ISSN 0974-3618 (Print)
0974-360X (Online)

www.rjptonline.org

RESEARCH ARTICLE

Potential Antiseptic of *Rhodomyrtus tomentosa* leaves extract on Healing wound in Male Wistar rats

Endang Sri Purwanti Ningsih[1], Noorlaila[1], Muhammad Ikhwan Rizki[2], Windy Yuliana Budianto[3]

[1]Department of Nursing, Health Polytechnic of Health Ministry Banjarmasin, Indonesia.
[2]Department of Pharmacy, Faculty of Science, Universitas Lambung Mangkurat, Banjarbaru.
[3]Department of Pediatric Nursing, Faculty of Medicine, Universitas Lambung Mangkurat,
Jl. Ahmad Yani Km.36, Banjarbaru 70712, Indonesia.
*Corresponding Author E-mail: **eenspn75@gmail.com**

ABSTRACT:

Background: The process of wound healing is influenced by various factors such as age, hormones, and wound care. Wound care is done to accelerate wound healing which can be done by various methods, one of them is traditional care. Traditional wound care can use medicinal plants. *Rhodomyrtus tomentosa* is a medicinal plant that has an antioxidant, anti-inflammatory, antitumor and antibacterial content. Thus this study aims to evaluate the effectiveness of the antiseptic solution of the Rodhomyrtus tomentosa leaf extract on wound healing in male Wistar rats. **Method:** this research is pure experimental research with post test only control group design. Thirty male white rats were divided into five groups, namely negative control, positive control, *Rhodomyrtus tomentosa* leaf extract 15%, 30%, and 60%. *Rhodomyrtus tomentosa* leaf extraction was carried out by maceration method with 70% ethano solvent. The extraction results are divided into 3 concentrations (15%, 30% and 60%). The wound healing process was evaluated by measuring the length of the wound manually from 0 to 10 days in each group. Meanwhile, the number of fibroblast cells was calculated through hematoxylin eosin (HE) staining and observed using an Olympus CX41 microscope with a 10x magnification and objective lens magnification in 3 fields. **Result:** There was a significant difference in the reduction in wound length (p =< 0,000) between the five experimental groups (*Rhodomyrtus tomentosa* leaf extract solution 15%, 30% and 60%, negative control and positive control. Solution of *rhodomyrtus tomentosa* leaf extract accelerated the increase in the number of fibroblasts compared to the negative control group (p = 0.003), but did not make a difference (p = 0.403) with the positive control group. *Rhodomyrtus tomentosa* leaf extraction solution had the same microscopic effect on the number of fibroblasts with a positive control group given 0.9% NaCl solution. **Conclusion:** There was a significant difference in the number of fibroblasts between all groups, but no difference in wound healing length.

KEYWORDS: Wound Healing, *Rhodomyrtus tomentosa*, Wound Length, Fibroblast**.**

INTRODUCTION:

Wound healing is a physiological process of body that runs dynamically, continuously, overlaps, and is programmed appropriately. The wound healing process involves many body cells and occurs in 3 phases, as inflammatory, proliferation and maturation (remodeling) phases[1,2].

This process is influenced by many factors such as local and systemic factors. Local factors are factors that directly affect the characteristics of the wound. This factor consists of oxygenation and infection. Meanwhile, systemic factors are factors that indirectly affect local factors. The systemic factors include age, hormones, stress, disease, and medication (treatment)[3,4].

Various wound care methods have been widely used locally and systemically to help the wound healing process. Different agents used for wound healing include antibiotics and antiseptics, antibacterial agents (hydrogen peroxide, eusol and collagenase ointments, wound healing promoters), several substances (tissue extracts,

Received on 14.04.2020 Modified on 25.07.2020
Accepted on 15.09.2020
Research J. Pharm. and Tech. 2021; 14(6):3143-3149.
DOI: 10.52711/0974-360X.2021.00548

vitamins, and minerals), as well as a number of medicinal plant products[5,6]. Medicinal plants are known to have effects on wound healing by a mechanism that is different from the mechanism of medicine. Medicinal plants are able to modulate wound healing by reducing the number of bacteria, increasing collagen deposition, increasing the amount of fibroblasts and fibrocytes. Various types of medicinal plants that are often used in wound healing such as *Aloe vera, Arctium lappa, Astragalus propinqus, Ampelopsis japonica, Rhodomyrtus tomentosa*, etc.[6,7].

