Saponin Content Analysis on Leaves and Petioles of *Carica pubescens* Lenne & K. Koch

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ABSTRACT

Carica pubescens is a tropical species that adapt to the plateau environment and low temperatures. Morphological, chemical content, and analysis of protein banding pattern on *C. pubescens* has been done, but more on the analysis of active compounds for pharmaceutical raw materials and its accumulation in the body of the plant has not been widely studied. This study aims to determine the content of saponin in leaf and petiole of *C. pubescens* in terms of absorbance values. The results showed that the leaf and petiole of *C. pubescens* positive for the saponins with the formation of stable foam for 60 seconds at 1.5 cm - 1.7 cm. The third positive samples containing saponins triterpene the ring test produces a brownish color. Isolation saponin by TLC shows the best ratio of eluent chloroform:methanol:water (14:6:1) compounds can be separated perfectly. Saponin absorbance values obtained two samples from Cangar area as follows: petiole samples amounted to 0.852 and leaf samples amounted to 0.686. The highest saponins found in organs petiole. Thus the petiole of *C. pubescens* has the potential to be used as a source of triterpene saponins which can be developed into a commercial herbal medicines.

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1. INTRODUCTION

Carica pubescens Lenne & K. Koch is one of the plants typical of the plateau. In Indonesia, this plant is commonly known as "karika", can be found in the Bromo and Cangar East Java, as well as the Dieng Plateau, Wonosobo-Banjarnegara in Central Java. *Carica pubescens* is Caricaceae familia members and thus have the same genus *Carica papaya* and has a high similarity in morphology. Presence of hair *(pubescens)* on the abaksial and petiole into the primary identifier in addition to the morphology of flowers, fruits, and branchs in the trunk when compared to the morphology of *Carica papaya.* Moreover, in contrast with *Carica papaya, Carica pubescens* thrives in places with a height of 1.400- 2400 meters above sea level (asl), low temperatures and high rainfall. Here morphology of *C. pubescens* shown in **Figure 1.**

Figure 1. Morphology *C. pubescens*

Allah SWT has created good herbs that can be taken the benefits by human as written in the Qur'an as follows: *7. And have they not seen the earth, how many We have made in the earth a variety of good herbs? 8. Verily in that is a Sign of Allah. And most of them do not believe. 9. and Your Lord is He, the Exalted in Might, the Merciful.* "(Al-Qur'an, ash-Shu'ara: 7-9)

Studi about medicinal plants and their potential for today's health has been developed. Morphological characters, antioxidant capacity, and analysis of protein banding pattern on *C.pubescens* has been done (Laily, 2011), but more about the use of the active compounds for medicinal raw materials and its conservation has not been widely studied. The fruit of this plant has been studied its content as a source of flavonoids (Minarno, 2014) while the seeds have been studied as a source of saponins (Supono, 2014). Flavonoid is closely related to antioxidant activity whereas saponins are compounds in the form of glycosides, both of them are widespread in plants.

Saponins form a steady foam when shaken (Harbrone, 1987), a complex group of natural compounds, with mass and large molecules, with wide uses (Burger et al., 1998). The structure of saponins causes saponins to be soap or detergent that saponins are called natural surfactants (the name saponins is derived from this main character ie "sapo" in Latin meaning soap) (Calabria, 2008; Hawley & Hawley, 2004).

Saponins include phytochemical compounds that can inhibit elevated blood glucose levels by inhibiting the absorption of glucose in the small intestine and inhibit gastric emptying. With the slowing of the emptying of the stomach, the food absorption will be longer, and blood glucose levels will improve (Bruneton, 1999; Matsuda et al 1999). Thus, saponin on *C. pubescens* potential as ingredient herbal medicine of Diabetes Mellitus.

Diabetes Mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia and carbohydrate metabolism, fat and protein abnormalities caused by insulin secretion abnormalities, insulin work or both (WHO, 2006). Chronic hyperglycemia in diabetes mellitus will be accompanied by damage, impaired function of several organs of the body especially the eyes, kidneys, nerves, heart, and blood vessels. Although diabetes mellitus found metabolic disorders of all the food sources of our bodies, the most important metabolic disorder is carbohydrate metabolism abnormalities. Therefore the diagnosis of DM is always based on high levels of glucose in blood plasma (Adam, 2000).

