DENTINO
JURNAL KEDOKTERAN GIGI
Vol IX. No 2. SEPTEMBER 2024

EFFECT OF GELAM LEAF EXTRACT (*Melaleuca cajuputi*) AND TRICALCIUM SILICATE CEMENT AS DIRECT PULP CAPPING

Emma Annahal Husma[1)], Sherli Diana[2)], Debby Saputera[3)], Maharani Laillyza Apriasari[4)], Tri Nurrahman[5)]
[1)] Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin, Indonesia
[2)] Department of Conservative Dentistry, Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin, Indonesia
[3)] Department of Prosthodontics, Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin, Indonesia
[4)] Department of Oral Medicine Dentistry, Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin, Indonesia
[5)] Department of Oral Surgery, Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin, Indonesia

ABSTRACT
Background: *Reversible pulpitis is mild pulp inflammation. The treatment is direct pulp capping. Tricalcium silicate cement has been better at inducing reparative dentin than other materials. Tricalcium silicate cement can cause inflammation 1-2 weeks after application, that natural ingredients need to improve the quality. Tricalcium silicate cement can be combined with galam leaf extract which contains anti-inflammatory properties by accelerating lymphocyte cells.* ***Purpose:*** *To analyzed the effect of gelam leaf extract (Melaleuca cajuputi) concentration of 100% and Tricalcium Silicate Cement on the number of lymphocyte cells on day 3 and 5 in direct pulp capping treatment.* ***Methods****: This research is a true experimental study with a post test-only with control design. This study used 24 Wistar rats, divided into 3 treatment groups with 2 different days, namely the group given a combination of Gelam leaf 100% + tricalcium silicate cement, tricalcium silicate cement as positive control, and without treatment as negative control.* ***Results****: One-way ANOVA test obtained p value=0.00<0.05, which indicates there is a significant difference between each group. Post Hoc Bonferroni test showed significant difference between galam leaf extract and tricalcium silicate cement, tricalcium silicate cement, and no treatment on days 3 and 5.* ***Conclusion:*** *The combination of galam leaf extract and tricalcium silicate cement can increase the number of lymphocyte cells on day 3 of pulp inflammation and reduce the number of lymphocyte cells on day 5 more effectively than tricalcium silicate cement and without treatment on days 3 and 5 so that the healing process is faster.*

Keywords : *Direct pulp capping, Gelam leaf extract, Lymphocyte cells, Tricalcium silicate cement.*
Correspondence: Emma Annahal Husma; Faculty of Dentistry, Lambung Mangkurat University, Jl. Veteran, Banjarmasin, Indonesia; email: annahalhusma@gmail.com

INTRODUCTION

The results of the Riset Kesehatan Dasar (Riskesdas) in 2018 showed that 57.6% of Indonesians experienced oral health problems. South Kalimantan has 59.6% oral health problems. One of the oral problems is caries.[1] Untreated caries causes bacterial invasion of the pulp. Pathogenic bacteria diffuse through the dentinal tubules, then the dental pulp is inflamed and secretes an immune response.[2,3]

The inflammatory phase is characterized by lymphocytes infiltrating the wound area along with neutrophils and macrophages. Lymphocytes produce transforming growth factor-β (TGF-β) which activates odontoblast-like cell proliferation and triggers reparative dentin formation.[4] Lymphocytes play a role in the chronic inflammatory phase. This phase occurs on day 3 and is marked by an increase the number of lymphocytes and peaks on day 5 during the proliferation phase. A decrease in inflammatory cells signals a new phase in the healing process, in addition to a shorter inflammatory process.[5,6]

Inflammation in the pulp caused by a stimulus that can return to a non-inflammatory state after the stimulus is removed is called Reversible pulpitis. Reversible pulpitis that is left untreated will cause further inflammation so treatment is required. The treatment for exposed vital pulp tissue is direct pulp capping.[7] Tricalcium silicate cement (Ca3SiO5 or C3S) is a direct pulp capping treatment medicament material that has been developed in the field of dentistry and has good biological properties.[8] One of the most important goals of direct pulp capping treatment is to reduce inflammation in the pulp. The disadvantage of Tricalcium Silicate Cement products (Biodentine;

