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Anticoagulant Activity of Ethanolic Extract Stingging Nettle from Biak Numfor

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Abstract

Stinging nettle (Laportea aestuans (Linn) Chew) have been used empirically as relieving pain such as stiffness, headache, abdominal pain, muscle aches, joints and bruises. The aims of this study was to determine the effect of ethanol extract of stinging nettle leaf as an anticoagulant that used human blood (A, B, O and AB) using Lee-white method and blood smear. The stinging nettle leaf samples were taken from Biak Numfor, made simplicia, and macerated by 96% ethanol. The anticoagulant activity of ethanol extract was tested with preliminary of 50, 100, 150, 200, and 250 ppm. Then test was followed by anticoagulant activity by Lee White method and eustek method (blood smear). The results showed that in the preliminary test the minimum concentration of extracts of 45 ppm was observed for 120 minutes. Extracts Laportea aestuans have anticoagulant activity against various human blood groups as well as positive controls of EDTA. Both of them can bind calcium that the blood clotting factors so blood did not freeze. Blood clotting activity by eustek method showed that the extract of Laportea aestuans seen in the microscopic blood cells did not freeze because the unrelated blood cells were intact and separated from each other.

Keywords: *Stinging nettle (Laportea aestuans (Linn) Chew), Anticoagulan, Papua*

INTRODUCTION

Anticoagulants are substances that are used to prevent blood clots that are commonly used in clinics and in laboratories [1] by inhibiting the function of several blood clotting factors [2]. Anticoagulants are needed to prevent the formation, expansion of thrombus with emboli and to prevent blood clots in vitro from laboratory tests or transfusions. From several studies regarding the effects of anticoagulants showed that anticoagulant effects derived from secondary metabolites of flavonoids [3], essential oils, and terpenoids. Epidemiological studies showed that compounds which function as platelet modulation are flavonoids [4].

There have been many empirical experiences related to the efficacy of stingging nettle leaves. It has been known that one of the properties of the leaves can stimulate blood circulation because the leaves contain flavonoids which have an effect as anticoagulants. Stingging nettle leaves are widespread in Kalimantan, Sulawesi, Maluku, Papua, and Papua New Guinea forests. In addition this plant is cultivated in India and Java [5]. Stingging nettle leaves are used by the people of Maluku, Papua and Papua New Guinea to threats various health complaints such as pain, stiffness, headaches, stomach aches, muscle - joint pain, and bruises [6]. These effect comes from the formic acid which in contained in "trichoma or thorn" gland found on the leaf surface. When the thorns hit the body, the acid of the gland ant is released and affects the occurrence of the body's pores. Widening of these pores that

stimulate blood circulation so that it becomes smooth. That is why the use of leaves is generally used to overcome aches or make people feel better [7].
Scientifically stingging nettle leaf plants from the Urticaceae family generally contain chemicals such as monoridine, tryptophan, histidine, alkaloids, flavonoids, formic acid and authraguinones. Based on [8] the Urticaceae family in Java consists of 22 genera and 76 species while in Papua only six species have been known namely *Laportea decumana*, *Laportea sinuata*, *Laportea interupta*, *Dendrocnide peltata*, *Laportea sp.*, and *Laportea aestuans* [9-10].

L. aestuans and *L. decumana* that have been studied scientifically with several pharmacological activities need to be developed [11-13] continuously including anticoagulant activity. This study aims to determine whether *Laportea aestuans* (L) Chew can be used as anticoagulants tested in human blood samples, namely in blood groups A, B, O and AB using the Lee-White method and blood smears.

MATERIAL AND METHODS

Material

L. aestuans leaves was obtained from Biak Numfor (Figure 1) and blood samples obtained from several volunteers who had blood types A, B, O and AB. This research was conducted from January to March 2018 in the Laboratory of Pharmacy, Faculty of Mathematics and Natural Sciences, Cenderawasih University.

