of turmeric (Curcuma longa Linn) found phytochemical content in the form of alkaloids, flavonoids, and tannins. The results of this study are not much different from the results of the research conducted (Alwafi Ridho Subarkah 2018); (Cobra n.d.) Turmeric (Curcuma longa Linn) reported that the sample had phytochemicals in the form of phenolics, saponins, flavonoids, tannins, triterpenoids, and alkaloids.

Table 3. Turmeric Phytochemical Screening Results (Curcuma longa Linn)

Phytochemicals	Test Method	Result
Alkaloid	Dragendorff	+
Steroid	Maeyer Salkowsky	+
Saponin	Aquadest Aquadest + Alcohol 96%	-
Flavonoid	FeCl3 5% NaOH 5%	+
Tanin	FeCl3	+

4.2 Wound Healing Activities

To evaluate wound healing in each group of mice, different test analyses were conducted in accordance with the results of the normality analysis of data from each of the wound healing parameters evaluated. The results of the analysis of the normality of the data can be seen in the table 4. From table 4. It can be seen that the wound contraction parameter data on day 6 and day 9 shows a normal data distribution, so the data analysis used for different tests is One Way Anova followed by Post Hoc Test Tukey HSD. Meanwhile, other parameters show an abnormal distribution of data so that different tests used are kruscal-wallis and mann-whitney tests. From the results of the analysis there was a significant difference in wound contraction between the control group and the other group.

Table 4. Results of Data Normality Analysis on Burn Healing Parameters

Wound Healing Parameters	*p-value*
Wound Contraction on the day -3	0.009
Wound Contraction on the day -6	0.311
Wound Contraction on the day -9	0.051
Wound Contraction on the day -12	0.022
Wound Contraction on the day -14	0.005
Period Epitelialisasi	0.005

Meanwhile, between the standard group and the group that was given turmeric ointment at all times of observation found a significant difference in the magnitude of

wound contraction. Meanwhile, the change in the concentration of Turmeric ointment to the large difference in wound contraction was insignificant at the beginning of the observation time, but the wound contraction difference between Turmeric ointment 5% and 10% began to be observed on the last day of observation, namely on the 14th day with a p-value of 0.008 < 0.05. Therefore Ha accepted, that there is a significant influence of turmeric ethanol extract with the healing process of grade II burns in Wistar rats. As one of the parameters of wound healing the results of different tests of wound contraction in each treatment group are shown in Table 5.

Table 5. Results of analysis of One Way Anova and Kruskal-Wallis with Wound Contraction as Wound Healing Parameters in The Treatment Group

Observation Time	**Wound Contraction (%)**				
	Control	**Standart**	**Turmeric ointment (Curcuma Longa Linn) 5 %**	**Turmeric ointment (Curcuma Longa Linn) 10 %**	**Score**
Hari Ke-3	4.12 (8.33)	0.00 (8.66)	12.14 (24.81)	10.52 (15.91)	**0.003****
Hari Ke-6	8.22 ± 5.21	18.12 ± 10.54	31.18 ± 7.28	34.17 ± 7.58	**0.012****
Hari Ke-9	7.59 ± 7.92	35.21 ± 8.05	46.15 ± 6.75	51.42 ± 8.42	**0.009****
Hari Ke- 12	9.22 ± (29.17)	45.36 (21.11)	64.14 (20.15)	66.57 (8.86)	**0.023****
Hari Ke- 14	27.17 (37.50)	82.61 (41.22)	76.77 (11.24)	77.29 (3.64)	**0.011****

* Data is presented in Median (Range). Different lowercase letters in the same column show significant differences in P values < 0.05

From the table data above it can be seen that the P-value of each test at each observation time is < 0.05, this shows that there is a significant wound contraction difference between each group at each unit of observation time. However, in the analysis of the different tests is not clearly explained between which groups there are significant differences. Therefore, the analysis continued with the Tukey HSD and Mann-Whitney Post Hoc Test to compare two groups at each unit of observation time so that it is met between groups whichever difference is significant wound contraction.