Rhodomyrtus tomentosa has been widely used as a medicinal plant in various countries such as China, Vietnam, Thailand, the Philippines[8,9], and including Indonesia. In Indonesia, especially in South Kalimantan, *Rhodomyrtus tomentosa* can be found growing wild in the forest or even cultivated by local communities outside their natural habitat. Based on preliminary studies, it is known that *Rhodomyrtus mentosa* is widely used by local people as a traditional medicinal ingredient for various diseases (urinary tract infections, diarrhea, and wound healing).

The 2015 Mordmuang study states that *Rhodomyrtus tomentosa* leaf extract has an antibacterial effect, so that it can improve the process of healing open sores[9]. In addition, several research results mention that the ethanol extract of *Rhodomyrtus tomentosa* leaves has antioxidant, anti-inflammatory, antitumor and antimicrobial properties. The content is known to be able to play a role in the process of wound healing[8,10,11,12,13]. Thus, this study aims to evaluate the effectiveness of the antiseptic solution of the leaves extract of *Rhodomyrtus tomentosa* on wound healing in male Wistar rats.

MATERIAL AND METHODS:

Animal and Research Design:

This study is an experimental study with a post test only control group design. This study used a white male Wistar strain male rat aged 12 weeks with a weight of 250-300g. The rats were acclimatized for 7 days at the Faculty of Pharmacology at Lambung Mangkurat University. During the acclimation process, ad libitum is given food and drink according to laboratory procedures.

Thirty rats were divided into five groups using the simple random sampling method. Group 1 is a negative control group, the mice were injured and the wound was allowed to heal naturally. Group 2 was a positive control group, the mice were injured and then treated wounds using NL solution twice a day for 10 days. Group 3 was the group of mice that were injured and treated with wounds using a solution of 15% *Rhodomyrtus tomentosa* leaf extract twice a day for 10 days. Group 4 treated the injured rats and then treated the wound using *Rhodomyrtus tomentosa* leaf extract solution 30% twice a day for 10 days. Group 5 was the group of mice that were injured and then wound care was done using a solution of *Rhodomyrtus tomentosa* 60% leaf extract twice a day for 10 days. This study has received ethical approval from the Research Ethics Commission of the University of Muhammadiyah Banjarmasin with No. 014 /UMB/KE/III/2019.

Rhodomyrtus tomentosa Leaf Extract:

Rhodomyrtus tomentosa adult leaves are wet sorted and washed. After that the sample is dried using an oven at 600C and the making of simplex powder using a blender. The extraction process was carried out according to the maceration method, 200g of simplicia powder was dissolved with 2000mL 70% ethanol for 24 hours and repeated for 3 days. Simplified powder immersion was filtered using filter paper and continued with the evaporation process using a rotary evaporator and oven at 600C. The extract obtained from the extraction results will then be blended into 3 (15g, 30g and 60g). Extracts that have been divided will be reprocessed with the addition of 0.5g kabopol, 10g of propyl glycol, 0.05g of nipagil, 0.01g of nipasol and 100mL of distilled water. The mixture of extract and solution is homogenized so that the extraction solution is obtained 15%, 30%, and 60%.

Phytochemical Analysis:

Phytochemical analysis of *Rhodomyrtus tomentosa* leaves was carried out with various methods such as Dragendroff for alkaloid compound test, foam test used as saponin test, ferric chloride used as identification of saponin compounds, gelatin test for identification of tannin compounds, alkaline reagent test as identification of flavonoid compounds, Libermann Buchard test and ferric chloride test were used as identification of saponin compounds, gelatin test for identification of tannin compounds, alkaline reagent test as identification of flavonoid compounds, Libermann Buchard test and salkowski's for steroid and terpenoid testing[12,13,14].

Wound Length Measurement:

The length of the wound is measured using a manual measuring ruler. Measurement is carried out on day 0 to day 7.