Preparation of plant extracts

Sample were cleaned and then aerated in open air and then dried using an oven at 50 ° C, dried stingging nettle leaves were smoothed into powder and sieved with no. 35. Simplicia was weighed 300 grams and macerated using 96% ethanol as much as 2 liters. Samples that have been soaked with ethanol are stored for 3 days and stirred occasionally. For 3 days soaking or 3 X 24 hours. Then the macerate obtained was collected and then evaporated with a solvent with Rotary Vacum Evaporator so that the concentrated extract was obtained.

Preparation of blood test samples

The blood sample (5 cc) was taken from the cubiti vein using a 5 ml / cc disposable syringe and sterile 22 G needle. Blood samples were obtained from 1 blood type A volunteer, 1 blood type B volunteer, 1 blood type O volunteer, and 1 blood group AB volunteer aged 20-27 years, with a healthy physical condition and no history of bleeding disease prolonged. It is assumed that volunteers have no hemostasis abnormalities.

Preparation of samples for testing

Ethanol extract obtained before the preliminary test is conducted to determine the minimum concentration range of extract to be used in 1 ml of blood. Extract concentration obtained as much as 45 ppm. Types of blood groups taken are A, B, AB, and O

Anticoagulant activity test

Tube Method (Modification of Lee and White)

Determination the period of blood clotting that was observed visually, the modified Lee-White method [14] was used. The normal blood clotting period in humans generally occurs between 3 - 18 minutes based on the normal blood clotting period [15]. Five clean blood tubes (BT) were prepared and labeled with BT 1 - 5. The blood taken from volunteers was poured 1 ml in each BT. BT 1 (containing blood without treatment), TB 2 (blood + 1 ml ethanol 96%), BT 3 (blood + EDTA 1 mg), BT 4 (blood + 1 ml extract of the minimum dry concentration + 1 mg EDTA) and BT 5 (blood + 1 ml dry extract of minimum concentration). This procedures was also done for each blood group A, B, AB and O three times repetition. The BT was then exported at the same time and the blood clotting time

was calculated using a stopwatch. After 3 minutes the tube is lifted and each test tube is tilted to see whether or not the freezing has occurred

Blood smear (Slide Method)

The effect of blood clotting can also be seen microscopically by eustrek technique. This method is carried out to look at the state of blood cells microscopically, according to the May mix method of Grunwald-Giemsa [16]. The sample used in this test was selected from one blood group which had the most significant anticoagulant activity effect. Five clean and non-fat objects glass (OG) were prepared and labeled with OG 1 - 5. OG 1 (control blood), OG 2 (blood + 96% ethanol), OG 3 (blood + EDTA), OG 4 (blood + EDTA), OG 5 (blood + extract).

Blood from the number 1 BT until the number 5 was taken as much as 20 µl each. The blood is topped with OG number 1 through number 5 in sequence. Droplets of blood on were touched with a cover glass so that the droplets of blood will be widen and the layer is thin to the edge of the OG. Preparations are fixed with ethanol solution until they cover the surface for 15 minutes and are air-dried to dry. Preparations are soaked in giemsa solution for 30 minutes and rinsed with water, then aerated until they dry. The results are observed under a light microscope and documented with the camera.

LITERATUR REVIEW

Stingging nettle is widely distributed throughout the world from tropical to temperate region especially Indonesia. Stinging nettle, is a perennial plant growing in temperate and tropical wasteland areas around the world. It belongs to Urticaceaea that grows 2 to 4 meters high. Stingging nettle or Daun Gatal (the Papuan people call it) is a plant whose have trichomes along the leaf and stem leaves [17]. This plant is widely used by people in Biak, Wamena, Sentani Papua Indonesia as an analgesic such as pain repief, soreness, stomach pain, and fatigue effectively [11, 18]

Stinging netlle or genus Laportea has 163 species spread all over the world (IPNI, 2016). It is found and reported (1) In Nigeria, L. Aestuans (phytochemical screening, toxicity, antibacterial, antioxidant [19]. In Cameroon (2) *L. ovafolia* (antiandrogenic, antioestrogenic, antioxidants, effects to monosodium glutamate in rats) [20-22]. In Philippines (3) *L. interrupta* (potential pre-gestational) [23]. In Europe (4) *L. canadiensis* (trichomes influence on the response of herbivores). This plant has many the benefits that pharmacological activities not only as pain-relief as Papuan known. But also it has activities such pharmacological antioxidant, anticancer, antibacterial, antidiabetic, antiandrogenik, antioestrogenik, antihypertensives, antiprostat, antacids veins, and others.

