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Antidiabetic Activity of *Peperomia pellucida* In Streptozotocin-Induced Diabetic Mice

(Aktivitas Antidiabetes Tanaman Peperomia pellucida pada Mencit Diabetes Terinduksi Streptozotozin)

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ABSTRACT

Background: Diabetes mellitus is a heterogeneous group of diseases in the form of disorders in the body's metabolism clinically. *Peperomia pellucida* herbs have phytochemical substances that potential as antidiabetes. **Objectives:** This study aims to compare the antidiabetic activity of ethanol extract and n-hexane fraction of *Peperomia pellucida* in diabetic mice model induced by streptozotocin. **Material and Methods:** This research was conducted on diabetic mice with 50 mg/kg bw of streptozotocin induction, which was then treated for 7 days with doses of 250 mg/kg bw of ethanol extract and n-hexane fraction of *Peperomia pellucida*, respectively. **Results:** The results showed that the ethanol extract and n-hexane fraction of *Peperomia pellucida* able to reduce the blood glucose levels in diabetic mice induced by streptozotocin. The n-hexane fraction of *Peperomia pellucida* can lower blood glucose levels as much 244.00 ± 18.99 mg/dL better than the ethanol extract, which is 99.50 ± 28.17 mg/dL. **Conclusions:** *Peperomia pellucida* herb has the potential to be developed as an antidiabetic agent.

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ABSTRAK

Latar Belakang: Diabetes melitus merupakan kelompok penyakit heterogen berupa gangguan metabolisme tubuh secara klinis. Tanaman herba *Peperomia pellucida* memiliki zat fitokimia yang berpotensi sebagai antidiabetes. **Tujuan**: Penelitian ini bertujuan untuk membandingkan aktivitas antidiabetes ekstrak etanol dan fraksi n-heksana *P. pellucida* pada mencit model diabetes yang diinduksi streptozotocin. **Bahan dan Metode**: Penelitian ini dilakukan pada mencit diabetes yang diinduksi streptozotocin 50 mg/kg bb, kemudian diberi perlakuan selama 7 hari masing-masing dengan dosis 250 mg/kg bb ekstrak etanol dan fraksi n-heksana *Peperomia pellucida*. **Hasil**: Hasil penelitian menunjukkan bahwa ekstrak etanol dan fraksi n-heksana *Peperomia pellucida* mampu menurunkan kadar glukosa darah mencit diabetes yang diinduksi streptozotocin. Fraksi n-heksana *P. pellucida* dapat menurunkan kadar glukosa darah sebanyak 244,00 ± 18,99 mg/dL lebih baik dibandingkan ekstrak etanol, yaitu 99,50 ± 28,17 mg/dL. **Kesimpulan**: Herba *Peperomia pellucida* berpotensi untuk dikembangkan sebagai agen antidiabetes.

Keywords: Antidiabetes, *Peperomia Pellucida*, Ekstrak etanol , Fraksi n-heksana.

INTRODUCTION

Diabetes mellitus is a heterogeneous group of diseases in the form of disorders in the body's metabolism clinically (Dianaly *et al*., 2017). A report from the International Diabetes Federation (2017) stated that around 425 million adults in the world with an age range of 20-79 years suffered by diabetes mellitus desease and it is estimated that in 2045 there are 629 million sufferers.

Chronic hyperglycemia in diabetes is associated with long-term damage, dysfunction or failure of several organs, especially the eyes (diabetic retinopathy), nerves (diabetic neuropathy), heart (cardiovascular), and kidneys (diabetic nephropathy) (Lim, 2014). Treatment of diabetes mellitus and its complications are chronic and lifelong treatment. Management of diabetes is currently carried out with lifestyle modifications or the use of antidiabetic agents such as metformin, sulfonylurea or thiazolidinediones. However, the use of these antidiabetic agents has not been able to achieve good glycemic control and has not been able to restore insulin sensitivity and prevent the degeneration of pancreatic beta cells as the only insulin-producing cells. In addition, long-term use of antidiabetic agents has been shown to possess side effects and tolerance (Rotenstein *et al*., 2012). Therefore, exploration natural sources in obtaining alternative diabetes treatment is very important (Patel *et al*., 2014).