Measurement of Fibroblast Cells:

Fibroblast cell measurements were carried out on the 10th day on 10 rats (2 mice from each group)[15]. The skin of the wound area is sliced (0.5cm) square, then the sample is fixed in NBF 10% for 24 hours. The skin is dehydrated using alcohol, clarified in xylol and made of paraffin block and cut at 5µm in size using a microtome. Sample pieces were stained using Hematoxylin Eosin (HE) to be examined under a microscope[16]. Measurement of fibroblast cells was observed using an Olympus CX41 microscope with a 10x magnification of

the eyepiece and a 10x objective lens in 3 visual fields.

Statistical analysis:

The results of data analysis for each group are presented in the mean. Shapirowilk test and Levene test are used to test normality and homogeneity of data. Kruskal Walis non parametric test was used to see the difference in closure length of the wound in each group. Meanwhile, the One-way ANOVA parametric test was used to see differences in the number of fibroblast cells from each group. Statistical analysis was performed with SPSS version 20.0 (SPSS Inc., Chicago, IL, USA), with a significance level of $p < 0.05$.

RESULTS:

Results of fitochemistry analysis from making antiseptic solution extract of *Rhodomyrtus tomentosa* leaf showed that describe molecule through color between each solution. The higher the concentration, the viscosity of the solution increases. High concentration of the extract also affects the color of the washing solution of the wound which is getting darker. Fitochemistry analysis was shown in figure 1.

Consist of	Reagen	Description	Results	Documentation
Flavonoid	NaOH	Yellow color	+	
Fenol	$FeCl_3$	Dark Green precipitate	+	
Tanin	Uji Gelatin	White precipitate	+	
Alkaloid	Dragendrof	Reddish Brown precipitate	+	
Saponin	Aquades	No foam	+	
Steroid	Lieberman Burcard	No brown ring	-	

Terpenoid	Salkowski	No gold ring	-	

Figure 1. Fitochemistry analysis was shown in each molecule.

1. Comparison of wound length in between normal group, and a solution extract of *Rhodomyrtus tomentosa* leaf with concentratio 15%, 30%, and 60%.

Acceleration in wound healing was found in the concentration group of 15% and 30% solution. While in the 60% solution concentration group there was a slowdown in wound healing compared to the normal group who were not treated. The research data that has been obtained later statistically to determine the difference in wound length using the *Rhodomyrtus tomentosa* leaf extract solution compared with the positive and negative control groups. The research data was tested by Shapiro-Wilk normality, but the results of test showed that the data were not normally distributed in each treatment group with a significance value of p <0.05. Then, researchers conducted a statistical test with the Kruskal Walls test.

Table 1 Differences in wound length in all groups

Wound length (n=250)	Mean	Chi Square	P
Negative (n=50)	159.41	41.401	<0.001
Positive (n=50)	98.75		
Solution 15% (n=50)	97.62		
Solution 30% (n=50)	109.77		
Solution 60% (n=50)	161.95		

Kruskal Wallis test results showed that value of p (Asymp. Significance) = <0,001 <α = 0.05 then Ho was rejected, which means there was a significant difference in average reduction in wound length in all groups. Then finding out which groups there are differences, post Hoc analysis on the Willis Crucial Test is the Mann Whitney Test. The Mann Whitney test results in all groups are shown in table 2 below:

Table 2 Overview of Post hoc Test results

No.	Group	Probability number (p)
1.	Negative and Positive	0,000
2.	Negative and solution 15%	0,000
3.	Positive and solution 60%	0,000
4.	Solution 15% and solution 60%	0,000
5.	Solution 30% and solution 60%	0,000
6.	Negative and solution 30%	0,001

From table 2 could be seen that there is a significant difference between the negative control group (untreated group) and positive control group (NaCL solution treatment) more than 10 days). Meanwhile, if compared with a 15% solution with a negative solution, there is a significant difference, which means that the wound condition given 15% *Rhodomyrtus tomentosa* leaf extract solution has the ability to close the wound faster than without treatment. Solution 15% solution of *Rhodomyrtus tomentosa* leaf extract has the same ability with NaCl solution in wound care.