Information on the organ where the accumulation of saponins in plants *C. pubescens* is required in order to use these plants as a source of saponins. Required knowledge about saponins in leaves and petioles as a potential herbal remedy in cases of DM. The amount of saponin absorbance value in UV-Vis spectrophotometry can be used as a reference to know the ratio of saponin content in plant organ. Thus this research needs to be done.

This study aims to know saponins on leaf and petiole *C. pubescens.* Results of this research expected to provide information about the potential of saponins contained in the various organs of plants *C. pubescens* growing in Indonesia as a diabetes drug raw materials. Such knowledge can help communities and researchers to obtain DM drugs while conserving these plants.

2. RESEARCH METHOD

This research is an observational research with the object is *C. pubescens* that were not given any treatment, but examined saponins qualitatively and quantitatively so that the research data presented descriptively. The study was conducted in August until November 2015. Activity of *Carica pubescens* sampling was conducted in Cangar area, East Java. Qualitative and quantitative analysis was conducted in Plant Physiology Laboratory of Biology Department and Chemical Laboratory of Chem Department, Faculty of Science and Technology, UIN Maulana Malik Ibrahim Malang.

The tools required for field sampling and laboratory analysis are: camera and oven plastic bags, jars extraction, a stirrer, a knife, 60-mesh sieve, rotary vacuum evaporator, a hair dryer, a UV-VIS spectrophotometer, test tubes, glass beaker, beakers, pipettes micro, analytical balance, capillary tube, plate TLC (Thin Layer Chromatography), TLC chamber, and a UV lamp. The materials required include: *C. pubescens* (from Cangar area), filter paper, p.a. methanol, distilled water, 2N hydrochloric acid, chloroform, alcohol 95%, concentrated acetic acid anhydride, and sulfuric acid.

a. Sample Preparation
1) Sample of *C. pubescens* taken from Cangar area, East Java
2) The sample is cleaned of dirt and dried
3) Weighed wet weight of sample
4) Oven to dry for approximately 5-6 days at 40° C
5) Weighed dry weight of the sample
6) Crushed and sieved using 60 mesh sieve to be simplicia powder

b. Sample Extraction
1) Maceration with p.a. methanol ratio of 1:5 sample weight

2) Simplicia were filtered to obtain first macerate
3) Remaceration with p.a. methanol ratio of 1:4 weight of the sample
4) Simplicia were filtered to obtain a second macerate
5) Macerate were evaporated with rotary vacuum evaporator
6) Extracts were incubated at 27° C to become viscous preparations

c. Preliminary Test

The saponin content test is qualitatively composed of foam test and color test.

1) Test foam
a) Weighed 0.3 gram extract later put in a test tube
b) Added 10 ml of distilled water and then shake strongly
c) 2N HCl was added, its form stable foam was observed for approximately 1 minute

2) Color test
a) Extract weighed 0.3 gram put into a test tube
b) Added 10 ml of chloroform
c) Heated in an incubator for 5 minutes while shaking / stirring
d) LB (Lieberman Burchad) reagent is added to form a brown ring on the surface of the tube

d. Isolation of Saponin by TLC Analytical

1) Activation of the TLC plate with the oven for 1 hour at 100˚C
2) Eluent made with chloroform:methanol:water (13:7:2) and (14:6:1)
3) Eluent included in the 10 ml chamber
4) Eluent saturation in the chamber for approximately 1 hour
5) Extract diluted 1000 ppm
6) TLC plat cut 2x10 cm size, lined, 1 cm for the top and bottom plate
7) The extract is bottled on a plate using a capillary pipe
8) Spotting was done as much as 5-8 times, every once spot waited until plate becomes dry/use hair dryer
9) The plates were inserted in the chamber and wait until the plate eluted to the mark of upper limit
10)Read the results of elution with 366 nm UV light

e. Isolation of Saponin by TLC Preparative

1) Activation of the TLC plate with the oven for 1 hour in 100º C
2) Eluent made with ratio composition of chloroform:methanol:water (14:6:1)
3) Eluent included in the 10 ml chamber
4) Eluent saturated in the chamber for approximately 1 hour
5) Extract diluted in 1000 ppm
6) TLC plat cut 5x10 cm size, lined, 1 cm for the top and bottom plate
7) Extract spotted on the plate using capillary tube
8) Spotting was done as much as 5-8 times, every once spot waited until the plate becomes dry or use hair dryer.
9) The plates were inserted in the chamber and waited until the plate was eluted until the upper limit mark.
10)Read the results of elution with 366 nm UV light.

f. Measurement of Saponin Compounds with UV-Vis Spectrophotometry

From the TLC results, the appropriate color is scraped. Results scrapings were added in the *tube* and added 1 ml of methanol, then *centrifuge* to separate the plate with the supernatant. The obtained supernatant then measured it absorbance using a 209 nm wavelength spectrophotometer by add 700 micro liters of isolate / supernatant. Saponin content data analysis was done qualitatively by comparing foam and color. Whereas quantitatively done by determining the best saponin absorbance value from two samples *of C. pubescens* based on measurement results of UV-Vis spectrophotometry.

