International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor (2018): 7.426

Comparison of the Effect of the Application of Jatropha Curcas Linn Sap Gel with Gelatinsponge on Fibrin Density after Tooth Extraction in Sparague Dawley Mice

Hadira[1], Hardianto Andri[2], Kasim Awin[3], Harlina Eva[4], Syamsudin Endang[5]

[1]Resident of Department of Oral and Maxillofacial Surgery Faculty of Dentistry, PadjadjaranUniversity, Bandung, Indonesia

[2, 3, 5]Department of Oral and Maxillofacial Surgery Faculty of Dentistry, PadjadjaranUniversity, Bandung, Indonesia

[4]Department of Patology Faculty of Veterinary, Bogor Agriculture Institute, Bogor, Indonesia

Abstract: ***The bleeding during and after tooth extraction requiring local haemostatic agent administration is bleeding due to capillary or arteriolar trauma to the tooth socket.The most commonly used local haemostatic drug is gelatin sponge derived from animal gelatin, which is porous, flexible, easy to absorb, non-oxygenic, non-allergic, stimulates the formation of blood coagulation and clogs the bleeding area. According to the WHO in 2013, 80% of the world's population still relies on traditional medicines which are mostly based on plants. J.c Linn castor plants contain flavonoids, and tannins which can accelerate the process of cessation of bleeding and formation of blood clots. The study was conducted to analyze the differences in the effect of J.c Linn castor gel application with gelatin sponge on fibrin density and blood clotting time. This study was a pure experimental study in 27 S. dawley mice which were randomly divided into 3 groups. Group 1 was the control, Group 2 was applied Jc Linn castor gel and Group 3 was applied gelatin sponge after tooth extraction. The clotting time was calculated and, after 5 minutes, nefrocted. Histological preparations were made with Hematoxylin-eosin staining, then the fibrin density was assessed under a microscope. Data was analyzed with non parametric kruskal wallis and one way Anova. The results of the kruskal wallis test obtained a p-value of 0.072 (p> 0.05) which means that there was no significant difference in fibrin density between the groups using Jc Linn castor gel and gelatin sponge gel. From the results of the oneway ANOVA test, p-values were obtained at 0,000 (p <0.05) which means that there were significant differences in blood clotting between the groups using castor gel and gelatin sponge. J.c Linn castorgel can increase the fibrin density and accelerate the formation of blood clots, therefore it can be used as a local haemostatic.***

Keywords: blood clot, fibrin, J.c Linn castor gel

1. Introduction

Bleeding can both occur during and after tooth extraction which is sometimes found in dental practice as well as Oral and Maxillofacial Surgery office. Complications of post tooth extraction bleeding can be caused by local or systemic factors, so prevention is important. If post tooth extraction bleeding occurs due to local factors, a dentist and Oral and Maxillofacial Surgeon must know the principles of bleeding management, in this case by applying pressure or suturing properly, and if necessary administering haemostatic agents locally. Post-extraction bleeding that requires local haemostatic agent administration is bleeding due to capillary or arteriolar trauma to the tooth socket.[1]

These local haemostatic drugs may vary, including alginic acid, natural collagen sponge, fibrin sponge, gelatinsponge, oxidized cellulose and bone wax. However, of the many types of local haemostatics, gelatinsponge is the haemostatic material that is most commonly used in dentistry because it is easily available, porous-shaped sponge, flexible and easy to absorb, non-antigenic and non-allergic. Gelatinsponge stimulates the formation of blood coagulation and clogs the bleeding area. [1,2]

According to the WHO in 2013, 80% of the world's population still relies on traditional medicines which are mostly plant-based.[3]Indonesian tropical forests are high in biodiversity. One of the traditional plants that are beneficial for treating diseases is Jatropha curcas Linn. Local residents of Surakarta believe and have used jatropha sap in wounds.[4] Researches on the use of plants and fruits that have efficacy for wound healing have been widely published, but the effect of J.c Linn castor gel application in stopping bleeding has never been published.[5,6,7]

J.c Linn castor gel contains various useful natural substances. Some of which are flavonoids and tannins contained in the sap. Both of these compounds play an important role in helping to cease the bleeding.[4] Flavonoids are generally pigments that exist in every part of the plant in the form of glycogen and aglycogen compounds so that they can accelerate the cessation of bleeding.[8] Tannin is a complex chemical compound consisting of various polyphenols, with the highest concentration found in almost every parts of Jatropha plants. Tannin compounds are astringent which have the ability to form a complex with macromolecules, especially proteins. The ability of these tannins can accelerate the process of stopping bleeding.[9,10]