2. Effect of *Rhodomyrtus tomentosa* leaf extract solution as a washing fluid for wounds in wistar rats based on parameters of the number of fibroblast cells:

Testing number of fibroblasts as a wound healing parameter, researchers conducted a histopathological examination on 10 rats (with each group there were 2 rats). Preparation Skin tissue was isolated from test animals on the 10th day. Mice were sacrificed with ether inhaled. The skin is cut 0.5cm from the edge of the burn and fixed in NBF 10% for 24 hours. The tissue was dehydrated with multilevel alcohol, clarified with xylol, blocked in paraffin, cut to 5µm in size using a microtome, and stained with the Hematoxylin-Eosin method[16]. Preparations were observed with magnification of the 10 × ocular lens and 10 × objective lens in 3 visual fields. Results observations fibroblast the number based on histological results conducted on rat tissue on the 10th day. Standard in calculating the number of fibroblasts refers to the journal[15]. The observations are presented in the table 3 and figure 2 below.

Table. 3 Average results on the number of fibroblasts on the 10th day

No.	Group	Replication	Amount of Fibroblast	Average Amount
1.	Negative	1	1	1
		2	1	
2.	Positive	1	4	3,5
		2	3	
3.	Solution 15%	1	4	4
		2	4	
4.	Solution 30%	1	2	2,5
		2	3	
5.	Solution 60%	1	2	2,5
		2	3	

From figure 3 it can be explained that the number of fibroblasts on the 10th day after treatment with *Rhodomyrtus tomentosa* leaf extract solution was seen in the composition of the solution 15% (4 cells) more than in the positive control group using NaCl solution (3.5 cells).

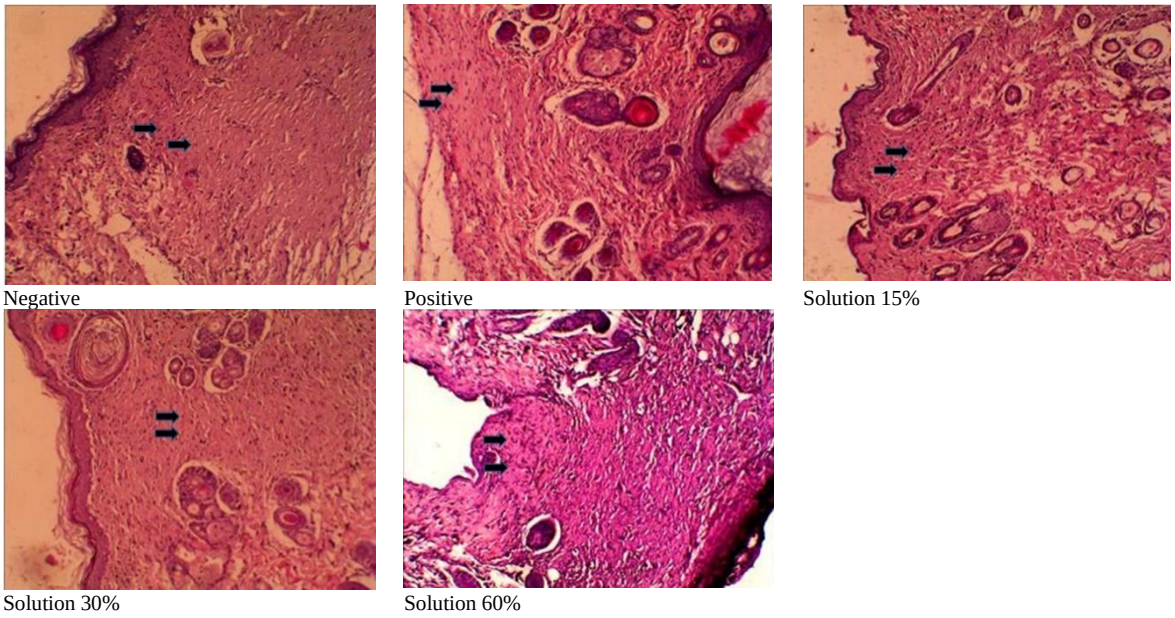

Fig2. Observation histopathology on 10th day. (Arrow showing Fibroblast)

Statistical testing to assess the significance of the measurement of fibroblasts in all groups, ANOVA analysis was performed by first conducting a data normality test. The data is normally distributed, so that the test can be continued. The next statistical analysis is the parametric test using the One Way ANOVA test. The results of the analysis show a significant value of p = 0.017 (P <0.05) which means that there is a difference in the number of fibroblasts in wastewater rat wounds between the normal, positive control, 15%, 30% and 20% solution groups. The analysis is then continued with the test LSD (least Significantly Different) is to find out significant differences between one group and another group