In Indonesia, reported that *Urticaceae* has been used as a traditional medicine to treat several infectious diseases. *Urticaceae* on Java island consist of 22 genera and 76 species [8] while on Papua, there was five species has been known ie *Laportea aestuans*, *Laportea decumana* [11], *Laportea sinuata*, *Laportea interupta*, *Dendrocnide peltata*, and *Laportea sp* [9-10, 24]

Stingging nettle *(L. aestuans)* reported that have many medical uses. The study showed that stinging nettle had potential in stem as an inflammatory agent; in leaf as an abortifacient, laxative, pain-killer, febrifuge, eye treatment, pulmonary, and stomatch troubles (leaf), and in flower to cure diarrhea and dysentery [25, 26]. *L. aestuans* consisted of saponins, tannins, flavanoids, phlobatanins, and ardiac glycosides [27]. The toxicity of crude extract leaf reported hexane faction was very toxic. The essensial oil from the plant is dominated by methyl salicylate and had

significant antioxidant and antimicrobial [13, 28-30].

RESULTS

Extracts was obtained by maceration method diffusion process occurs. The solvent will enter and penetrate the cell then removing active substances. Because of the differences concentration in the cell, the active substance will come out. Therefore, the maceration process is influenced by the size of the simplicia particles, where the smaller the simplicia particle size, the greater the surface area of the simplicia in contact with the solvent. But it must be noted, if the particle size is too small it will affect the screening process. The liquid macerate obtained was concentrated to obtain a thick extract and separated between the solvent and the active compound in the stingging nettle leaves. Extract were obtained from 300 grams of stingging nettle leaves resulting as much as 10.90 grams concentrated extract. So percent recovery about 3.63%.

Figure 1: *Stingging nettle from Biak*

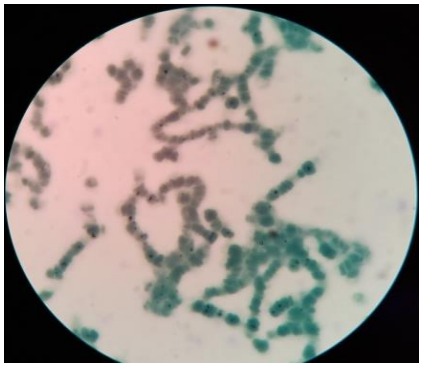
The period of anticoagulant or blood clotting activity of A, B, AB, and O from each volunteer is shown in Table 1 where the blood clotting period of control at BT 1 experienced coagulation of each donor who had a different blood type. The freezing period (coagulation) of each donor experienced a time difference with an average of 6 - 9 minutes.