Septodont) is that it causes inflammation to continue after application, on day 7 to day 14.[9]

Plants can be used as alternative herbal ingredients for a treatment because they have the advantages of cost-effectiveness, easy availability, low toxicity, and little microbial resistance.[10] One plant that has potential as a medicine is Galam (*Melaleuca cajuputi* Subps. *Cumingiana Barlow*). Galam is a plant that can be used for wound treatment because it has a high anti-inflammatory content.[11,12]

The galam plant (*Melaleuca cajuputi* Subsp. *Cumingiana Barlow*) is widely found in South Kalimantan and can be used as herbal medicine. The study by Wardhani, et al., (2018) stated that the results of the phytochemical test of galam leaves contained alkaloids, saponins, polyphenols, and flavonoids. The total phenol content in galam leaf extract is 16.5254 mgGAE/mg extract, while the flavonoid content of galam leaf extract is 0.3438 mgQE/g extract.[12] The compound content proves that galam leaves have anti-inflammatory, anti-bacterial, and anti-oxidant activities.[13]

Based on the description above, tricalcium silicate cement can cause ongoing inflammation, which can inhibit the proliferation process and wound healing will take longer. Galam leaf extract has anti-inflammatory properties, so it can be a supporting material for tricalcium silicate cement.

METHODS

This study uses a type of true experimental in vivo laboratory research conducted on wistar rat teeth. The research design used was post test only control group design. Plant determination and extract preparation were carried out in the FMIPA laboratory of ULM Banjarbaru, the treatment of experimental animals was carried out in the biochemistry laboratory of FK ULM and the preparation of histology preparations was carried out in the Anatomical Pathology Laboratory of Ulin Hospital Banjarmasin. This research was declared ethically feasible by Ethics Commission Faculty of Dentistry ULM No. 109/KEPKG-FKGULM/EC/X/2023.

The materials of this study were galam leaves (*Melaleuca cajuputi* Subsp. *Cumingiana Barlow*), 70% ethanol, Tricalcium Silicate Cement (Septodont, Saint Maur-des-Fossées, France) and Glass Ionomer Cement type III fuji IX. The anesthetic materials used were Xylazine HCL (*Rompun*®, Bayer, Leverkusen, Germany) and Ketamine (*Ketalar*®, Warner Lambert, Ireland) (65mg/kg body weight). Materials for making HPA preparations and staining were 10% formaline buffer, 2% nitric acid decalcification solution, xylol, paraffin, and Haematoxylin Eosin (HE) stain.[5]

The research tools in this tudy were syringe, micromotor, handpiece, round bur edenta, ball applicator, plastic filling instrument, handscoon, mask, spatula agate, paper point, paper pad, blender, gram scale, stirrer, cup, measuring cup, filter paper, a set of extract maceration tools, vacuum oven dryer, rotary evaporator, water bath, and equipment for making preparations, as well as experimental animal adaptation equipment.[5]

Galam (*Melaleuca cajuputi*) leaves weighing 1 kg were weighed and washed. Clean galam leaves were cut into pieces and dried in an oven at 50°C. Galam leaves were pulverized using a blender to form simplisia powder. Simplisia was soaked with 70% ethanol as much as 1L at room temperature for 3x24 hours. The resulting solution from soaking was evaporated at 60°C with a rotary evaporator to become an extract. The extract was then evaporated using a waterbath until a thick extract was obtained.[5] The galam leaf extract was mixed with tricalcium silicate cement that had been mixed in a 1:1 ratio using a small disposable spatula.