Table 4: ANOVA and LSD Test Analysis Results on the 10th day fibroblast level

Group	Negative	Positive	Solution 15 %	Solution 30 %	Solution 60 %	Annova
Negative		0,006 **	0,003**	0,041 **	0,041 **	0.17
Positive	0,006 **		0,403 *	0,127 *	0,041 **	
Solution 15 %	0,003**	0,403 *		0,041 **	0,041 **	
Solution 30 %	0,041 **	0,127 *	0,041 **		1 ,0 *	
Solution 60 %	0,041 **	0,041 **	0,041 **	1 ,0 *		

**Significally different

From the table 4 above it can be seen that the solution of 15% *Rhodomyrtus tomentosa* leaf extract has a significant difference with the negative control group (p = 0.003), meaning that the 15% *Rhodomyrtus tomentosa* leaf extract solution is more effective in increasing the number of fibroblasts compared to the negative control group (without treatment). But when compared with the positive control group there was no difference in the number of fibroblasts. However, if we look at the difference in the 15% solution of *Rhodomyrtus tomentosa* leaf extract compared to the 60% solution, it appears that the 15% solution is more effective in increasing fibroblasts (p = 0.041). This is also consistent with observations on the parameters of wound length where at a concentration of 60% the effect of wound closure is longer compared to other groups.

DISCUSSION:

There is a significant difference between the negative control group (the group without treatment with the positive control group (NaCl solution treatment). If seen from macroscopic data, the positive control solution has a faster wound closure than the normal group (negative control). Meanwhile, if compared to 15% solution with Negative dick solution shows that there is a significant difference which means that the wound condition given 15% *Rhodomyrtus tomentosa* leaf extract solution has the ability to close the wound faster than without treatment. This means that *Rhodomyrtus tomentosa* leaf extract solution can also be used as an alternative for wound washing, especially at a concentration of 15% solution. The ability of *Rhodomyrtus tomentosa* leaf as one of the ingredients used as medicine / wound healing material is caused by the presence of several ingredients in it. *Rhodomyrtus tomentosa* leaf contain flavonoids, tannins, saponins and triterpenoids which help accelerate

the wound healing process[16]. Isolation of flavonoid compounds contained in the leaves of *Rhodomyrtus tomentosa* plant (*Rhodomyrtus tomentosa* has been carried out by maceration extraction using methanol[17].

Flavonoids can accelerate wound healing by slowing the onset of cell necrosis, increasing the strength of collagen fiber, preventing cell damage[18,19]. Flavonoid-rich extracts in R. Tomentosa significantly increase the activity of antioxidant enzymes in rat serum. Methanol extract from the leaves of *Rhodomyrtus tomentosa* (R. Tomentosa) showed significant anti-inflammatory effects in vitro and in vivo, by inhibiting the production of inflammatory mediators (nitric oxide, NO and prostaglandins, PGE2[10]. *Rhodomyrtus tomentosa* leaf also have tannin components. Tannins in *Rhodomyrtus tomentosa* leaf turned out to have antioxidant activity that is useful in healing wounds. Antioxidants are those that play a role in the capture of free radicals that can damage cell membranes. Antioxidants also have an effect in reducing inflammatory mediators so that the inflammatory process can soon end to the next phase, namely the phase of nuclear proliferation and tissue repair[20].

Wound healing in the group that received *Rhodomyrtus tomentosa* leaf extract solution can also be caused by the presence of antibacterial content in it. Rough ethanol extraction from R. tomentosa shows good antibacterial activity against Gram-positive bacteria. Isolation of rhodomyrtone from ethanol extract using bioassay fractionation revealed the active compound to be a type of acylfloroglukinol which is a natural antibiotic for staphylococcal infections of the skin. This compound shows strong in vitro activity against various Gram-positive bacteria, including antibiotic-resistant strains[21,22]. Tomentosa ethanol and rhodomyrtone extracts have strong activity against Staphylococcus aureus and Staphylococcus epidermidis. In addition to direct antibacterial activity, antibacterial agents that are able to prevent or interfere with biofilm formation and are active against biofilm-forming organisms will be very beneficial in the treatment of infection[23,24,,25].