The identification of chemicals from medicinal plants provides an opportunity in the development of new drugs, one of which is an antidiabetic agent. One of the plants that has potential to be developed as an antidiabetic agent is *P. pellucida*. The herbs contain phytochemical groups such as alkaloids, flavonoids, saponins, terpenoids, steroids and glycosides (Raghavendra and Kekuda, 2018). *P. pellucida* in the form of dietary supplement by mixing dry powder of *P. pellucida* with standard feed can reduce blood glucose levels of alloxan-induced diabetic rats (Hamzah *et al*.,

2012). Docking studies on constituents of *P. pellucida* to the aldose reductase receptor showing yohimbine as a potent bioactive compound associated with antidiabetic activity (Akhilas *et al.*, 2012).

Based on literature studies, the research is needed to determine the active components that act as antidiabetic in vivo. Ethanol is a solvent used to attract polar and non-polar active compounds and n-hexane solvent is able to attract only non-polar compounds. Therefore, this study aims to compare the antidiabetic effect between ethanol extract and the n-hexane fraction of *P. pellucida* plants in streptozotocin-induced diabetic mice models.

MATERIALS AND METHODS

Materials

The plants were taken from the area around Jember and identified at Biology Laboratory, Faculty of Science and Applied, Ahmad Dahlan University, Glucose test and test-strips (Easy Touch GCU®), streptozotocin (Bioworld®), technical Na-CMC (Brataco), 0.9% NaCl solution, 5% dextrose (Otsuka Pharnaceutical Co, Ltd.) and aquadest. Male mice were taken from Veterinary laboratory, Faculty of Dentistry, University of Jember. Ethical cleareance was obtained from Health Research Ethics Committee STIKES dr. Soebandi Jember with the number 076/EC/KEPK/VII/2020.

Methods

Sample preparation

A total of 1 kg of herbs powder *P. pellucida* was macerated using 10 L of 80% ethanol for 5 days, then filtered using a vacuum filter. The residue was macerated again using 2 L of 80% ethanol for 24 hours. The obtained maserate was concentrated until thick using a vacuum rotary evaporator at a temperature of 60^{o}C.

10 grams of ethanol extract were divided into 10 tubes added with distilled water and dissolved in 5 ml of n-hexane solvent with a ratio (2: 1: 2), then vortexed and centrifuged until 2 phases were obtained, namely the supernatant phase as the soluble hexane fraction and the precipitate phase as n-hexane insoluble fraction. The supernatant which is the n-hexane fraction is separated from the precipitate and placed in a separate container. The sediment phase is added with n-hexane, vortexed and centrifuged again until it is clear and the green color of the supernatant is dissapeared.

Phytochemical screening of ethanol extract and n-hexane fraction

Phytochemical screening consists of identification of polyphenols, flavonoids, saponins and alkaloids. Identification of polyphenols was used FeCl3 reagent. The positive polyphenols are indicated by the formation of a blackish blue, green or turquoise color. Identification of flavonoids was carried out using the ammonia vapor method. Positive flavonoids will appear yellow color on filter paper. Saponin identification was carried out by adding 10 ml of heated aquadest, then cooled and shaken vigorously for 10 minutes. Positive results are indicated by the formation of foam as high as 1-10 cm. The foam is lost if added 1 drop of 2N HCl. Alkaloid identification was carried out using dragendrof reagent. a positive result of alkaloids will appear orange color and precipitation.

Diabetic-induced in mice

Male mice aged 6-8 weeks were placed in groups in cages at room temperature 25 ± 1ºC. During the study, the need for food and drink was maintained in excess. Before testing, the mice were checked for normal blood glucose levels. On day 0, mice were induced with streptozotocin at a dose of 50 mg/kg bw (body weight) intra-peritoneally. On the day 10, the development of hyperglycemia mice was examined. Blood samples were taken through the tail by injuring the tail of the mice. Blood glucose levels were measured by glucose test. If after 10 days, the blood glucose levels is more than 200 mg/dL, the mice have become diabetic.

Treatment of experimental animals

A total of 20 mice were used and divided into 4 groups with 5 mice per group. Group I (normal control, a healthy mice). Group II (diabetic control, were diabetic mice treated with 0.5% Na-CMC), Group III (the treatment group given ethanol extract at a dose of 250 mg/kg bw), Group IV (the treatment group given the hexane fraction at a dose of 250 mg/kg bw. The treatment was carried out orally for 7 days. On day 8, all mice were fasted and their blood sugar levels were measured.

Data analysis

To identify the significant differences from the data set obtained from this study, the blood glucose level data after induction were statistically analyzed using paired t-test on SPSS 17.0. Blood glucose level data after treatment were statistically analyzed using the One Way-Anova test.