In Indonesia, jatropha can almost be found across the country. The benefits of Jatropha plant are not limited to producing biofuels, but also for lubricating oils, raw materials in the manufacture of high-quality soaps; raw materials in insecticide, fungicide and molluscida industries,

Licensed Under Creative Commons Attribution CC BY

as well as for anti-tumor drugs. The chemical contents of castor plants are triacontranol, alpha-amirin, cholesterol, beta-sitosterol, 7-keto-betasitosterol, stigmasterol, stigmata-5-en-3-beta-7-alfadiol, vitexin, isovitexin, and cyanide acid (HCN). The leaves contain saponin, flavonoida, tannin, epigenin, vitexsin and polyphenol compounds. The stems contain sponin, flavonoida, tannin and polyphenol compounds. The sap contains tannins, saponins and flavonoids. The seeds contain various alkaloids, saponins, and a kind of toxic protein called Kursin, as well as 35–45% fat oil, which consists of various palmitic, stearic, and curkanolic acid triglycerides.[11,12,13,14]

Phenol compounds include various compounds originating from plants which have the same characteristics, namely aromatic rings, containing one or two hydroxyl groups. Phenol compounds tend to dissolve easily in water because they generally bind to sugar as a glycoside. Phenol compounds include simple phenol compounds such as monophenol with one benzene ring found in legumes, sinamic hydroxy acids group (ferulic acid and cafeate), flavonoids and its glycosides (catechins, proanthocyanins, anthocyanidin, and flovonol) and tannins which are phenol complex compounds with high molecular weight.[14,15,16]

2. Research Method

The object of this research wasSparague Dawley mice weighing 200 mg - 300 mg. The castor sap gel was made at the pharmaceutical laboratory of the Faculty of Veterinary Medicine - Bogor Agricultural Institute. Gelatinsponge came from animal gelatin. Gelatinsponge can be used as a haemostatic to control bleeding.

The population in this experimental study was Sprague Dawley white mice from the Center for Tropical Biopharmaca Studies LPPM-IPB and was kept at the Educational Veterinary Hospital (RSHP) FKH-IPB.

The sample size was determined based on the objective of the research, which was to see the difference in the effects between one sample group and another, for which the sample size formula was used as follows:

$$n = 2\left[\frac{(Z\alpha + Z\beta)}{X1 - X2}\right]S^2$$

$$n = 2\left[\frac{(1{,}96 + 0{,}84)}{2{,}5 - 1{,}6}\right]0{,}69^2$$

$$n = 9{,}22 \approx 9\ sampel$$

In this study the 95% confidence level (Zα = 1.96) from the normal distribution table, and 80% power test (Zβ = 0.84) were obtained. Using the above formula, n = 17 was obtained with a drop out of 10%, so that for each group 9 samples were needed, which were divided into 3 treatment groups so that the total sample was 27. Simple Random Sampling used a lottery model.

The inclusion criteria for S. dawley white mice in this experiment were male, healthy according to the Veterinarian, had an average body weight of 250 grams ± 25 mg, and were 8 weeks old. The research conducted was a pure experimental study conducted on experimental animals of S.Dawley DE white mice. The independent variables were Jc Linn jatropha and gelatinsponge. The dependent variables were the density of fibrin and the time of blood clotting. The controlled variables were the method of extracting teeth in the mouth of experimental animals, the age of experimental animals, and the physical state of the socket after tooth extraction.

Research Methods: S.dawley white mice as experimental animals had been quarantined for one week, aiming to adapt to the environment and special feed. The experimental animals were divided into 3 groups, groups I, II and III. Group I was the control group, group II was applied the castor J.c Linn gel and group III was applied gelatinsponge, after tooth extraction. Then the experimental animals were anesthetized using ketamine hirdochloride at a dose of 45 mg/kgBW and 0.35 mg / kgBW of mice intramuscularly in the abdominal muscle. Extraction of maxillary central incisors was carried out in each group.