In this study the group of 60% concentration solution actually shows an obstacle in the closure of the wound length. The administration of drugs to external preparations is also influenced by the release of inflammatory mediators around the wound[26,27]. If the area around the wound is too much covered by other chemicals in too large quantities, then the wound's healing ability can decrease because it can be considered a foreign substance[28,29]. It also correlates from various theories which state that, the dose of the drug is not always linear with pharmacological activity, especially drugs that are not affected by the drug's binding to the receptor. Drug dosages that are too high can also cause toxic effects because each drug has a limit of minimum and maximum drug levels that affect drug therapy[30,31,32].

Fibroblasts are one indicator in tissue repair. The higher the value of fibroblasts, the higher the ability to repair tissue. The results in the table show that a 15% solution can increase fibroblasts in the tissue, higher than the positive control group. The 15% solution has the highest fibroblast score compared to other concentrations. Fibroblast concentration increases because it is affected by the release of basic fibroblast growth factor (bFGF) which triggers migration and proliferation of fibroblasts[33,34]. *Rhodomyrtus tomentosa* leaves contain tannins which can increase migration and proliferation of fibroblasts through increased bFGF release. This has an effect on the increase in fibroblasts when administering a 15% solution[35,36]. In line with this research, it can also be found that the concentration of *Rhodomyrtus tomentosa* leaf extract can also be increase fibroblast levels[37,38].

The number of fibroblasts on the 10th day after being treated with *Rhodomyrtus tomentosa* leaf extract solution showed the most amount in the composition of the solution 15% (4 cells) more than in the positive control group using *NaCl* solution (3.5 cells). Statistical analysis of the One Way ANOVA test showed a significant value of $p = 0.017$ ($P < 0.05$) which meant that there were differences in the number of fibroblasts in wastewater rat wounds between normal, positive control, 15%, 30% and 20% solution groups. Through the perspective in this study, it is expected that a wound specialist nurse is expected to comprehend the concept of angiogenesis comprehensively. Clinical nurses with good insight into angiogenesis must be able to choose the best dressing and suitable for wound healing. Utilizing materials developed based on evidence based in the management of acute and chronic wounds will accelerate the formation of growth factors that induce angiogenesis, and blood vessel production.

CONCLUSION:

There was a significant decrease in wound length between the five groups. There were significant differences in the number of fibroblasts between the five experimental groups (administration of 15%, 30% and 60% *Rhodomyrtus tomentosa* leaf extract solution, negative control and positive control groups. 15% *Rhodomyrtus tomentosa* leaf extraction solution did not have a significant difference in wound length but had a significant effect microscopically similar to the number of fibroblasts with a positive control group given 0.9% NaCL solution.

CONFLICT OF INTEREST:

The authors declare no conflict of interest.

REFERENCES:

1. Bullers S, Berry H, Ingham E, Southgate J. The resolution of inflammation during the regeneration of biological scaffolds by human tissue. Journal of Tissue Engineering and Regenerative Medicine. 2012;6: 218-218
2. Pesce M, Patruno A, Speranza L, Reale M. Extremely low frequency electromagnetic field and wound healing: Implication of cytokines as biological mediators. European Cytokine Network. 2013; 24:1-10. DOI: 10.1684/ecn.2013.0332
3. Kasuya A and Tokura Y. Attempts to accelerate wound healing. Journal of Dermatological Science. 2014: 76 (3): 169–172.
4. Fan D, Takawale D, Lee J, and Kassiri Z. Cardiac fi- broblasts, fibrosis and extracellular matrix remodeling in heart disease. Fibrogenesis and Tissue Repair. 2012: 5 (1): p 15.
5. Bainbridge P. Wound healing and the role of fibroblasts. Journal of Wound Care. 2013; 22: 407-412.
6. Raina R, Prawez S, Verma PK, Pankaj NK. Medicinal plants and their role in wound healing. Vet Scan. 2008; 3(1): 1-24
7. Lai T.N.H, Andre C, Rogez H, Mignolet E, Nguyen T.B.T, Larondelle Y. Nutritional composition and antioxidant properties of the sim fruit (*Rhodomyrtus amentosa*). Food Chem. 2015: 168: 410-416.
8. Zhang Y, Li W, Jiang L, Yang L, Chen N, Wu Z, Li Y, Wang G. Cytotoxic and anti-inflammatory active phloroglucinol drivates from *Rhodomyrtus tomentosa*. Phytochemistry. 2018: 153: 111-119.
9. Mordmuang, A., and Shankar, S. Effects of *Rhodomyrtus tomentosa* Leaf Extract on Staphylococcal Adhesion and Invasion in Bovine Udder Epidermal Tissue Model, 8503–8517. 2015. https://doi.org/10.3390/nu7105410
10. Jeong D, Yang W.S, Yang Y, Nam G, Kim J.H, Noh H.j, Lee S, Kim T.W, Sung G.H, Cho J.Y. In vitro and in vivo anti-inflammatory effect of *Rhodomyrtus tomentosa* methanol extract. J. Ethnopharmacol. 2013: 146: 2015-213.
11. Lai T.N.H, Herent M.f, Quentin-Leclercq J, Nguyen T.B.T, Rogez H, Larondelle Y, Andre C.M. Piceatannol, a potent bioactive stilbene, as major phenolic component in *Rhodomyrtus tomentosa*. Food Chem. 2013; 138: 1421-1430
12. Liu H.X, Chen K, Tang G.H, Yuan Y.F, Tan H.B, Qiu S.X. Isolation and biomimetic total synthesis of tomenttododiones A-B, terpenoid-conjugated phloroglucinols from the leaves of *Rhodomyrtus tomentosa*. RSC Adv. 2016: 6: 48231-48236.
13. Wu P, Ma G, Li N, Deng Q, Yin Y, Huang R. Investigation of in vitro and in vivo antioxidant activities of flavonoids rich extract from berries of *Rhodomyrtus tomentosa*(Ait.) Hassk. Food Chem. 2015: 172: 194-202.
14. Gayathri V, and Kiruba D. Phytochemical analysis of leaf powder extracts of *Rhodomyrtus tomentosa*. International Journal of Current Research. 2014: 6(5):6527-6530.
15. Ohashi, T., Iizuka, S., Ida, H., and Eto, Y. (2008). Reduced α-Gal A enzyme activity in Fabry fibroblast cells and Fabry mice tissues induced by serum from antibody positive patients with Fabry disease. Molecular Genetics and Metabolism, 94(3), 313–318. doi:10.1016/j.ymgme.2008.03.008
16. Lydia J., Sudarsanam D. Total phenol and total tannin content of Cyperus rotundus L. and its medicinal significance. Research J. Pharm. and Tech. 2012;5(12): 1500-1502.
17. Mescher, A.L. Janqueira's Basic Histology Text and Atlas. 14th Edition. Mc Graw-Hill Education, New York. 2016.
18. Amit Tapas, Dinesh Sakarkar, Rajendra Kakde. The Chemistry and Biology of Bioflavonoids. Research J. Pharm. and Tech. 2008;1(3): 132-143.
19. Rachana Mishra, D L Verma. Principal Antioxidative Flavonoids from Rosmarinus officinalis Grown in the Hills of Central Himalaya. Research J. Pharm. and Tech. 2011;4(3): 476-479.
20. Doloksaribu R. 2009. Isolation of Flavonoid Compounds from Harimonting Plants (*Rhodomyrtus tomentosa* W.Ait). Medan: Universitas Sumatera Utara
21. D. Singh, S. J. Daharwal, M. Rawat. Hydrogels- A Potent Carter in Wound Healing. Research J. Pharm. and Tech. 2008; 1(1): 6-13.