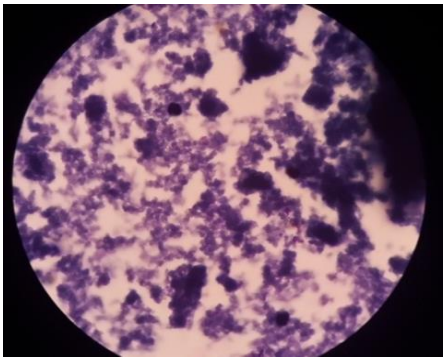
Table 1: *Blood Clotting Period (BCP)*

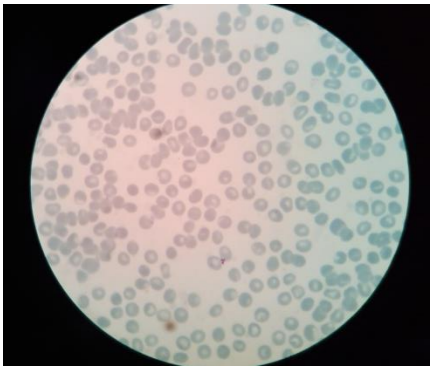
Blood type	BT 1 Blood controle (minutes)	BT 2 Blood + Ethanol (minutes)	BT 3 Blood+ EDTA (minutes)	BT 4 Blood + ELA (minutes)	BT 5 Blood + Extract (minutes)
A	7'.07"	28'.03"	> 120	> 120	> 120
B	8'.10"	23'.56"	> 120	> 120	> 120
AB	9'.02	26'.55"	> 120	> 120	> 120
O	6'.48"	32'.03"	> 120	> 120	> 120

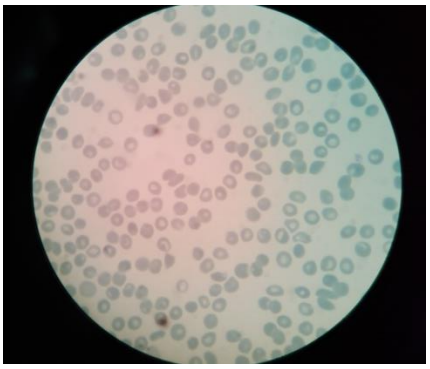
Note:
BCP : Blood Clotting Period
ELA : EDTA and L. aestuans extract
> 120 : More than 120 minutes

Based on Figure 2 which is a preparation from the first tube, where the blood smear preparations are not treated or is blood control can be seen images of blood cells that are not separated but bind to each other but do not experience damage seen from blood cells still intact this is due to blood cells having undergone clotting (coagulation). According to Sofian (1950) the blood that freezes blood cellswas attached to one another [31]. Junqueira *et al.*, (1997) stated that platelets in clots with clotting blood appear solid and in groups [32].


Blood smear preparations control (A)


Blood smear preparations given ethanol (B)


Blood smear preparations given EDTA(C)


Blood smear preparation (D)

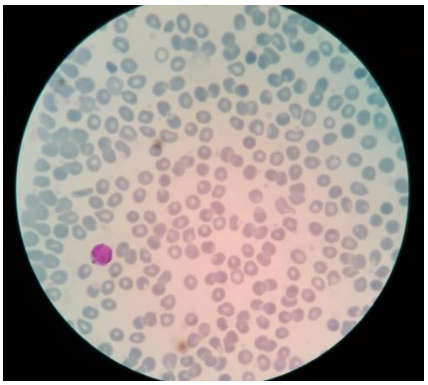

Blood smear preparations given extract (*E*)

Figure 2: *Blood smear in variation of threats*

DISCUSSION

Preliminary Test

The preliminary test observed for 120 minutes (2 hours) aims to determine the minimum concentration range of the extract to be used in 1 ml of blood. At a concentration of 50 ppm showed the anticoagulant effect, the concentration series is made smaller to obtain the concentration range of the extract to be used. The concentration series was made to 45 ppm, 40 ppm, 30 ppm, 20 ppm, and 10 ppm. At a concentration of 45 ppm still showed anticoagulant effects but at concentrations of 40 ppm, 30 ppm, 20 ppm and 10 ppm no longer showed anticoagulant effects until the concentration of drinking extract obtained was 45 ppm.

Anticoagulant Activity

Control blood is blood that is not given any treatment. Bitthell (1993) states that normal blood clots occur in a time range of 3-18 minutes [15]. Control blood samples taken were still within the normal blood

clotting period. Blood clots occur by changing the prothrombin plasma protein to thrombin. Thrombin is an enzyme that catalyzes fibrinogen, a protein that dissolves into insoluble fibrin [16]. In a few seconds fibrin polymerizes into a structured network from the long threads of fibrin that travel in all directions this net captures the blood elements that are shaped and forms a clot. This shows that blood samples of volunteers freeze to normal limits.