This research used 24 wistar rats with the criteria of healthy rats, body weight 200-250 gr, age 3-4 months, having non-carious and non-anomalous left maxillary molar teeth. Wistar rats were acclimatized for 7 days in the laboratory. Wistar rats were divided into 6 groups consisting of the combination group (treatment) on day 3 and day 5, the tricalcium silicate cement group (positive control) on day 3 and day 5, and the no treatment group (negative control) on day 3 and day 5. Wistar rats were anesthetized intramuscularly on the lower thigh with ketamine and xylazine using a 0.2 ml syringe. Class I cavities were prepared on the occlusal surface with a round bur using a low speed handpiece until perforation of the pulp chamber occurred. The cavities were irrigated using sterile saline solution and then dried with cotton pellets. Sterile paper points were used to prevent bleeding.

Groups 1 and 4 (n=8) were applied galam (*Melaleuca cajuputi*) leaf extract and tricalcium silicate cement, groups 2 and 5 (n=8) were applied tricalcium silicate cement and groups 3 and 6 (n=8) were untreated. The material was applied to the cavities up to the pulp surface using a ball applicator. The cavities were then filled with glass ionomer cement (GIC). Experimental animals in groups 1, 2, and 3 were sacrificed on day 3 while groups 4, 5, and 6 were sacrificed on day 5. After the rats were sacrificed, jaw bone was taken from the interdental area of the left maxillary molar tooth. The dead wistar rats were wrapped in cloth and buried.

Tissue pieces were dissolved for ± 4 days in 10% formalin buffer at room temperature then placed in 2% nitric acid solution for decalcification and rinsed with running water. Tissues that have been softened are carried out the processing stage and continued with the embedding process. The tissue was sliced using a microtome in parallel according to the long axis of the tooth. Pieces of tissue sheets were floated on a waterbath at a temperature of 40º-50º C, then placed on a labeled object glass and the preparation was ready for haematoxylin-eosin (HE) staining. Preparations that

have been stained, dried using tissue and covered with deck glass and then observed and calculated using a light microscope with a total magnification of 400x and 5 fields of view.[5]

The calculation's outcomes were analyzed using the *Shapiro-wilk* test for normality and *Levene's Test* for homogeneity. Data that were normally distributed and varied equally were then subjected to parametric tests using *One Way Anova* to see whether or not there were significant differences between treatment groups, followed by Bonferroni Post Hoc test to see which treatments had significant differences.

RESULTS

The average number of lymphocyte cells in the teeth of Wistar rats with pulp inflammation is shown in Table 1.

Table 1. The Mean of Lymphocyte Cells All Treatments.

Treatment Groups	Day-	Mean of Lymphocyte	Std. Deviation	N
Galam leaf extract + TSC	3	11.15	1.007	4
	5	8.38	1.240	4
TSC	3	8.55	1.265	4
	5	6.36	0.457	4
Without treatment	3	4.61	0.704	4
	5	5.73	0.403	4

Notes:
TSC : Tricalcium Silicate Cement

Table 5.1 shows that the group given the combination of galam leaf extract (*Melaleuca cajuputi*) and tricalcium silicate cement had the highest average number of lymphocyte cells on day 3 compared to other treatment groups, while the group without treatment had the lowest average number of lymphocyte cells on day 3. The treatment group given a combination of galam leaf extract (*Melaleuca cajuputi*) and tricalcium silicate cement was more effective in increasing the number of lymphocyte cells in dental pulp of Wistar rats that have inflammation when compared to the positive control and negative control groups. The average number of lymphocyte cells on days 3 to 5 showed an effect on the decrease in the number of lymphocyte cells in the combined treatment group of galam leaf extract (*Melaleuca cajuputi*) and tricalcium silicate cement and the positive control group, while there was an increase in the number of lymphocyte cells in the negative control group.

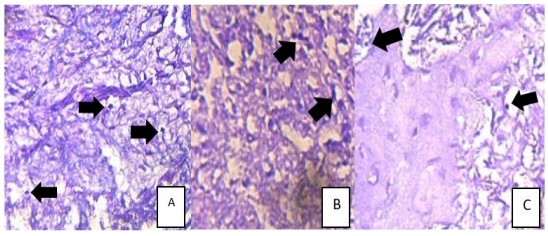

Figure 2. Histopathological Overview of Male Wistar Mice (Rattus norvegicus) Lymphocyte Cells on 3rd day in group: a) Galam leaf extract + TSC; b) Positive control; c) Negative control.