RESULT AND DISCUSSION

Phytochemical Screening

Phytochemical screening includes identification of polyphenols, flavonoids, alkaloids, and saponins. The results of the phytochemical screening test are presented in table 1. Phytochemical screening aims to provide an initial figure of the class of secondary metabolite compounds present in the fraction. Phytochemical screening of the extract and fraction showed that the ethanol extract contained polyphenols, flavonoids, alkaloids and saponins, while the n-hexane fraction contained saponins and alkaloids. The ethanol extract contains more polyphenols, while the n-hexane fraction contains more alkaloids.

Table 1. Phytochemical screening of ethanol extracts and n-hexane fraction of *P. pellucida*

Phytochemical Compounds	Reagent	Sample	
		Ethanol Extract	n-hexane Fraction
Polyphenol	$FeCl_3$	++	-
Flavonoid	Ammonia	+	-
Alkaloid	Dragendrof	+	+
Saponin	HCl	+	++

Observation of Experimental Animal Blood Glucose Levels

Diabetes mellitus is a chronic disease due to disorders of the metabolism of carbohydrates, proteins and fats which is a health problem for most people around the world. This disease is caused by decreased insulin secretion, decreased insulin sensitivity or both (Dipiro *et al*., 2016). Antidiabetic drugs currently in use can lower blood glucose levels. This has been proven by experimental and clinical data so that the drug is used as a diabetes mellitus therapy, but not all of these drugs have a direct effect on pancreatic beta cells (Nakatsuma *et al*., 2015).

In this study, ethanol extract and n-hexane fraction were used as an antidiabetic given to mice after streptozotocin induction. The experimental animal model for diabetes was used 20 male Babl/C strain rate aged 6-8 weeks and divided into 4 groups. Before being used for research, an adaptation was carried out for 1 week. Mice that have met the requirements were given streptozotocin at a dose of 50 mg/kg bw by intraperitoneally (i.p). on day 0. 15 mice were fasted for 4 hours and then given intraperitoneal injection of streptozotocin at a dose of 50 mg/kg in a freshly prepared citrate buffer to induce diabetes. Blood glucose levels were measured on day 10, diabetic mice were indicated by blood glucose levels> 200 mg/dL (Hajiaghaalipour *et al*., 2015). Blood glucose data before and after induction can be seen in table 2 and figure 1.

Table 2. Blood glucose levels before and after diabetes induction

Groups	Number of mice	Blood Glucose Levels (mg/dL) (X±SE)	
		Day-0	Day-10
Normal	5	108.50±13.21	70.50±17.69
Diabetic	15	79.27±5.97	324.45±50.17*

Day-0, all groups were homogeneous (p>0.05)
*) Day-10 of blood glucose levels data differed significantly on the day-0 (p<0.05)

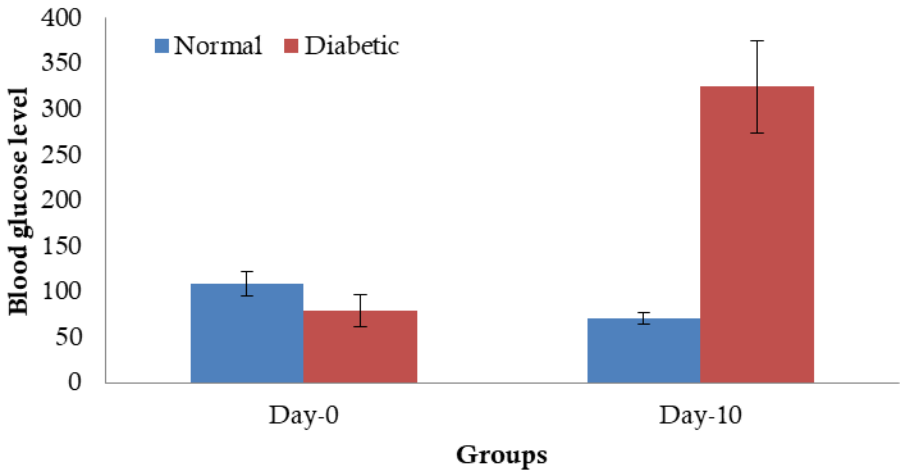

Figure 1. Diagram of pre-induction and post-induction blood glucose levels

The levels of blood glucose induced by mice on the day-10 increased significantly from 79.27 ± 5.97 mg / dL to 324.45 ± 50.17 mg/dL. After the statistical test was carried out, it was obtained p <0.05, which means that the injection of streptozotocin was able to significantly increase blood glucose levels compared to the normal group on the day-10.