Extraction of maxillary incisivus was carried out in group I which was a control group without treatment. After tooth extraction, bleeding in the socket was left untreated. Every 30 seconds a blood clot was picked up with a stick until a fibrin thread appeared and the blood clot formed was recorded by using a stopwatch, then nefrocted after 5 minutes. Extraction of the maxillary Incisivus was performed in group II. Immediately after tooth extraction in group II, 0.05 cc Jc Linn castor gelwas applied by using a disposable syringe 1 cc in the socket. Every 30 seconds a blood clot was picked up with a stick until a fibrin thread appeared and the blood clot formed was recorded by using a stopwatch, then nefrocted after 5 minutes. The extraction of the right upper jaw incisivus was carried out in group III.Immediately after tooth extraction in group III, a 0.2 x 0.2 cm gelatin sponge was applied in the socket. Every 30 seconds a blood clot was picked up with a stick until a fibrin thread appeared and the blood clot formed was recorded by using a stopwatch, then nefrocted after 5 minutes. Necropsy was performed on experimental animals in groups I, II and II in the 5th minute, then the socket tissue of the extracted tooth was taken and soaked in a 10% BNF for 3 days. Furthermore, tissue decalcification was carried out in 10% nitric acid solution for 72 hours. The next step is cutting the tissue and processing it for making histopathological preparations, followed by dehydrating, embedding, until Hematoxyllin-eosin staining. An assessment of fibrin density in each histological preparation was carried out.

In this study the researchers modified the slide method using glass objects with experimental animal tooth sockets because there was an application of experimental materials. After tooth extraction, bleeding occurred in the socket. Every 30 seconds the blood was picked up using a stick and the time when fibrin thread appeared was recorded.

Hematoxilin-Eosin was used to color the tissue. Histological observations were carried out using a binocular light microscope with 4x, 10x, 20x and 40x magnifications. The density of Fibrin that filled the socket was assessed by a scoring system. The fibrin density assessment method was a modification of the Greenhalgh's scoring system, because

Licensed Under Creative Commons Attribution CC BY

the observation data had an ordinal scale in the form of a score. Observations were made by dividing the field of view into 4 equal areas

The scoring of fibrin density assessment is as follows:
0 = no fibrin threads are formed
1 = a slight density of fibrin threads is seen
2 = a moderate density of fibrin threads is seen
3 = a heavy density of fibrin threads is seen
4 = socket is filled with fibrin threads.

The study was conducted in the Division of Pathology in the Department of Reproductive and Pathology Clinics of FKH-IPB and RSHP FKH IPB. The study was conducted on February - April 2018.

3. Results

Data were analyzed using SPSS 20. Figure 4.1 microscope observation shows the wall of the socket with the presence of blood clots surrounded by fibrin thread in the tooth socket of the mice in the control group. Figure 4.2 shows the presence of blood clots and fibrin thread in the tooth socket of the mice after the application of Jc Linn gel. Figure 4.3 shows the presence of a blood clot with fibrin thread in the tooth socket of the mice after the application of gelatinsponge.

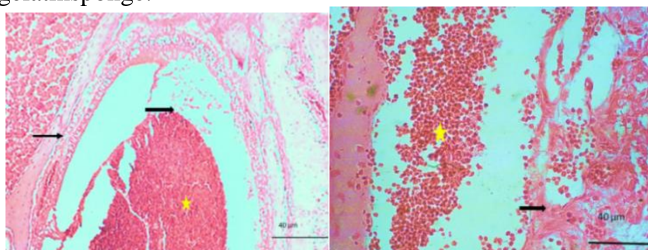

Figure 4.1: Histology of tooth socket of the control group mice. Sockets are filled with blood clots (stars), Wall sockets (arrows), Fibrin threads (thick arrows) Hematoxyillin-eosin staining. 40 μm bar

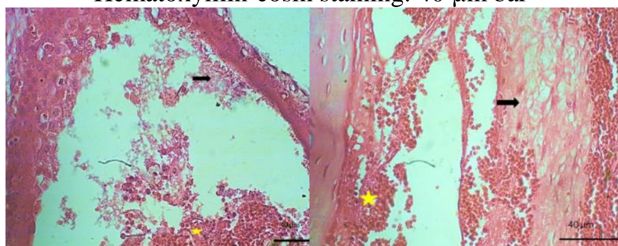

Figure 4.2: Histology of tooth sockets of the mice which were given J.c Linn sap gel.. Sockets are filled with blood clots (stars), Wall sockets (arrows), Fibrin threads (thick arrows) Hematoxyillin-eosin staining. 40 μm bar.

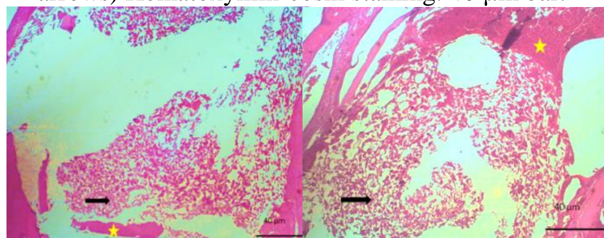

Figure 4.3: Histology of tooth sockets of the mice which were givengelatinsponge.Sockets are filled with blood clots (stars), Wall sockets (arrows), Fibrin threads (thick arrows) Hematoxyillin-eosin staining.40 μm bar.

