International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.7, No.4, pp 601-605, 2014-2015

Burn Wound Healing Effect of Trembesi (*Samanea saman*) Leaves Extract Gel on Rats (*Rattus novergicus*)

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Abstract: Trembesi (*Samanea saman*) leaves is used by Gorontalo society as burn wound healing. It contains tannin, flavonoid, saponin, steroid, glikosida, and terpenoid.
The aim of this study was to determine effective concentration of trembesi (*Samanea saman*) leaves extract against burn wound healing on rat (*Rattus novergicus*). Trembesi leaves extract was gained by maceration using methanol then trembesi leaves extract formulated into gel dosage form. Gel, contained trembesi leaves extract, was made into three concentrations such as 6%, 9%, and 12%. Gel basis was negative control and bioplacenton was positive control. After that, back skin of rat was injured by hot induction tool. The result of this study showed that group, content of 6% extract, described the healing effect average on thirteenth days. Meanwhile, gel, content of 9% extract, gave healing effect on eleventh days and 12% of extract showed that the average of healing process occurred on ninth days. From the result of one way ANOVA and Duncan test depicted that gel which gave burn wound healing the best was 12% of trembesi leaves extract. It means that the trembesi leaves extract containing saponin was one of active compounds which can accelerate collagen formation. It had function to accelerate wound healing process.
Keywords : burn wound, extract, gel, trembesi leaves.

Introduction

In Indonesia, there are some various types of plant which have been utilized as natural product sources traditionally. However, recently technology advances and modern sciences are not able to replace traditional drugs. It can be seen from natural product research have been developed and investigated by pre-clinic and clinic test for public health.

Trembesi (*Samanea saman*) plant, which is many found in city center in Gorontalo, is utilized by people as burn wound healing medicine. Some people do not know the function of trembesi leaves because they lack of experience to use it optimally. The use of trembesi leaves extract is not practical unless it is used on skin directly because it can cause irritation on skin. Therefore, this extract can be developed and formulated in other dosage form such as gel so that it can be applied easily on skin surface.

The result of phytochemical analysis data stated that trembesi leaves extract contained tannin, flavonoid, saponin, steroid, glycoside and terpenoid. Saponin is one of active compounds accelerating collagen formation i.e. structural protein having a role to heal wound. Whereas flavonoid is phenol group having function as antiseptic and be able to inhibit arachidonic acid release and inflammation mediator.[1]

The one of infection causes on burn wound can be caused by bacteria such as Streptococcus or Staphylococcus and negative gram microorganism.[2] According to Raghavendra research stated that methanol extract of trembesi leaves 0.002% (106 CFU/mL) was able to inhibit *Escherichia coli* having inhibition zone 8.87 mm, *Staphylococcus aureus* 18.37 mm, *Pseudomonas aeruginosa* 10.18 mm and *Streptococcus faecalis* 9.75 mm.[3] Besides that, previous research had proven that trembesi extract cream 8% had inflammation effect and gave wound healing which were equal with bioplacenton.[4]

Based on this background, trembesi leaves extract was formulated in gel dosage form having aim to know effectiveness of trembesi leaves extract gel against burn wound healing on rats.

Materials and Methods

TrembesiLeaves Preparation

Trembesi leaves was cleaned by water and dried in the room without direct sunlight. After that, samples were cut into small and blended till obtained trembesi leaves powder.

Sample Extraction

Trembesi leaves powder 1.26 kg was put into maceration container then added methanol 9.5 L after that, closed for 3 days and stirred every day then filtered by filter paper (filtrate I). Residue of sample was extracted by methano for 3 days then filtered (filtrate II). Further filtrate I and II were collected then evaporated by rotary evaporator on 60^{o}C till obtained viscous extract and dried into oven 40^{o}C till gained dry extract.

Procedure of TrembesiLeaves Extract Formulation

Carbopol 940 was dispersed into warm water for 15 minutes then stirred 346 rpm for 10 minutes till form gel (mixture I). After that, gel was checked pH 6.5 – 7. Meanwhile, other container containing methyl paraben was dissolved into propylene glycol then stirred till homogeny (mixture II). After that, mixture between methyl paraben and propylene glycol were included into mixture of carbopol 940 gel and added trembesi leaves extract and TEA then stirred with using stirrer 300 rpm for 10 minutes and checked pH of trembesi leaves extract gel.

This procedure was repeated by using other concentrations of extracts i.e. 9% and 12%.

Experimental animals

Fifteen rats were divided into 5 groups of treatment which each group consisted of 3 rats. Group I, rats were induced by hot metal plate for 5 minutes then affixed on dorsal of rat for 2 seconds. After that, dorsal of rats were given trembesi leaves extract gel 6% for one day and measured wound diameter each day. This treatment was done for two days.Meanwhile, for group II and III were treated the same thing with group I but different concentration of extract i.e. 9% and 12%. Otherwise, group IV and V were negative and positive control which only utilized gel basis and bioplacenton® with the same thing of group I, II, and III.