Blood Glucose Levels on Pre-Treatments and Post-Treatments in Mice

The treatment was carried out for 7 days, blood glucose levels were measured on the day 8. The pre-treatmet blood glucose levels in each treatment group can be seen in table 3 and figure 2.

Table 3. Pre-treatment and post-treatment blood glucose levels

Groups	Pre-treatment blood glucose levels (mg/dL)	Post-treatment blood glucose levels (mg/dL)
Normal control	101.25±1.38	98.00±8.95*
Diabetic control	200.25±19.80	462.00±24.09
Diabetic + ethanol extract	353.00±85.12	253.50±58.54*
Diabetic + n-hexane fraction	475.50±46.49	231.50±41.91*

*) Different meaning with the diabetic group ($p<0.05$)

Based on table 3. the blood glucose levels of mice before treatment showed that the healthy group was 101.25 ± 1.34 mg/dL and the diabetes treatment group was> 200 mg/dL. Blood glucose levels of diabetic mice treated with ethanol extract ordered at a dose of 250 mg/kg bw for 7 days were different from the diabetes group. This shows that the administration of ethanol extract and n-hexane fraction at a dose of 250 mg/kg bw shows a significant improvement in blood sugar levels.

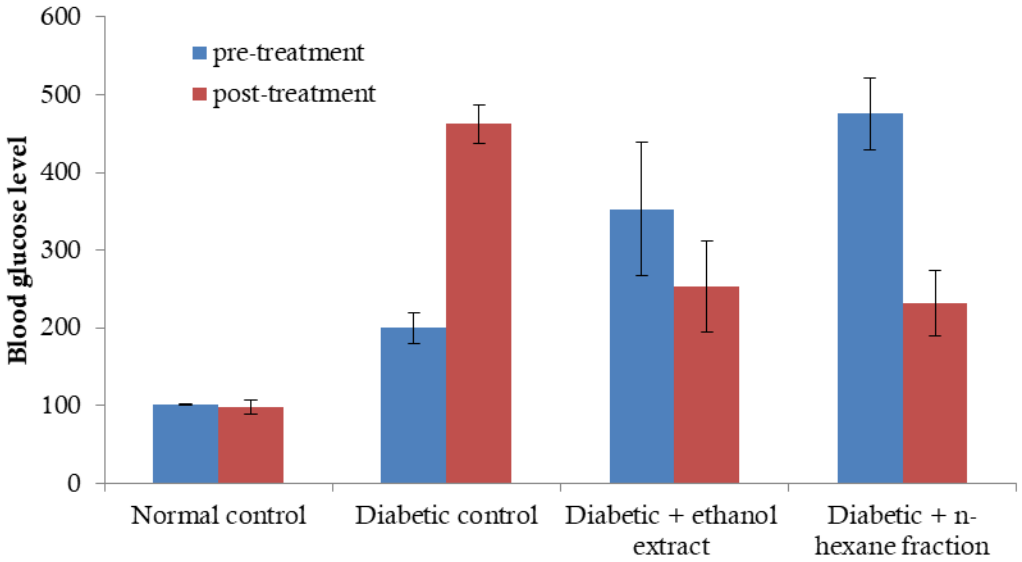

Figure 2. Diagram of pre-treatment and post-treatment blood glucose levels

Observation of Decreasing Blood Glucose Levels of Experimental Animals

The decrease in blood glucose levels in each treatment group can be seen in table 4. The results of the decrease in blood glucose levels are shown in Table 4. where from the data, it can be seen that the administration of Na-CMC (diabetic control) for 7 days caused the increasing of glucose levels, while the ethanol extract and n-hexane fraction at a dose of 250 mg/kg bw decreased blood glucose levels. The largest decrease in blood glucose levels was 244.00 ± 18.99 mg/dL, by the treatment of n-hexane treatment dose of 250 mg/kg bw.