The mean results of the assessment of fibrin density after tooth extraction in S.dawley mice are presented in table 4.1.

Table 4.1: The Mean of Fibrin Density

Groups	Fibrin Density			
	Mean	St.Deviation	Minimum	Maximum
Control	2.2	0.4	2.0	3.0
Jatropha Sap Gel	3.0	0.8	2.0	4.0
GelatinSponge	2.7	0.6	2.0	4.0

Source: SPSS 20 primary data processing, 2018

To find out whether there are statistically significant differences in fibrin density in at least one treatment group, a comparison test with the non-parametric kruskal wallis test was carried out, table 4.2.

Table 4.2: Fibrin Density Kruskal Wallis Test Results

Test Statistics [a,b]

	Kerapatan Fibrin
Chi-Square	5.273
df	2
Asymp. Sig.	.072

a. Kruskal Wallis Test
b. Grouping Variable: Kelompok

Source: SPSS 20 primary data processing, 2018

The results of the kruskal wallis test obtained a p-value of 0.072 (p> 0.05) which means that there was no significant difference in fibrin density between the groups using Jc Linn castor gel and gelatinsponge gel. Because the results of the comparison test showed no significant differences, further testing using Mann Whitney was no longer necessary.

The mean results of post-extraction blood clotting time calculation in Sprague Dawley mice are presented in Table 4.3.

Table 4.3: The Mean of Blood Clotting Time

Groups	Blood Clotting			
	Mean	St.Deviation	Minimum	Maximum
Control	2.8mnt	1.2	1.3mnt	5.3mnt
Jatropha sap gel	1.2mnt	0.1	1.0mnt	1.4mnt
Gelatin Sponge	2.3mnt	0.2	2.1mnt	2.7mnt

Source: SPSS 20 primary data processing, 2018

Before being tested with a one way ANOVA test, the data was first tested for normality and homogeneity. The following is the calculation result of the normality and homogeneity test of post-extraction blood clotting, table 4.4.

Table 4.4: Blood Clots Normality and Homogeneity Test

Groups	Test for Normality*			Test of Homogeneity**	
	Statistic	*Df*	*p value*	*Levene*	*p value*
Control	0.877	9	0.245	4.207	0.053
Jatropha sap gel	0.956	9	0.770		
GelatinSponge	0.827	9	0.041		

Source: SPSS 20 primary data processing, *)*shapiro wilk*
**) *Levene Test*

The data is said to have a normal distribution if the p value is greater than 0.05, otherwise it is said to be abnormal if p value is less than 0.05. Meanwhile the data is said to be homogeneous if the p value of the homogeneity test is

Licensed Under Creative Commons Attribution CC BY

greater than 0.05 and vice versa. The test results showed that data from the three treatment groups are normally distributed and have homogeneous variances. Therefore the hypothesis testing was done using the oneway ANOVA test. One way ANOVA test results are presented in table 4.5.

Table 4.5: Blood Clotting One Way Anova Test Results

	ANOVA				
	Sum of Squares	Df	Mean Square	F	p value
Between Groups	12.530	2	6.265	13.399	0.000
Within Groups	11.222	24	0.468		
Total	23.7522	26			

Source: SPSS 20 primary data processing, 2018

The oneway ANOVA test results obtained a p-value of 0,000 (p <0.05) which means that there were significant differences in blood clotting between groups using castor gel, sponge gelatinand the control group.

Post hoc tests employed the Tukey HSD test. The results of the Tukey HSD further test are presented in table 4.6.

Table 4.6: The Results of The Tukey HSD Further Test

Multiple Comparisons

Dependent Variable: PembekuanDarah

Tukey HSD

(I) Kelompok	(J) Kelompok	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Kontrol	Gel getah jarak	1.65333*	.32235	.000	.8483	2.4583
	Gelatine Sponge	.63111	.32235	.145	-.1739	1.4361
Gel getah jarak	Kontrol	-1.65333*	.32235	.000	-2.4583	-.8483
	Gelatine Sponge	-1.02222*	.32235	.011	-1.8272	-.2172
Gelatine Sponge	Kontrol	-.63111	.32235	.145	-1.4361	.1739
	Gel getah jarak	1.02222*	.32235	.011	.2172	1.8272

*. The mean difference is significant at the .05 level.