3. RESULTS AND ANALYSIS

Firdous et al. (2009) proves that saponins act as antidiabetes. After histopathology examination, known that saponins are able to regenerate the pancreas which causes an increase in the number of pancreatic β cells and the islands of Langerhans that insulin secretion will increase. Increased secretion of insulin will help decrease blood glucose levels. Pigreas β cell regeneration occurs because of the quiescent cells in the pancreas that have the ability to regenerate.

Some examples of saponins in plants include diosgenin, and botogenin from the genus Dioscorea. Hekogenin, manogenin, and gitogenin of Agave species. Sarsapogenin and smilagenin from the genus Smilax. Sarmentogenin from the genus Strophantus. Sitosterol from plant oil. Family Liliaceae, Amaryllidaceae, and Dioscoreaceae contain sapogenin. Similarly in Apocynaceae (Sirait, 2007). Thus, in this study investigated the Genus Caricaceae saponins, namely *C. pubescens* on two organs of plants that grow in Cangar area.

3.1. Preparation of extract of *Carica pubescens* Lenne & K. Koch

Sampling fruit from Cangar area, East Java. Samples obtained from Cangar area with leaf wet weight of 1117 grams and petioles with wet weight of 1291 grams. The literature study has been conducted to obtain extraction methods, qualitative and quantitative tests samples of leaf and petiole *of C. pubescens.*

The process of drying fruit samples *Carica pubescens* Lenne & K. Koch is done by using an oven with a temperature of ± 40ºC for 5-6 days so that the desired substances are protected from possible damage. Samples were subsequently dried mashed with a 60 mesh sieve and weighed to determine the dry weight in the form of powder. Samples from Cangar area have dry weight of leaves of 220 gram and petioles with dry weight of 50 gram. The extract obtained by extracting the active compounds from botanicals using methanol pro analys. The method applied to the extraction of leaves and petioles of *Carica pubescens* Lenne & K. Koch is maceration. Fruit maceration is performed by the process of extracting simplicia at room temperature (26ºC to 28ºC), so that the substances contained in the simplicia are relatively safe. The maceration is done by using the weight ratio of the sample: solvent = 1:5 for 24 hours. Furthermore, remaceration is done by using the weight ratio of the sample:solvent = 1:4 for 24 hours. Then, the extract was concentrated with a rotary vacuum evaporator and incubated at 27°C to become viscous preparations. From the extraction process, obtained condensate from Cangar area of 11 grams of leaf extract and 7.5 grams of leaf extract.

3.2. Preliminary test content saponin extract of *Carica pubescens* Lenne & K. Koch

The preliminary test to determine the saponin content is qualitatively carried out by the method described by Suharto et al. (2012). This test is performed to ensure the qualitative presence of saponins contained in leaf and petiole samples from area *C. pubescens* Cangar area.

a. Test Foam

Saponin when shaken will foam. The ability to lower surface tension is due to saponin molecules consisting of hydrophores and hydrophils. The hydrophobic part is the aglycons, the hydrophilic part is the glycine. It tastes bitter. Most of the saponins reacts neutral (water soluble), some are acidic (water-soluble), some are reacting alkaline. Aglikon saponin is called sapogenin. Sapogenin is difficult to dissolve in water. Saponin may be a compound having one sugar chain or two mostly branched sugar chains (Sirait, 2007).

C. pubescens condensed extract as much as 0.3 grams included in the test tube. In the foam test used aquades as solvent and 2 N hydrochloric acid as it reagent.