For BT 2 (blood + ethanol), it was shown that blood clots had occurred with an average freezing time in the 23 – 32 minutes (Table 1), for each donor. These results indicated that ethanol tending to have coagulation activity. The occurrence of a blood anticoagulation process was also shown in blood samples added with EDTA in BT 3 tubes and blood samples added with EDTA and ELA (BT 4). At BT 3 and 4 which can be see in Table 1. The freezing period for blood groups A, B, AB and O in the blood added with 1 mg of EDTA dried and blood samples that have been added with 1 mg of dried EDTA and ELA did not occur blood clotting with a clotting period observed more than 120 minutes (> 120). At BT 5 (extract + blood each blood group A, B, AB, and O) did not show any blood clotting activity. This shows that the blood from each of them volunteered after ethanol extract of *Laportea aestuans* had anticoagulant activity.

EDTA is an anticoagulant used in hematocrit examination. EDTA is also used in the examination of hemogtrobin levels, blood cell counts, reticulocytes, blood type and smear blood preparations. EDTA anticoagulants can be used in two forms, namely in the form of solution or liquid and in the form of dry matter or solids. The use of EDTA anticoagulants is 1 mg / 1 ml of blood. EDTA functions as an anticoagulant that binds Ca^{2+} ions so that the blood clotting process does not occur [33]. EDTA has a function as an anticoagulation that binds calcium ions so there is no process in blood clotting. In this test it can be seen that *L. aestuans* extract has properties similar to EDTA, both of which can bind to one of the blood clotting factors, namely calcium, so the blood does not freeze.

Selection of time for 120 minutes (2 hours) to observe the blood sample, because the time of 120 minutes is a time determination in which all blood clotting factors will not form, so that blood cannot freeze or not coagulate. It was stated that it will be held for 2 hours until the desired effect occurs, namely the blood does not freeze, and the work of anticoagulation will last about 4-6 hours.

From the results of phytochemical testing carried out, stingging nettle contained flavonoids, alkaloids, saponins and tannins [11, 13]. From several previous studies on the effects of anticoagulants and based on epidemiological studies, it has been shown that compounds that function as platelet modulation are. Flavonoids are one type of antioxidant that can inhibit platelet adhesion, aggregation and secretion [34]. Based on these observations, the extract from *L. aestuans* is assumed to be used as a drug preparation for thrombolysis, high blood pressure and heart disease. Anticoagulants were used in those who had a heart attack caused by thrombosis or clots in the coronary arteries. Anticoagulation in medicine, can be used both in the laboratory and in the clinic, for blood transfusion, surgery, and preventing thromboembolism [35].

Blood clotting activity can also be seen with eustrek technique (blood smear) which is to see the condition of blood cells microscopically [16]. The blood samples tested came from one of the blood type O volunteers. The results of microscope photos showing blood cells after being treated can be seen in Figure 2.

In the preparation of the second tube of figure 2 the blood added with ethanol, the results obtained indicate the occurrence of blood clots as well as preparations from the first tube, but red blood cells or erythrocytes no longer have a form where the cell wall is destroyed. Ethanol contains toxic substances in the blood, so that blood cell membranes can no longer withstand external pressure, which causes blood cells to rupture or lysis [36].

From figure 2 C, D and E which are preparations from tubes number 3, 4, and 5, it can be shown that blood cells do not experience clots because the unrelated blood cells are still intact and separate from one another. In non-freezing blood cells are generally round like a coin, yellowish and have no core [37]. In blood smear preparations that do not freeze platelets appear round and not in groups, have the same size with each other and have an empty core.

CONCLUSION

Stingging nettle leaf extract (*Laportea aestuans* (L) Chew) used a minimum concentration of extract obtained from a preliminary test of 45 ppm after a laboratory study for 120 minutes. The results stated that extracts from Laportea aestuans have activity as anticoagulants against various human blood groups (A, B, O and AB).