Table 4. Post-treatment reduction of blood glucose levels

Groups	Decrease of post-treatment blood glucose levels (mg/dL)	Percent decrease of blood glucose levels (%)
Normal control	- 3.25±4.71	- 3.21
Diabetic control	+ 261.75±43.89	+ 130.71
Diabetic + ethanol extract	- 99.50±28.17	- 28.19

Diabetes + n-hexane fraction	-	244.00±18.99	-	51.31

P. pellucida is a plant originating from tropical America. Empirically, herbal herbal plants used for the treatment of diabetes and gout by drinking boiled water all parts of the plant, as fresh vegetables or by grinding all parts of the plant and then affixed to the sick for headaches, fever and colic (Kinho *et al*., 2011).

Several studies related to antidiabetic activity have been carried out. The ethanol extract at a dose of 40 mg/kg bw had a better antidiabetic effect than hexane extract in diabetic mice with sucrose administration (Togubua *et al*., 2013). Administration of the 40 mg/kg bw dose of the extract possessed an effective reduction in blood sugar levels compared to the 20 mg/kg bw and 80 mg/kg bw (Salma *et al*., 2013). In addition, ethanol extract of the extract can also reduce the blood sugar levels in mice by induction of alloxan (Purwati, 2019).

In this study, it is known that the components contained in ethanol extract of *P. pellucida* are polyphenols, flavonoids, saponins and alkaloids. Whereas the n-hexane fraction of *P. pellucida* only contains saponins and alkaloids (Table 1). Other research states that *P. pellucida* contain alkaloid compounds, tannins, saponins, flavonoids, calcium oxalate, fats, glycosides, carbohydrates, phenolics, steroids, triterpenoids, proteins, amino acids and essential oils (Patel *et al*., 2014). Saponins are chemical compounds from plants that are classified as triterpenoids and have been widely reported to have antidiabetic activity. The antidiabetic activity of triterpenoids is thought to increase insulin expression. The use of phytotherapy such as flavonoids in the management of diabetes is thought to be related to the effect of antioxidants and modulation of glucose transporter through increased GLUT-2 expression in pancreatic beta cells and increased expression and promotion of GLUT-4 translocation through PI-3K/Akt, CAP/Cb1/TC10 and AMPK pathway (Hajiaghaalipour *et al*., 2015). On the other research stated that the alkaloid fraction rich in isokuinolin significantly decreased gluconeogenesis in mouse hepatocytes as with insulin use and increased insulin secretion in RINm5F cells equivalent to that of tolbutamide (Patel and Mishra, 2011). Alkaloids also have an antidiabetic effect by increasing GLUT 4, glucokinase activity and PPARγ peroxisome (Aba and Asuzu, 2018).

The n-hexane fraction of *P. pellucida* showed a decrease in blood glucose levels better than the ethanol extract (Table 4.). The results of phytochemical screening showed that the n-hexane fraction contained more alkaloids than the ethanol extract. This is in accordance with research which states that yohimbine compounds which are included in the alkaloid group are the most active compounds as antidiabetics from *P. pellucida* (Akhilas *et al*., 2012). Other research states that the alkaloid compound dissolve in n-hexane solvent is piperine (Mgbeahuruik *et al*., 2018).

Antidiabetic studies of piperine showed that 9 out of 10 piperine derivatives had higher antidiabetic activity compared to standard rosiglitazone. This inherited antidiabetic mechanism is thought to be related to PPARγ agonists (Kharbanda *et al*., 2016).

CONCLUSION

The ethanol extract and the n-hexane fraction of *P. pellucida* were able to reduce the blood glucose levels in diabetic mice with streptozotocin induction. The decrease in blood glucose levels in diabetic mice with n-hexane was higher than the ethanol extract.

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CONFLICT OF INTEREST

Author declares no conflict of interest

REFERENCES

Aba, P. E., Asuzu, I. U. (2018). Mechanisms of actions of some bioactive anti-diabetic principles from phytochemicals of medicinal plants: A review. *IJNPR*. 9(2):85-96

Akhilas, S., Aleykutty, N. A., Manju, P. (2012). Docking Studies on *Peperomia pellucida* as Antidiabetic drugs. *Int J Pharm Pharm Sci.* 4. suppl 4:1-2

Dianaly ,T. A., Dudley, K. S., Selen, C. M. (2017). The Ldl Receptor-Related Protein 1: At The Crossroads Of Lipoprotein Metabolism And Insulin Signaling. *J Diabetes Res. 2017: 8356537*.