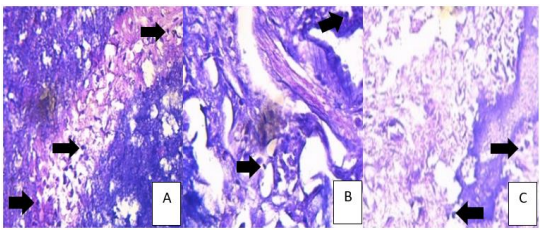

Figure 3. Histopathological Overview of Male Wistar Mice (Rattus norvegicus) Lymphocyte Cells on 5th day in group: a) Galam leaf extract + TSC; b) Positive control; c) Negative control.

The results of lymphocyte cell counts were followed by data processing and analysis with the Shapiro-Wilk test for normality test and Levene's test for homogeneity test. The results of the Shapiro-Wilk test in all treatment groups obtained sig. ($p>0.05$), so it was concluded that the data in all treatment groups were normally distributed. Levene's Test results obtained sig value. = 0.066 ($p>0.05$) which means that the data varies homogeneously or the variance is the same.

Data that are known to be normally distributed and vary equally, then proceed with parametric analysis using the One-way ANOVA test with a confidence level of 95%. The average number of lymphocyte cells varies significantly between treatment groups, as indicated by the One-way ANOVA test findings, which had a sig value of 0.00 ($p<0.05$). Data analysis was continued to see which treatments had significant differences. The results of the Bonferroni Post-Hoc test can be seen in table 2.

Table 2. *Post-Hoc Bonfferoni* results of Lymphocyte cells all treatments (*p<0,05 = there is a significant difference)

	Galam leaf extract + TSC	Tricalcium Silicate Cement	Without Treatment
Galam leaf extract + TSC		0.012	0.000
Tricalcium Silicate Cement	0.012		0.013
Without Treatment	0.000	0.013	

The results of the Bonferroni Post-Hoc test with a sig value. ($p<0.05$) showed significant differences in all treatment groups. The galam leaf extract and tricalcium silicate cement groups with tricalcium silicate cement (positive control) showed significant differences in lymphocyte cell counts as shown in Table 2. The galam leaf extract and tricalcium silicate cement groups with the no treatment group (negative control) showed significant differences. The table also shows that the tricalcium silicate cement group (positive control) and the no treatment group (negative control) had significant differences.

DISCUSSION

The results showed that in the treatment group the combination of galam leaf extract (*Melaleuca cajuputi*) and tricalcium silicate cement had a significant effect as a direct pulp capping material as evidenced by the increase in the number of lymphocyte cells on day 3 and decreased on day 5. This is due to the content of metabolite compounds from galam leaf extract in the form of polyphenols, flavonoids, alkaloids, and saponins which have anti-inflammatory properties.[12,13]

The chronic inflammatory phase starts on day 3 and is characterized by an increase of lymphocyte cell count. Lymphocytes produce transforming growth factor-β (TGF-β1) which induces odontoblast-like cell proliferation and triggers reparative dentin formation.[4,5] Lymphocyte cells will reach the maximum number on day 3 and decrease gradually. The average number of lymphocyte cells on day 3 in the treatment group of the combination of galam leaf extract and tricalcium silicate cement was higher than the tricalcium silicate cement group, as well as the group without treatment. This is due to the biological effects of tricalcium silicate cement and the content of secondary metabolite compounds in galam leaf extract in the form of flavonoids.[12]

Tricalcium silicate cement can stimulate TNF-α and IL-6 as pro-inflammatory cytokines higher than other direct pulp capping materials such as Mineral trioxide aggregate (MTA).[14] IL-6 plays a role in lymphocyte cell formation.[15] The study by Wardhani, et al., (2018) states the results of the phytochemical test of galam leaf extract contain flavonoids. Flavonoids can stimulate lymphocyte proliferation and Interleukin-2 (IL-2) production. The production of IL-2, IL-4, and IL-1 will stimulate the proliferation and differentiation of T cells and B cells.[12,16] This is in accordance with the results of a study that showed the combination of galam leaf extract (*Melaluca cajuputi*) and tricalcium silicate cement was more effective in stimulating lymphocyte cell activation.