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REFERENCES

1. Gandasoebrata R. Hematologi. In: Gandasoebrata R. Penuntun Laboratorium Klinik. 7th Edn. Jakarta: Dian Rakyat. 1992: 159.
2. Rosmiati H, Gan VHS. 1995. Antikoagulan, Antitrombotik, Trombolitik dan Hemostatik dalam: Farmakologi dan Terapi. 4th Edn. S. Gan, R. Setiabudi, U. Sjamsuddin, Z.S. Bustani, (editor). Jakarta: Farmakologi FKUI. 1995.
3. Hidayat R. Efek antikoagulan ekstrak buah mahkota dewa (Phaleria macroparpa) pada mencit putih jantan. Skripsi. Fakultas Farmasi, Universitas Andalas .2007
4. Shahriyari L, Yazdanparast, R. Antiplatelet and antithrombotic activities of *Artemisia dracunculus* L. leaves extract. Pharmacology Online Inst. Biochem. 2009; 1:217-228.
5. Winduo, SE. Indigenous Knowledge of Medicinal Plants in Papua New Guinea. Canterbury: University of Canterbury. 2003
6. World Health Organization [WHO]. Medicinal plants in Papua New Guinea. Manila: World Health Organization, regional office for the Western Pacific. 2009.
7. Puro dan Imam. Kajian Ativitas Antibakteri Daun Gatal (Laportea decumana (Roxb.) Wedd.) dan Daun Benalu Cengkeh. Skripsi. 2012.
8. Backer CA, Bakhuizen van den, Brink JRC. Flora of Java. Groningen: N. V. P. Noordhoff. 2nd Edn. 1965.
9. Perdana BY, Putra AP, Primanisa A. Uji toksisitas daun jelatang *(Laportea sinuata Blume)* terhadap larva nyamuk *Aedes aegipty*. Universitas Andalas. [Cited 2019 5 March]. 2016
10. Simaremare ES, Holle E, Yabansabra YR, Budi IM, et al. Analisis Perbandingan Efektifitas Antinyeri Salep Daun Gatal Dari Simplisia *Laportea Aestuans (L)* Chew dan *Laportea Decumana (*Roxb) Wedd. Pharmacy. 2015; 12 (1):1-10.
11. Simaremare ES. 2014. Skrining Fitokimia Ekstrak Etanol Daun Gatal (*Laportea decumana (Roxb.) Wedd*). Pharmacy. 2014;11(01): 98-107.
12. Simaremare ES, Holle E, Gunawan E, et al. Toxicity, Antioxidant, Analgesic, and Anti-inflamantory of Ethanol Extract of *Laportea aestuans* (Linn.)