In leaf extract, after added 10 ml of aquades and shaken strongly then added 2N chloride acid, formed foam that do not lost with a height of 1.5 cm for approximately 60 seconds. Foam test results on leaf extract samples as shown in **Figure 2 (a).** Whereas, in the extract of petiole, after added 10 ml of aquades and shaken strongly then added 2N hydrochloric acid, formed foam do not lost with a height of 1.7 cm for approximately 60 seconds. The result of foam test on leaf extract samples as shown in **Figure 2 (b).**

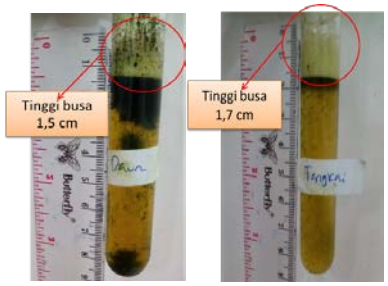

Figure 2. (a) Test results on samples of foam leaf extracts **(b)** Foam test results on leaf extract samples from Cangar area

The foam is formed because the saponin compound has a physical property that is easily soluble in water and will cause foam when shaken. On structure micelles, polar groups are facing outside while nonpolar groups are facing inside. This circumstances which looks like foam.

b. Color Test

Color test performed on all samples showed a positive result. In this color test can also known that saponin group contained in the extract. According to Steinegger and Hansel in Sirait (2007), saponins are divided into 2 groups:

1) Steroid sterols, when hydrolyzed forms a sterol compound.
2) Saponin triterpen, when hydrolyzed forms triterpen compounds.

Tschsche, Wollf, and Gerlach in Sirait (2007) state that saponins are divided into 3 groups:

1) Spirostanol Saponins
   Also called neutral saponins. Example: sarsasapogenin
2) Saponin triterpena
   Also called saponin acid. Example: oleanolic acid
3) Saponin sterols

Also called basic saponins, is divided into two types, namely:

a) Type of demysin or solanine, example: solanidine, solatubin.
b) Tomatin type, example: tomatidin.

Color test results on samples of leaf extract *of C. pubescens* in Cangar area show positive results. The color of the brown ring is an indication of the presence of triterpene saponins, as shown in **Figure 3 (a).** Next, test results of a sample of color on the petiole *of C. pubescens* extract from Cangar region also show positive results saponin. The color of the brown ring is an indication of the presence of triterpene saponins, as shown in **Figure 3 (b).**

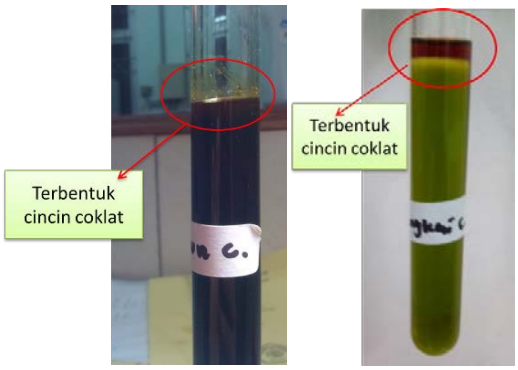

Figure 3. The color test results on **(a)** leaf extract **(b)** petiole extract samples from Cangar area

All of extracts with the addition of chloroform and LB reagent showed different color on the extract of the petiole from Cangar area, which is greenish while the other two extracts are more brownish. Nevertheless the final result on the color test of the samples yields the same color, that is the brownish ring on the surface of the tube. Thus it is known that the samples indicate the existence of triterpen saponins. Based on a previous study of saponin compounds stating that samples after added LB reagents will produce a purple-brown ring indicating the presence of triterpenes and green-blue saponins for steroidal saponins.

3.3. Isolation of Saponin with Thin Layer Chromatography (TLC)

Separation of saponins from the extract *of C. pubescens* in this study using TLC (Thin Layer Chromatography). TLC is a method often used to separate components of compounds in an extract. A good eluent ratio is known based on laboratory trials. The elution results with eluent of chloroform: methanol: water (13: 7: 2) are compound do not completely separated then tailing occurs. The results can be seen in **Figure 4.**

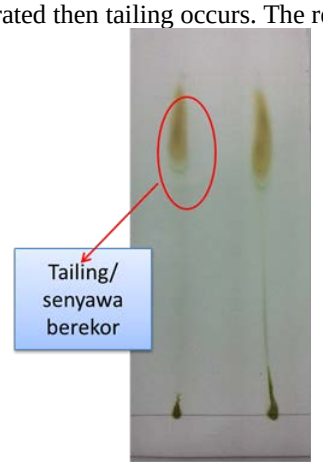

Figure 4. Compounds not completely separated by chloroform eluent: methanol: water (13: 7: 2)

Next was eluted elution with eluent of chloroformt: methanol: water (14: 6: 1), and the compound was completely separated. From these results then performed irradiation with 366 nm UV lamp (**Figure 5).**