Based on the results of the study, there was a decrease in the number of lymphocyte cells in the treatment group of the combination of galam leaf extract (*Melaleuca cajuputi*) and tricalcium silicate cement and the tricalcium silicate cement group as a positive control on day 5. A decrease in the number of lymphocyte cells indicates healing has entered the next stage. This indicates that the inflammatory phase will be faster and wound healing will be shorter.[6] This is due to the anti-inflammatory properties of tricalcium silicate cement and the content of secondary metabolite compounds from galam leaf extract in the form of polyphenols, flavonoids, alkaloids, and saponins.[4,13]

Polyphenols are the highest compound content in galam leaf extract. The results of the phytochemical test conducted by Wardhani, et al., (2018) stated that the total phenol content in galam leaf extract *(Melaleuca cajuputi*) was 16.5254 mgGAE/mg extract..[12] Phenolic metabolites of galam leaves showed inhibitory activity of cyclooxygenase-2 (COX-2) enzyme and reduced the levels of several cytokines such as TNF-α, IL-1β, IL-6, and prostaglandins.[17] Phytochemical test research by Wardhani, et al., (2018) states that galam leaf extract contains flavonoids as much as 0.3438 mgQE/g extract.[12] Flavonoids act as anti-inflammatory agents that can decrease capillary permeability and reduce edema and prevent the production of inflammatory mediators including histamine and prostaglandins. Flavonoids can inhibit cyclooxygenase (COX) activity thereby reducing the release of arachidonic acid.[18] Flavonoids also play a role in regulating cell function by stimulating the production of TGF-β1 as one of the factors that will induce odontoblast-like cell proliferation and trigger the formation of reparative dentin.[4]

Based on the results of phytochemical tests by Thamrin, et al., (2016) it was found that galam leaves (*Melaleuca cajuputi*) contained alkaloid metabolite compounds.[19] One of the main targets of alkaloids as anti-inflammatory is to inhibit pro-inflammatory cytokines (IL-1, IL-6, and TNF-α) and the enzyme cyclooxygenase-2 (COX-2).[20] Saponins exhibit anti-inflammatory activity in acute and chronic inflammation. The anti-inflammatory activity that occurs is the inhibition of prostaglandins and histamine. Prostaglandin inhibition occurs because saponins inhibit the cyclooxygenase (COX) enzyme pathway and reduce the production of pro-inflammatory substances.[21]

The process of reducing several cytokines by secondary metabolite compounds owned by galam leaf extract will cause the inflammatory phase to last shorter and minimize the occurrence of tissue damage due to inflammation.[13] Studies conducted by Silvestro (2020) are in line with the results of the study, namely extracts from the galam plant (*Melaleuca cajuputi*) can reduce pro-inflammatory cytokines so that they have anti-inflammatory effects.[22]

Tricalcium silicate cement has anti-inflammatory properties by increasing TGF-β1 secretion.[4] TGF-β1 is a cytokine with a mechanism of action that stimulates the differentiation of odontoblast-like cells that play a role in the formation of reparative dentin.[8] This is supported by a study by Fonseca TD, Silva J, et al., (2019) which showed that the tricalcium silicate cement group can

modulate the inflammatory response and stimulate osteoblast differentiation by stimulating the cytokine transforming growth factor-β (TGF-β).[13] This is aligns with the results of the study, namely a decrease on day 5 of the number of lymphocyte cells because they have entered the next phase, so that the healing process is shorter.