Chew. Journal of Chemical and Pharmaceutical Research. 2018; 10(5):16-23.
13. Simaremare ES, Yabansabra YR, Gunawan E, et al. Uji Mutu Fisik Sediaan Salep Daun Gatal *(Laportea decumana (Roxb.) Wedd.)* Sebagai Kandidat Antinyeri. Galenika. 2016; 3(2): 55-60.
14. Gandasoebrata R. Urinalisis dalam Penuntun Laboratorium Klinik. 10th Edn. Jakarta: PT Dian Rakyat. 2001.
15. Bithell TC. The Diagnostic Approach to the Bleeding Discordes. In Lee RG, Bitthell, TC, Foerster J, Athens JW, Lukens JN. Wintrobe's Clinical Hematology. 9th Edn. Malvern, Pennsylvania: Lea and Febiger. 1993; 2:1301-1324.
16. Geneser F. Buku Teks Histologi. 1st Edn. Jakarta: Binarupa Aksara. 1994.
17. Ahmed MKK, Parsuraman K. U*rtica dioica* l., (*Urticaceae*): A Stinging Nettle. Systematic Reviews in Pharmacy. 2014; Vol 5(1):6-8.
18. Holle E, Simaremare ES, Budi IM, et al. Evaluasi, Uji Aktivitas, Dan Pengembangan Produk Salep Daun Gatal Papua Varietas Biak. 2015. Jurnal Biologi Papua. Prosiding Seminar Nasional Biologi PBI ke-XXIII. Jayapura 8-9 September 2015
19. Okere SC, Elekwa I. Studies on the Invitro Antioxidan *Laportea aestuans*. 2014.
20. Njina SN, Tagne RS, Telefo BP, et al. Anti-androgenic, anti-oestrogenic, and antioksidan activities of aqueous extract of *Laportea ovalifolia* on adult rats. Internasional Journal of Phytomedicine. 2016; 8: 257-266
21. Tchinda CF, Voukeng IK, Beng VP, et al. Antibacterial activities of the methanol extract of Albizia adianthifolia, Alchornea laxiflora, Laportea ovaliafolia and three other Cameroonian plants against multi drug resistan gram negative bacteria. Saudi Journal of Biological Sciences. 2016
22. Nzeugeum KWM, Dongmo MOL, Lienou LL, et al. 2015. Effect of Laportea ovalifolia (urticaceae) on Monosodium glutamate induced obese rats. African Article of Integrated Health. 2015; 5(2): 48-53.
23. De Gusman JA, Beltran RJL, Rubio R, et al. 2015. Potential of pre-gestational intake of Laportea interrupta L. (Stingging nettle) leaf decoction as an aid for fetal-materal health. Asian PMK. Asific Journal of Reproduction. 2015; 4(2): 85-90.
24. Mom SA, Langi MA, Kainde RP, et al. Ilmu Kehutanan Universitas Samratulangi. Manado. 2016.
25. Elujoba AA, Odeleye OM, Ogunyemi CM. Review-Traditional medicine development for medical and dental primary health care delivery system in Africa. Afr. J. Tradit. Complement. Altern. Med. 2005; 2: 46-61.
26. Smith GM, Clegg CK, Grivetti L. Int J food Sci Nutr. 1996; 47:2-7.
27. Essiett UA, Edet NI, Bala DN. Asian J Plant Sci Res. 2011; 1(2):35-42.
28. Okereke SC, Elekwa I. J of Environmental Sci, Toxicology and Food Technology. 2014: 33-41.
29. Oloyede KG, Ayanbadejo OE. Phytochemical, toxicity, antimicrobial, and antioksidan screening of extract obtain from *Laportea aestuans* (Gaud). J. Med Sci. 2012; 14(2): 51-59.
30. Simaremare ES, Ruban A, Runtuboi D. Aktivitas Antibakteri Ekstrak Etanol Daun Gatal (*Laportea aestuans* (L) Chew). Jurnal Biologi Papua. 2017; 9(1):1-7
31. Sofian A. Ilmu Urai Tubuh Manusia. Jakarta: Penerbit Teragung. 1950.
32. Junqueira LC, Carneiro J, Kelley RO. Histologi Dasar. 8th Edn. Jakarta: Penerbit Buku Kedokteran. 1997.
33. Widmann FK. Tinjauan Klinis Atas Hasil Pemeriksaan Laboratorium, Terjemahan Kresno SB, Gandasoebrata R, Latu T. Jakarta: EGC. 1994.

34. Widowati W. Potensi fraksi aktif antioksidan kacang Koro *(Mucuna pruriens)* dalam pencegahan aterosklerosis. Laporan Hasil Penelitian Hibah Bersaing Tahun Anggaran 2007/2008. Universitas Katolik Soegijapranata, Semarang. 2007.
35. Macnair, Anti Coagulan. 2011. Cited 2019 March 11. Available on http://news.bbc.co.uk.id.mk.gd/2/hi/health/medical_notes/ab/990593.stm.
36. Pindan A. Sitotoksik Rhizopora mucronata dan Aktivitas Koagulasinya Dalam Darah. 1998.
37. Pearce EC. Anatomi dan Fisiologis Untuk Para Medis, 29th Edn. Jakarta: PT. Gramedia Pustaka Utama. 2006: 141-142.