Ethical clearance was obtained from Institutional animal Ethical Committee of Medicine faculty.

Statistical analysis

The results are expressed as the mean SEM for each group. Statistical differences were evaluated using a one way analysis of variance (ANOVA) followed by LSD test. Results were considered to be statistically significant at $p<0.01$.

Results

The Percentage of burn wound healing (table 1) showed that wound healing was counted from first day which can be seen the end of inflammation, F1 showed burn wound healing on the fifth day, F2 on the fourth day, F3 on the third day, F4 on the sixth day, and F5 on the fifth day. Overall burn wound healing totally, which is the fastest, was gel of trembesi leaves extract 12% (F3) on the ninth day.

Table1.The percentage of burn wound healing

Formula	Rat	Observation the day														
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
F1	1	0	- 10,6	- 31,8	- 7,5	**5,8**	16,9	64	69,1	80,9	90,3	92,8	97,3	99,5	**100**	100
	2	0	- 30,6	- 44	- 22,6	**1,4**	17,7	47,9	61,4	87,2	92,2	96,2	99,1	**100**	100	100
	3	0	- 9,9	- 26,6	- 8,5	- 15,9	**18,5**	55,6	76,4	78,4	90,2	95,9	98,4	**100**	100	100
F2	1	0	- 7,3	- 14,9	**21,7**	27,9	39,6	65,2	89	96,2	99,6	**100**	100	100	100	100
	2	0	- 11,7	- 19,3	**12,4**	23,9	38	71,7	86,4	98,4	99,8	**100**	100	100	100	100
	3	0	- 16,8	- 23,2	**4,4**	16,9	35,8	58,1	81,2	94,5	99,1	99,8	**100**	100	100	100
F3	1	0	- 20,2	**5,4**	22	36	65,6	81,7	95,1	99,4	**100**	100	100	100	100	100
	2	0	- 25,6	**5,6**	20,1	36,9	67,8	85,3	97,6	**100**	100	100	100	100	100	100
	3	0	- 11,5	**14,8**	37,5	46	70,2	88,7	98,4	**100**	100	100	100	100	100	100
F4	1	0	- 5,5	- 35	- 25,8	- 15,4	**2,7**	25,2	47,7	69,3	82,5	91,2	94,4	98,7	99,7	**100**
	2	0	- 41	- 54,5	- 34,5	- 18,9	**4,1**	30,5	40,6	59,2	83,2	93	97,4	99,4	**100**	100
	3	0	- 24,5	- 41,7	- 12,6	**9,3**	11,87	29,9	45	64,9	79,2	91	96,1	98,9	99,9	**100**
F5	1	0	- 4,27	- 25,4	- 7,2	**28,6**	42,2	71,4	80,9	87,1	96,1	**100**	100	100	100	100
	2	0	- 12,4	- 3	**8,8**	32,8	58,2	72,3	80,9	92,3	98,6	**100**	100	100	100	100
	3	0	- 27,6	- 46	- 1,4	**19,1**	51,3	68,5	89,5	95,9	**100**	100	100	100	100	100

The result of wound diameter observation (figure 1) showed that diameter of burn wound can be calculated the average of burn wound diameter with measurement time interval each 24 hours. The graph showed that each formula of F1 – F5 had the average of the same length of the initial injury i.e. 1.4 cm on the first day. The treatment of F1 – F5 had different effectiveness of wound healing such as treatment F5 on the ninth day. Otherwise, the treatment of negative control had healing effectiveness on the thirteenth day. Meanwhile, the treatment group (F1, F2, and F3) indicated that the existence of different healing effectiveness occurred on the eighth day which group F3 had showed effectiveness of burn wound healing totally. Whereas Group F2 had begun wound healing on the tenth day and the twelfth day for group F1. It means that trembesi leaves extract contained active compound which was able to remedy burn wound. Otherwise, group F4 containing gel basis (negative control) can also heal burn wound because gel basis can give cooling effect on burn wound so that it can accelerate healing process. Besides that, the body had natural ability to protect and restore itself.[6]

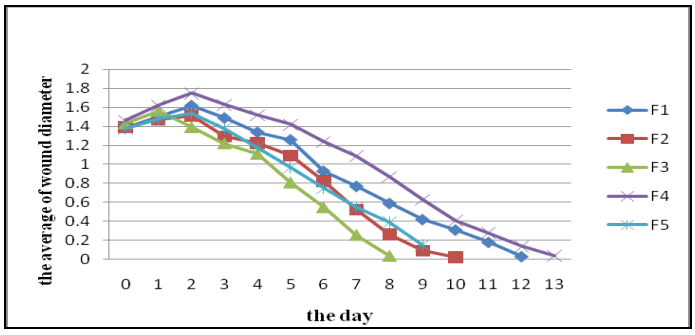

Figure 1. Graph of wound diameter change

Based on figure 2 can be seen that treatment group (F1 – F5) experienced inflammation process which occurred edema on the skin so that the wound became bigger than initial injury but the end of wound healing process of treatment groups were different each group. Group F3 showed that the end of inflammation and wound healing totally was the fastest compared to group F2, F5, and F1. Otherwise, the percentage of wound healing, which was the slowest, was showed by group F4. So, the treatment of group F3 gave effect of burn wound healing which was bigger than group F1 and F2.