Dipiro, R. L., Talbet, G. C., Yee, G. R., Maztake, B. G., Weels, L. M. (2016). *Pharmacotheraphy, a Patophysiologic Approach, Ed. 9th,* New York: Mc Graw-Hill Co. pp 1333-1367

Hajiaghaalipour, F., Khalilpourshbafi, M., Arya, A. (2015). Modulation of Glucose Transporter Protein by Dietary Flavonoids in Type 2 Diabetes Mellitus. *Int J Biol Sci*. 11(5): 508-524

Hamzah, R. U., Odetola, A. A., Erukainure, O. L., Oyegbemi, A. A. (2012). *Peperomia pellucida* in Diets Modulates Hyperglycemia, Oxidative Stress and Dyslipidemia in Diabetic Rats. *JAD*. 2012: 135-140

International Diabetes Federation. (2017). IDF Diabetes Atlas 7 Edition. Brussels: International Diabetes Federation. *http://www.diabetesatlas.org/*. {Diakses: 16 September 2020}

Kharbanda, C., Alam, M. S., Hinna Hamid, H., Javed, K., Bano, S., Ali, Y., Dhulap, A., Pasha, M. (2016). Novel Piperine Derivatives with Antidiabetic Effect as PPAR-γ Agonists. *Chem Biol Drug Des*. 88(3):354-62.

Kinho, Julianus, Arini, Diah, I. D., Tabba, Supratman, Kamma, Harwiyaddin, Kafiar, Yermias, Shabri, S., Karundeng, Mood C. (2011). Traditional Medicinal Plants in North Sulawesi Volume 1. Manado: Balai Penelitian Kehutanan Manado. ISBN: 978-602-98144-1-5.

Lim, A. H. (2014). Diabetic nephropathy – complications and treatment. *Int J Nephrol Renovasc Dis*. 7: 361–381.

Mathew, M. and Harindran, J. (2018). Antioxidant And Free Radical Scavenging Activity Of *Peperomia Pellucida* (L.) Kunth: An *In Vitro* Study. *WJPR*. 7(17):2018.

Mgbeahuruike, E. E., Fyhrquist, P., Vuorela, H., Julkunen-Tiitto, R., Holm, Y. (2018). Alkaloid-Rich Crude Extracts, Fractions and Piperamide Alkaloids of Piper guineense Possess Promising Antibacterial Effects. *Antibiotics*, 7:98

Nakatsuma, A., Kiriyama, Y., Kino, K., and Ninomiya, M. (2015). Diabetes drugs that protect pancreatic β cells. *Integr Mol Med*. 3(1): 467-472

Patel, M. B., Mishra, S. (2011). Hypoglycemic Activity of Alkaloidal Fraction Of Tinospora Cordifolia. *Phytomedicine*. 15;18(12):1045-52

Patel, S. B., Santani, D., Patel, V., Shah, M. (2014). Anti-diabetic effects of ethanol extract of *Bryonia laciniosa* seeds and its saponins rich fraction in neonatally streptozotocin-induced diabetic rats. *Pharmacognosy Research*. 7(1):92-100

Purwanti, E. (2019). Effect of Plant Extract (*Peperomia pellucida*) on Kidney Histopathology and Blood Glucose Levels in Male Mice Induced by Alloxan. Skripsi. Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung, Bandar Lampung

Raghavendra H., Kekuda P. (2018). Ethnobotanical Uses, Phytochemistry And Pharmacological Activities of *Peperomia Pellucida* (L.) Kunth (Piperaceae)-A Review. *Int J Pharm Pharm Sci*, 10, Issue 2: 1-8

Rotenstein, L. S., Benzamin, B. A., Shivers, J. P., Yarchoan, M., Close, J., Close, K. l. (2012). The Ideal Diabetes Therapy : What Will It Look Like: How Close Are We?. *Clin Diab*. 30(2): 44-53.

Salma, N., Paedan, J., Mamuat, L., Togubu, S. (2013). Antihyperglycemic of Suruhan Plant Extract (*Peperomia Pellucida* [L.] Kunth) Against Wistar Rats (*Rattus Norvegicus* L.) Induced by Sucrose. *Jurnal Ilmiah Sains*. 13(2): 116-123.

Togubua S., Momuat L., Jessy E. Paendonga, Salma N. (2013). Antihyperglycemic Activity of Ethanol and Hexane Extracts of Suruhan Plant (*Peperomia pellucida* [L.] Kunth) in Hyperglycemic Wistar Rats (*Rattus norvegicus* L.). *Jurnal MIPA Unsrat Online*. 2 (2) 109-114