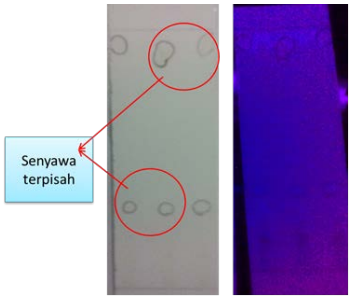

Figure 5. Compound separately with chloroform eluent: methanol: water (14: 6: 1): **(a)** without UV light irradiation; **(b)** with a UV light irradiation of 366 nm

3.4. Isolation of Saponin with Thin Layer Chromatography (TLC) Preparative

The results of analytical TLC showed that the best eluent to separate the compounds in the extracts *C.pubescens* is chloroform: methanol: water (14: 6: 1). Therefore this eluent is used for preparative TLC. There are several color spots showing that the extract contains several compounds. The result of leaf sample can be seen in **Figure 6 (a)** and results of TLC from petiole sample can be seen in **Figure 6 (b)**

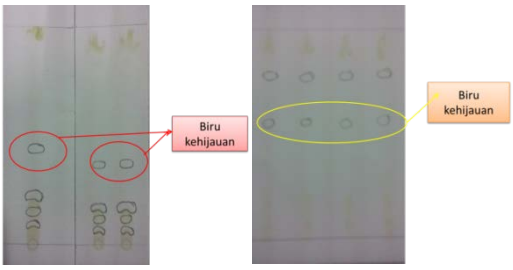

Figure 6. Result TLC of **(a)** leaf sample **(b)** petiole sample

In both of samples carried out by spraying the plate with LB reagents followed by heating using a *hair dryer* obtained a bluish green color patches. Wagner et al. (1984) states that a simplicia is said to contain saponins when spraying with LB gives a blue, violet, sometimes red or yellow brown on visible light. Rf values obtained are 0.275-0.375 (Suharto et al., 2012).

3.5. Measurement of Saponin Compounds with UV-Vis Spectrophotometry

From the preparative Thin Layer Chromatography (TLC) then suitable color was scraped as an isolate for examination of absorbance value at 209 nm wavelength with UV-Vis spectrophotometer. It is known that the absorbance value of saponin at the petiole is 0.852 and the leaf is 0.686. Thus the petiole has the highest saponin absorbance value.

Phytochemicals of Caricaceae and *C. pubescens* are generally unstudied, therefore, need to be explored sustainable information. In view of the process of making powder simplicia, the ratio value of wet weight to dry weight of powder is as follows: leaf samples are 5.08; petiole samples are 25.82. This shows the lowest water content found in the sample of the petiole.

Low water content may affect secondary metabolites contained in a plant organ. In line with Sirait's (2007) opinion that the physical plant consists mostly of water, the water content reaches more than 90% in leaves, flowers, fruits (many watery fruits), and underground parts of the plant. In poor tissue storage organ, water content decreases to about 50% ie on the skin and wood. The least water is seed, generally containing ± 10%. The chemical compounds of the most abundant plant are small molecular chemical compounds with limited spreading, ie secondary metabolites. Included in this case is saponin content.

With the known presence of saponins in plant organs *C. pubescens* then these plants could potentially be used as a source of raw material for medicine in the treatment of Diabetes Mellitus. WHO recommends the use of traditional medicines, including herbs, in the maintenance of public health, prevention and treatment of diseases, especially for chronic diseases, degenerative diseases, and cancer. The use of traditional medicine, in general, is considered relatively safer than the use of modern medicine, with a record of the rules and rules in its use. Therefore, traditional medicine has relatively fewer side effects than modern medicine in proper and rational use (Purwanto, 2014).

Allah SWT is the only God who creates all sickness, but He also shows the method of healing. As stated by Rasulullah SAW: "Allah does not decrease the disease unless He lowers the medicine for him" (Narrated by Bukhari). Rasulullah SAW asserted that every disease there is a cure and can be cured by permission of Allah SWT, except aging and death. While the variety of drugs have been provided (created) by the Allah SWT. Similarly, the theory and practice of treatment, in outline and detail has been exemplified Rasulullah SAW. "Every disease has a drug, when the drug is about the disease then he is healed by the permission of Allah (HR Muslim).