Source: SPSS 20 primary data processing, 2018

Based on the results of Tukey HSD further test, it was shown that blood clotting in the control group was significantly different from blood clotting in the castor sap gel group (sig <0.05), but not significantly different from blood clotting using gelatin sponge (sig> 0.05). Blood clotting in the castor sap gel group was significantly different from the blood clotting of the control and the gelatinsponge groups (sig <0.05).

Blood clots that occur if there is alveolar bone bleeding due to trauma to the capillaries or arterioles in the tooth socket begins with vascular vasoconstriction, platelet adhesion, and activation of collagen, causing blood flow to slow down and blood clots to occur. Platelets adhere to the injured subendothelium, then platelets aggregations take place to form platelet plaques that clog the wound and stop the bleeding. This blockage is temporary. At the same time, this activation of the coagulation system will form fibrin which will replace platelet plaque blockage and is permanent in a certain time. When fibroblasts have formed, fibrin will undergo complete lysis by fibrinolysis.[17]

Fibrin is a protein in the form of thread fibers that are not soluble in plasma in the process of clotting or blood clotting. Fibrin is derived from fibrinogen which changes due to the activity of thrombin enzymes. Jc Linn castor sap gel adds to the density of fibrin because the castor gel has flavonoids and tannins, which are thought to be chemical compounds capable of increasing the density of fibrin through a gel mechanism that is bound to blood fibrin, causing the entrapment of trombosit in the castor sap gel, so that the fibrin becomes more stable. Besides, it is also suspected that the gel functions as a bleeding clog so that the fibrin thread gets stronger or more numerous.

Gelatin sponge comes from animal protein (gelatin), with a porous, flexible and easy to absorb sponge. In this study, gelatin sponge was attached to the tooth socket, so that platelets were activated and trapped in gelatin. Gelatin sponge functions as a platelet barrier by binding to fibrin tissue to capture platelets, causing bleeding to stop faster so that blood clots occur quickly and form stable fibrin threads.

Analysis of differences in blood clotting time of the three groups of experimental animals found that the blood clotting time of J.c Linn gel application group was shorter than the control group and gelatin sponge application group. This is presumably due to the presence of chemical elements in the J.c linn castor sap namely flavonoids and tannins which are procoagulant that accelerate blood clotting (Anonym, 2010).

The blood clotting mechanism works on the extrinstic pathway. In this pathway, factor VII (proconvertin) will be activated into fVIIa in the presence of ion calcium (fIV) and tissue factor (fIII), which is released from the wall of the blood vessel that is injured. Only these three factors (fVIIa, Calcium ion and tissue factor) are needed to activate fX into fXa through the extrinsic pathway. Activation of this extrinsic pathway in a very short time will produce a small amount of thrombin which can form fibrin. [18,19,20] In this study, the sap content of the Jc linn castor plant along with blood clotting factors in the extrinsic pathway work together in complex reactions that help accelerate the blood clotting process.

Flavonoids and tannins are thought to play a role in inhibiting local synthesis and production of vasodilatory prostaglandin I2 (prostacyclin), which causes the process of contracting local blood vessels (vasocysts) to be faster

Licensed Under Creative Commons Attribution CC BY

(Salawu et al, 2008). Tannin is one of the components responsible for thromboxane A2 and 5-hydroxytryptamin (serotonin) secretion (Rochrbach, 2007 and Sari et all,2013). Thromboxane A2 and serotonin are compounds secreted due to the response to platelet activity that attaches to the walls of damaged blood vessels. Serotonin has a function as a strong vasoconstrictor whereas thromboxan A2,apart from functioning as a vasoconstrictor, also plays a role in the process of platelet activation (Guyton, 2014).[21,22,23]

4. Conclusion

Application of Jatropha Curcas Linn castor sap gel can increase the density of fibrin and accelerate the formation of blood clots.

References

[1] Fragiscos,FD. 2007. Perioperative and Post-operative Complication. Dalam Fragiscos, FD " *Oral Surgery*" Selected Reading,hlm.74,181-188. Berlin : Elsevier Balaji, S.M. 2013. *Texbook Of Oral and Maxillofacial Surgery.* India: Elsevier.

[2] Santhos, K. M P.2016. *Department of Oral and Maxillofacial Surgery*. India: Saveetha Dental College and Hospital

[3] Arunachalam K. PT. 2013. Anti inflammatory, wound healing and in-vivo antioxidant properties of the leaves of *Ficus amplissima* Smith. J.jep.145;139-145.