The untreated group as a negative control showed the lowest average number of lymphocyte cells compared to the combination group and the positive control group. Tissue repair in Wistar rat teeth that are not given direct pulp capping material will be slower so that there is no influence on the pattern of lymphocyte infiltration and there is a possibility of more antigens in this group. The negative control group illustrates the state of the number of lymphocyte cells that appear in accordance with the body's immune response during the inflammatory phase. This is indicated by an increase in the average number of lymphocyte cells on day 5 because it enters the proliferation phase.[5]

The results lead to the conclusion that there is an effect of the combination of galam leaf extract (*Melaleuca cajuputi*) and tricalcium silicate cement on increasing the number of lymphocyte cells on day 3 and decreasing the number of lymphocyte cells on day 5 in the dental pulp of Wistar rats that experience inflammation compared to the tricalcium silicate cement group and without treatment.

REFERENCES

1. Riset Kesehatan Dasar. Laporan Provinsi Kalimantan Selatan Risekdas [Internet]. Laporan Riskesdas Nasional 2019. 2020. 493 P.
2. Octiara E. Response Immune In Caries And Role Of Odontoblast In Immune Response. Dentika Dent *J*. 2016;19(1):83–8.
3. Said F, Salamah S. Hubungan Konsumsi Air Sungai (Fe) Dengan Rata-Rata Angka Dmf-T Pada Masyarakat Desa Mekar Sari Kecamatan Tatah Makmur Kabupaten Banjar Provinsi Kalimantan Selatan Fahmi. J Skala Kesehat. 2017;8(1):1–13.
4. Hidayatullah G, Herniyati H, Putri LSD. Efek Seduhan Kopi Robusta Terhadap Jumlah Limfosit Pada Daerah Tarikan Ligamen Periodontal Gigi Marmut (*Cavia Cobaya*) Yang Diinduksi Gaya Mekanis Ortodonti. Pustaka Kesehat. 2020;7(3):182 pp.
5. Zayyan AB, Nahzi MYI. Pengaruh Ekstrak Kulit Manggis (*Garcinia Mangostana*) Terhadap Jumlah Sel Limfosit Pada Inflamasi Pulpa. J Kedokt Gigi Univ Lambung Mangkurat. 2016;I(2):151–7.
6. Riyani NJ, Pasaribu R, Mardiyantoro KF. Evaluasi Jumlah Limfosit Pasca Aplikasi Lendir Bekicot (*Achatina Fulica*) Pada Soket Tikus Wistar (*Rattus Norvegicus*) Address : Sinnun Maxillofac J. 2021;03(01):42–9.
7. Panbiyani R, Kusuma ARP, Feranisa A. Perbandingan Jumlah Odontoblast-Like Cells Medikamen Kaping Pulpa Studi Terhadap Tikus Putih (*Rattus Norvegicus*) Galur Wistar. Univ Islam Sultan Agung Semarang. 2020;109–17.
8. Deviyanti S. Semen Trikalsium Silikat Sebagai Bahan Alternatif Untuk Penatalaksanaan Hipersensitif Dentin (Kajian Pustaka). *J Ilm Dan Teknol Kedokt Gigi*. 2017;13(1):12.
9. Rizkia A, Kusuma P, Praptiningsih RS, Nurhapsari A, Aisyah HA. Comparison Of The Number Of Macrophages In The Rat Reversible Pulpitis After Application Of Biodentin And Siwak Extract Application Of Biodentin And Siwak Extract. *Medali J*. 2022;4(1):6–11.
10. Anuradha B, Mensudar R, Mitthra S, Ganesh A, Simon A. Holistic Endodontics. J Ayurvedic Herb Med. 2017;3(4):229–33.