22. Falodum A, Igbe I, Erharuyi O, Agbanyin O. J., 2013. Chemical Characterization, Anti inflammatory and Analgesic Properties of Jatropha Multifida Root Bark. Nigeria J. Appl. Sci. Environ. Manage. Sept 2013;7(3): 357- 362
23. Dharmendra Raghuvanshi, Nilesh Gupta, U.K. Jain, A.S. Raghuvanshi, Ajay Patel. Evaluation of Wound Healing Activity of Bark Extract of Artocarpus heterophyllus. Research J. Pharm. and Tech. 2010;3(4): 1283-1284.
24. Limsuwan S, Hesseling-Meinders A, Voravuthikunchai SP, Van Dijl JM, Kayser O. Potential antibiotic and anti-infective effects of rhodomyrtone from *Rhodomyrtus tomentosa* (Aiton) Hassk. on Streptococcus pyogenes as revealed by proteomics. Phytomedicine 2011;18(11): 934-40.
25. Miguel López-Lázaro, 2009, Distribution and Biological Activities of the Flavonoid Luteolin, Mini-Reviews in Medicinal Chemistry, Department of Pharmacology, Faculty of Pharmacy, University of Seville, Spain, 9, 31-59
26. Devi P, Merlin NJ, Madhumitha B, Meera R. Wound healing property of *Aerva lanata* leaves extract. Research J. Pharm. and Tech. 2009; 2(1): 210-211.
27. Nilesh Gupta, UK Jain. An Update- Prominent Wound Healing Property of Indigenous Medicines-: A Review. Research J. Pharm. and Tech. 2011;4(2): 203-213.
28. A. Saravana Kumar, Avijit Mazumder, J. Vanitha, M. Ganesh, K. Venkateshwaran, V. S. Saravanan, T.Sivakumar. Antibacterial Activity of Methanolic Extract of Sesbania Grandiflora (Fabaceae). Research J. Pharm. and Tech. 2008;1(1): 59-60.
29. Saising J., Ongsakul M., Piyawan S and Voravuthikuncha. *Rhodomyrtus tomentosa*(Aiton) Hassk. Ethanol extract and rhodomyrtone: a potential strategy for the treatment of biofilm-forming staphylococci. Journal of Medical Microbiology 2011. DOI10.1099/jmm.0.033092-0. 2011.
30. Andriany A., Tahir T., Sjattar ES Ake J and Nuru H. Wound healing angiogenesis: A perspective of nurse. Global Health Management Journal. 2019; 3(1): 1-3.
31. Thomas, G., Fundamentals of medical chemistry, John Wiley and Sons Ltd, New York. 2003,
32. Wermuth, C., The Practice of Medicinal chemistry, Third edition, Elsevier Ltd, London. 2008,
33. Li WW, Tsakayannis D, Li VW. Angiogenesis: a control point for normal and delayed wound healing. Contemp Surg. 2003;1(2): 5-11.
34. Prem Shankar Misra, V. Ravichandiran, M. Vijey Aanandhi. Design, Synthesis and In Silico Molecular Docking Study of N-carbamoyl-6-oxo-1-phenyl-1, 6-dihydropyridine-3-carboxamide derivatives as Fibroblast growth factor 1 inhibitor. Research J. Pharm. and Tech. 2017; 10(8): 2527-2534.
35. Shedoeva A, Leavesley D, Upton Z, and Fan C. Wound Healing and the Used of Medicinal Plants. Evidence-Based Complementary and Alternative Medicine. 2019. DOI:10.1155/2019/2684108
36. AA Baravkar, RN Kale, RN Patil, SD Sawant. Pharmaceutical and Biological Evaluation of Formulated Cream of Methanolic Extract of *Acacia nilotica* Leaves. Research J. Pharm. and Tech. 2008; 1(4): 480-483.
37. Na-phatthalung, P., Chusri, S., Suanyuk, N., and Voravuthikunchai, S. P. In vitro and in vivo assessments of *Rhodomyrtus tomentosa* leaf extract as an alternative anti-streptococcal agent in Nile tilapia (*Oreochromis niloticus* L.), (May), 430–439. 2018.https://doi.org/10.1099/jmm.0.000453
38. Niah, R., Baharsyah, R. N., Tangi, K., and Utara, B. Potensi Ekstrak Daun Tanaman *Rhodomyrtus tomentosa* (Melastoma malabathricum L.) Di Daerah Kalimantan Sebagai Antibakteri Staphylococcus aureus, *4*(1), 36–40. (2018).Retrievedfrom http://jurnal.akfarsam.ac.id/index.php/jim_akfarsam/article/view/138
39. Zhang X.L, Guo Y.S, Wang C.H, Li G.Q, Xu J.J, Chung H.Y, Ye W.C, Li Y.L, Wang G.C, Two triterpenoids from the roots of *Rhodomyrtus tomentosa*. Chem Lett. 2016: 45: 368-370.