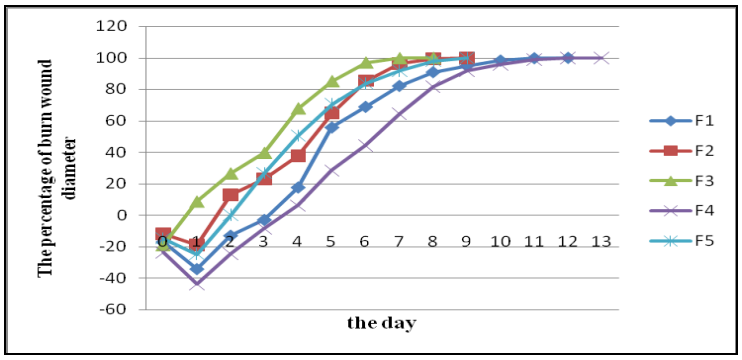

Figure 2. Graph of burn wound healing percentage

Discussion

Burn wound is a form of damage or tissue loss which caused by contacting of heat sources such as fire, hot water, chemical materials, and electricity.[5] The symptoms of burn wound are heat and redness which can be resolved by cooling the injured area or decreasing inflammation, preventing infection, giving opportunity epithelial cells to do proliferation and closing the wound surface. For decreasing inflammation need a material which can detract inflammation or materials having function as astringent and materials which can stimulate collagen formation.[5] The one of materials having function above is trembesi leaves extract gel which utilized by Gorontalo people to treat burn wound.

The burn wound healing process can be divided into 3 phase i.e. inflammation phase, proliferation phase, and closure phase.[5] Inflammation phase occurred on skin after inducing heat metal. It can be seen that wound diameter became bigger, redness and edema. The beginning of wound healing phase occurred rubor, heat, turgor, and dolor.[7]

The result of wound diameter change was analyzed by Kolmogrovsmirnov test to know influence treatment against test animal condition. The statistic result showed that data of wound diameter change had normal distribution and variation. It means that the use of sample had the same variation. Further test in this research was ANOVA test to know significant difference of treatment group after that continued LSD test to know different treatment on each group.

Based on ANOVA test can be described relationship between formula and closure wound completely. From statistical analysis result showed that F arithmetic > F table on significance 5% and 1%. This result proved that giving of trembesi leaves extract gel was different in accelerating burn wound healing on rat and was able to cure burn wound. Meanwhile, the value of degrees of freedom error was 10. It means that this research data can be relied its accuracy because according to Gomez (2007) stated that the result of F test can be relied it accuracy unless it have the value of degree of freedom error minimal 6.

The LSD test result of effectiveness of trembesi leaves extract gel against burn wound healing on rat showed that group F1 had healing effectiveness which was not different with group F4. Besides that, it can be also seen that group F2 had healing effectiveness which was not different with group F5 but group F3 had healing effectiveness which was different compared to other formulas. This means that group F3 contained higher concentration of active compounds which was capable to decrease inflammation faster than other formulas. Besides that, gel basis which was also able to reduce inflammation gave cooling effect on the skin. In other words, trembesi leaves extract contained one of active compounds such as flavonoid having function as inflammation effect. According to Loggia et all (1986) stated that flavonoid can inhibit arachidonic acid release until no releasing inflammation mediator.[9]

Further test results of LSD for curing wound completely showed that F5 had wound healing time which was not different with F2. Otherwise, F3 had wound healing time which was very different compared to other formulas. It means that F3 contained active compound which can stimulate collagen formation and cover the wound faster than F1, F2, and F5. Regarding this, one of active compounds of trembesi leaves extract was saponin which has an important role to spur collagen formation i.e. structural protein having function to accelerate wound healing process.[9,10]

Meanwhile, trembesi leaves extract also contained antiseptic materials which can kill or prevent microorganism growth on wound. In fact, the wound did not experience chronic infection.[5]Overall, the formulation of trembesi leaves extract in gel dosage form can remedy burn wound on rat and the best formula effect of burn wound healing was shown by formula 3 (12% of trembesi leaves extract) because F3 gave burn wound healing effect faster than other formulas including bioplacenton® gel .

Acknowledgements

The authors are highly thankful to sponsor State University of Gorontalo and some students of Pharmacy Department, Faculty of Health and Sport Sciences for the generous financial support.

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