Ibn Qayyim Al-Jauziyah said that the phrase of the Prophet "every disease there is medicine" gives the spirit and strength of the soul of the sick and the doctors who treat it, they are encouraged to seek medicine and examine it. As for the patient when he was convinced that there must be a medicine that can cure his illness, then he has the spirit to heal. Feelings of despair are lost because of open expectations. If his soul is strong, then the spirit increases, the stamina that supports his body is also increased so as to overcome even expel the disease. Similarly, for doctors, if you already believe that every disease must have the cure, he must continue to seek drugs from a disease and continue to do research (Al-Jauziyah, 2013).

Thus no exception on research using *C. pubescens* as a source of saponins can open new horizons treatment of Diabetes Mellitus.

4. CONCLUSION

Conclusion of this research is the petioles have the highest saponin absorbance value. Petiole of *C. pubescens* has the potential to be used as a source of triterpene saponins which can be developed into a commercial drug experienced.

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REFERENCES

[1] Adam, J. M. F., 2000, Klasifikasi dan Kriteria Diagnosis Diabetes Melitus yang Baru, Cermin Dunia Kedokteran, ISSN; 0125-913X No. 127, 37-40
[2] Al-Jauziyah, Syekh Ibnu Qayyim. 2013. *Rahasia Pengobatan Nabi SAW*. Mitrapress
[3] Al-Qur'an
[4] Bruneton, J. 1999. Flavonoid. Dalam: Pharmacognosy : Phytochemistry medical plants, edisi 2. France : Lavoisier Publishing. p. 310-327.
[5] Burger, I., Burger, B, V. Albrecht, C. F. Spicies, H. S. C. and Sandor. P. 1998. Triterpenoid saponin From Bacium gradivlona Var. Obovatum Phytochemistry. 49. 2087-2089.
[6] Calabria, L.M. 2008. *The Isolation and Characterization of Triterpene Saponins from Silphium and the Chemosystematic and Biological Significance of Saponins in The Asteraceae*. ProQuest
[7] Firdous, M., Koneri, R., Sarvaraidu, C.H., dan Shubhapriya, K.H. 2009. NIDDM Antidiabetic Activity Of Saponins Of Momordica Cymbalaria In Streptozotocin-Nicotinamide NIDDM Mice. Journal of Clinical and Diagnosis Research 3: 1460-1465.
[8] Harbrone. J.B. 1987. Metode Fitokimia : Penuntun Cara Moderen Menaganalisis Tumbuhan. Terbitan Kedua. ITB : Bandung
[9] Hawley, Ts. & Hawley, R.G. 2004. *Flow Cytometry Protocols*. Humana Press, Inc
[10] Laily, AN. 2011. Karakterisasi *Carica pubescens* Lenne & K.Koch Berdasarkan Karakter Morfologi, Kapasitas Antioksidan, dan Pola Pita Protein di Dataran Tinggi Dieng, Jawa Tengah. *Tesis.* Surakarta: Pascasarjana UNS
[11] Matsuda H., Li Y., Yamahara J., Yoshikawa M. 1999. Inhibition of Gastric Emptying by Triterpene Saponin, Momordin Ic, in Mice: Roles of Blood Glucose, Capsaicin-Sensitive Sensory Nerves, and Central Nervous System. http://jpnet.aspet journals.org/cgi/content/full/289/2/729, 25 Desember 2006
[12] Minarno, Eko Budi. 2014. Skrining Fitokimia dan Kandungan Total Flavanoid pada Buah *Carica pubescens* Lenne & K. Koch di Kawasan Bromo, Cangar, dan Dataran Tinggi Dieng
[13] Purwanto, Budhi; editor, Hanifah Fitriani. 2014. *Buku Ajar Ilmu Keperawatan Berbasis Herbal*. Yogyakarta: D-Medika
[14] Sirait, Midian. 2007. *Penuntun Fitokimia dalam Farmasi*. Bandung: Penerbit ITB

[15] Suharto, M. A. P., Edy, H. J., Dumanauw, J.M. 2012. Isolasi dan Identifikasi Senyawa Saponin dari Ekstrak Methanol Batang Pisang Ambon (*Musa paradisiaca* var. sapientum L.). Parmachon Journal. I(2), 82-92.
[16] Supono, 2014. Potensi Ekstrak Biji Karika (*Carica pubescens*) Sebagai Larvasida Nyamuk *Aedes aegypty*. *El-Vivo.* Vol 2, No 1
[17] Wagner, H.M.; Bladt, S.; Zgainski, E.M. 1984. *Plant drug analysis*. New York: Springer-Verlag,
[18] World Health Organisation. Diabetes Mellitus : Report of a WHO Study Group. World Health Organisation. Geneva-Switzerland. 2006. S5-36.