[4] Sukandar, E.Y. 2016. Tren dan Paradigma Dunia Farmasi, Industri-Klinik Teknologi Kesehatan, disampaikan dalam oral ilmiah Dies Natalis ITB, http://itb.ac.id/fokus_file/orasi-ilmiah-dies-45.pdf [17/04/ 17].

[5] Zubair, M..E A., Nybom, H., Renvert, S., Widen, C., & Rumpunen, K.. 2012. *Effects of Plantago major L. leaf extracts on oral epithelial cells in a scratch assay.* Journal of Ethnopharmacology, 141(3), 825-830.

[6] Majewska I, Darmach E G. 2011. Proangiogenic activity of plant extracts in accelerating wound healing — a new face of old phytomedicines. Actabp.58;449-460.

[7] Shim K M, Choi S H, Jeong M J, Kang S S. 2007. *Effects of Aucubin on the Healing of Oral Wounds*. In vivo.21;1037-1042.

[8] Narayana, KR., Reddy, MS, Chaluvadi,M.R., Krisna, D.R. 2001. Bioflavonoid Classification, Pharmacological, Biochemical Effect and Therapeutic Potential. *Indian journal of Parmacology*. 33 : 2-16.

[9] Hassanpour, S., Naser, MS., Behrad, E., Farhad, BM. 2011. Plants and secondary metabolites(tannin). *International Journal of Forest, Soiland Erosion (IJFSE)*.1(1): 47-53.

[10] Goswami A, dkk. 2014. Bleeding Disorder in Dental Practice : A diagnostic Overview. *Journal of ICDRO*.6(2): 143-150.

[11] Sinaga, E. Jetropa curcas l, jarak pagar. Pusat Penelitian dan Pengembangan Tumbuhan Obat http://bebas.vlsm.org/v12/artikel. 07/04/17.

[12] Ema, S., Martanto. M., Haryono, S., Jubhar, C.M. 2014. Manfaat Jarak Pagar (Jatropha Curcas) Sebagai Obat Tradisional. Seminar Nasional.hlm 9-1.1.

[13] Setyadji, M., Susiantini, E. 2005. Pembuatan metil ester (bio-diesel) dari minyak jarak pagardan metanol dengan katalisator natrium hidroksida. ISSN. hlm:13

[14] Pratiwi, S.I. 2008. Aktivitas antibakteri tepung daun jarak (jatropha curcas l.) pada berbagai bakteri saluran pencernaan ayam broiler secara in vitro.. 10/05/17.

[15] Nurmillah, O.Y. 2009. Kajian aktivitas antioksidan dan antimikroba ekstrak biji, kulit buah , batang dan daun tanaman jarak pagar (jatropha curcas l.). 10/05/17.

[16] Sumanto., Syakir., Allorerung, D., Purwani, J. 2011. Kompos kulit jarak pagar sebagai sumber kalium potensial. Prosiding seminar nasional inovasi perkebunan..hlm:130-134

[17] Prana, M.S.2006. Budidaya jarak pagar (jatropha curcas l) sumber biodiesel, menunjang ketahanan energi nasional. Jakarta: Lipi press.hlm:36-3.

[18] Metha A B dan Victor H A. 2006. At a Glance Hematologi. 2nd. Ed. Jakarta. Erlangga.

[19] Najwa, Z.D. 2015. Sistem Hemostasis, Dalam Najwa ZD "Pendekatan Diagnosis Pasien dengan Kelainan Perdarahan", Selected Reading, hlm 4-50. Bandung.

[20] Jay, P.M. 2011; Complication in Oral and Maxillofacial Surgery " Management of Hemostasos and Bleeding Disorgers in Surgical Procedures", hlm 387-393.Elsevier Inc

[21] Salawu O.A., Aliyu M., Tijani A.Y. 2008. Haematological Studies on The Ethanolic Stem Bark Extract 0f Pterocarpus Erinaceus Poir (Fabaceae). African Journal of Biotechnology vol. 7 (9) :1212-15

[22] Sari R.P., Sampurna., Danis P. 2013. Pengaruh Sari Buah Kurma (Phoenix dactylifera)terhadap Waktu Perdarahan. Sains Medika vol. 5 (1): 20-2.

[23] Guyton A.C, Hall J.E. 2014. Buku Ajar Fisiologi Kedokteran. Jakarta : Penerbit Buku Kedokteran EGC

Licensed Under Creative Commons Attribution CC BY