Table 6. Results of Epithelial Period Comparison in Each Treatment Group

Treatment Group	**Epithelialization Period***	**Value P**
Control	22 (2)[a]	
Standard	18 (2)[b]	0.028
Turmeric Ointment 5%	20 (2)[b]	
Turmeric Ointment 10%	20 (2)[b]	

* Data is presented in Median (Range). Different lowercase letters in the same column show significant differences in P values < 0.05

In addition to wound contraction, another parameter that was also evaluated in assessing burn healing was the epithelialization period, the results of different tests from

the epithelial period of each treatment group can be seen in table 6. It can be seen that there was a significant difference in the epithelial period of the standard group, turmeric ointment of 5% and 10% of the control group. However, in the turmeric ointment group and the standard group, there was no difference in epithelialization periods. This is evident from the value $P < 0.05$ (Value $P = 0.028$). Based on the results of the above research, it can be seen that there are significant differences in wound contraction parameters and epithelial periods of each treatment group.

The results of this study are supported by research (Muthia Milasari 2019), entitled The Effect of Yellow Turmeric Extract Ointment (Curcuma Longa Linn) On Wound Healing In White Rats (Rattus norvegicus). Declaring the administration of yellow turmeric extract ointment concentrations of 10%, 20%, and 30% affects the healing speed of wounds in treated rats and based on observations of anatomical pathology and post hoc tests with LSD showed that yellow turmeric extract ointment 10% is the best preparation in the wound healing process where significant differences from each group are evident on day 7.

Turmeric extract helps speed up the wound healing process due to curcumin compounds that have uses as anti-inflammatory and antioxidants that can accelerate epithelialization, cell proliferation, and collagen synthesis (Thangapazham, Sharma, and Maheshwari 2007). For the 10% wound closure group occurred on the 12th day, 20% of the wound closure occurred on the 14th day, and for the 30% group, the wound closed perfectly on the body's natural 15th day against a wound or infection characterized by redness or erythema, swelling or edema, heat, and pain. As an anti-inflammatory, curcumin contained in turmeric is known to inhibit the enzyme cyclooxygenase (COX-2) and is also effective in inhibiting the enzyme lipooxygenase (LOX) where both enzymes play a role in the inflammatory process.

Essential oils from turmeric rhizomes also showed anti-inflammatory effects in mice and suppressed arthritis, edema of the hands or feet. According to Akram (2010) Curcumin is an antioxidant as powerful as vitamins C, E and beta-caroten (Akram et al. 2010). The more the amount of extract added in the preparation of the preparation, the more the number of bases will decrease. Naibaho et al (2013) It states that the absorption of drugs in ointment preparations (percutaneous absorption) depends not only on the physical nature of medicinal substances but also on the properties of carriers and also skin conditions. Percutaneous absorption of a drug is affected by the concentration of the drug, the area of the membrane where the preparation spreads, the degree of solubility of the medicinal ingredients in both oil and water, the hydration effect of the skin, the time the drug sticks to the skin. The results of testing the effect of turmeric extract ointment with hydrocarbon base faster in the wound healing process. Yellow turmeric extract ointment with a hydrocarbon base has greater scattering power. Scattering power indicates the preparation's ability to spread on the skin. The wider the surface of the skin where the preparation spreads, the absorption of the medicinal ingredients will increase (Naibaho, Yamlean, and Wiyono 2013).

V. Conclusion

The results of phytochemical screening in turmeric found phytochemicals in the form of flavonoids, alkaloids, and tannins. The healing effects of burns possessed by Turmeric ointment are both 5% and 10% and nebacetin ointment as standard show significant differences. Where the wound contraction rate of turmeric ointment is 10% better than nebacetin ointment as standard. But in both turmeric ointments both 5% and 10% and

nebacetin ointment as standard did not show significant differences in the parameters of epithelial period.

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