11. Putri KV, Novitasari I, Wardatina H, Tangkeallo L, Dawud A, Isnaini. Aktivitas Penyembuh Luka Gel Ekstrak Metanol Bunga Galam (*Melaleuca Cajuputi* Subsp. *Cumingiana* (Turcz.) *Barlow*) Pada Tikus Wistar. J Agromedicine Med Sci. 2022;8(1):34–8.
12. Wardhani K, Roro R, Alicia A, Akhyar O. Skrining Fitokimia , Aktivitas Antioksidan , Dan Buah Tanaman Galam Rawa Gambut (*Melaleuca Cajuputi* Roxb) Screening Of Phytochemical, Antioxidant Activity And Total Phenolic- Flavonoid Of Leaves And Fruit Extract Of Galam Rawa Gambut (Melaleuca Cajupu. Quantum J Inov Pendidik Sains. 2018;9(2):133–43.
13. Silvestro L, Barçante F, Vitor K, Murata K, Teresa M, Souza G, Henrique O & Freitas K. Free Radical Biology And Medicine *Melaleuca Leucadendron* (L .) L . Flower Extract Exhibits Antioxidant And Photoprotective Activities In Human Keratinocytes Exposed To Ultraviolet B Radiation. Free Radic Biol Med. 2020;159(7):54–65.
14. E Silva, P., Lucisano, M. P., Consolaro, A., Sousa-Neto, D., & Silva LAB. Subcutaneous Tissue Reaction And Gene Expression Of Inflammatory Markers After Biodentine And Mta Implantation. *Braz Dent J*. 2022;33(1):41–56.
15. Da Fonseca TS, Silva GF, Guerreiro-Tanomaru JM, Delfino MM, Sasso-Cerri E & Tanomaru-Filho M. Biodentine And Mta Modulate Immunoinflammatory Response Favoring Bone Formation In Sealing Of Furcation Perforations In Rat Molars. Clin Oral Investig. 2019;23(3):1237–52.
16. Ningrum DP., Widyarti S. Pengaruh Pemberian Ekstrak Etanol *Annona Muricata* Linn. Terhadap Peningkatan Jumlah B220 Pada Mus Musculus. J Biotropika. 2014;2(5):269–72.
17. Khotimah SN, Muhtadi A. Review Artikel: Beberapa Tumbuhan Yang Mengandung Senyawa Aktif Antiinflamasi. *Farmaka*. 2016;14(2):28–40.
18. Ismardianita E, Nasrul E, Hemiawati M. Effect Of Ethanol Extract Of Myrmecodia Pendens On Tgf-B1 Expression And Osteoblast Cells After Tooth Extraction (Experimental Research On *Cavia Cobaya*). J Dentomaxillofacial Sci. 2017;2(3):150–4.
19. Kissinger K, Yamani A, Thamrin GA, Muhayyah R. Bioprospecting Of Kerangas Forest As Natural Medicine Material Sources: Screening Phytochemistry Compound Of Kerangas Forest Tree Species. J Wetl Environ Manag. 2016;4(2):13–9.
20. Bai R, Yao C, Zhong Z, Ge J, Bai Z. Discovery Of Natural Anti-Inflammatory Alkaloids: Potential Leads For The Drug Discovery For The Treatment Of Inflammation. Eur J Med Chem [Internet]. 2021;213:113165.
21. Luliana S, Susanti R, Agustina E. Uji Aktivitas

Antiinflamasi Ekstrak Air Herba Ciplukan (*Physalis Angulata* L.) Terhadap Tikus Putih (*Rattus Norvegicus* L .) Jantan Galur Wistar Yang Diinduksi Karagenan Antiinflammatory Activity Test Of Aqueous Extracts Herb Of Ciplukan (Physalis Angul. Trad Med J. 2017;22(12):199–205.

22. Silva LS, Perasoli FB, Carvalho KV, Vieira KM, Paz Lopes MT, Bianco De Souza GH, Santos OD & Freitas KM. *Melaleuca Leucadendron* (L.) L. Flower Extract Exhibits Antioxidant And Photoprotective Activities In Human Keratinocytes Exposed To Ultraviolet B Radiation. Free Radic Biol Med [Internet]. 2020;159(July):54–65.