EFFECT OF ETHANOL EXTRACT OF JAMBLANG ACEH (*Syzygium cumini*) IN DIABETIC MICE (*Mus musculus*) AND ITS POTENTIAL AS ANTI-DIABETIC AGENT

Ayu Nirmala Sari*, Januardi, Diky Setya Diningrat*****

**Department of Biology, Faculty of Science and Technology, Universitas Islam Negeri Ar-Raniry, Banda Aceh, Indonesia, ayunirmala79@gmail.com*
***Department of Biology, Faculty of Science and Technology, Universitas Islam Negeri Ar-Raniry, Banda Aceh, Indonesia, januardi14sainsbiologi@gmail.com*
****Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan Indonesia, dikysd@unimed.ac.id*

Email Correspondence: dikysd@unimed.ac.id

Received: November 5, 2019 Accepted: May 2, 2020 Published: June 30, 2020

Abstract : This study was designed to evaluate the anti-diabetic activity of the ethanol extract of *Syzygium cumini* leaves in alloxan-induced diabetes mice. The anti-diabetic activity of EDS was investigated in mice (*Mus musculus* SW.) Alloxan-induced diabetes. The effect of ethanol extract of *Syzygium cumini* leaves on normal blood glucose levels and oral glucose tolerance tests were studied in normoglycemic mice while the anti-diabetic effect was evaluated in alloxan-induced hyperglycemic mice. Ethanol extract of *Syzygium cumini* leaves (200 and 400 mg/kg) is given orally for 21 days. Glibenclamide (5 mg/kg, oral for 21 days) is used as a reference standard. Giving ethanol extract of *Syzygium* leaves causes a significant decrease in blood glucose levels in normoglycemic and hyperglycemic mice and also increases glucose tolerance test. Ethanol extract of *Syzygium* leaves reduces glycosylated hemoglobin levels, lactate dehydrogenase, and creatinine kinase in alloxan-treated mice. Ethanol extract of *Syzygium* leaves also improves TBARS oxidative stress parameters, catalase and superoxide dismutase activity and glutathione levels. The ethanol extract of *Syzygium cumini* leaves shows anti-diabetic activity through increased insulin secretion and this effect can be attributed to the content of flavonoids and phenolic compounds present in the ethanol extract of *Syzygium cumini* leaves.
Keywords : Ethanol extract; *Syzygium cumin*, Antidiabetic, phytochemical

Abstrak : Penelitian ini dirancang untuk mengevaluasi aktivitas anti-diabetes dari ekstrak etanol daun *Syzygium cumini* (L) Skeels (EDS) pada mencit diabetes yang diinduksi aloksan. Aktivitas anti-diabetes EDS diselidiki pada mencit (*Mus musculus* SW.) diabetes yang diinduksi aloksan. Pengaruh ekstrak etanol daun *Syzygium cumini (EDS)* pada kadar glukosa darah normal dan uji toleransi glukosa oral dipelajari pada mencit normoglikemik sedangkan efek antidiabetik dievaluasi pada mencit hiperglikemik yang diinduksi aloksan. EDS (200 dan 400 mg/kg) diberikan secara oral selama 21 hari. Glibenclamide (5mg/kg, oral selama 21 hari) digunakan sebagai standar referensi. Pemberian EDS menyebabkan penurunan signifikan dalam kadar glukosa darah pada mencit normoglikemik dan hiperglikemik dan juga meningkatkan uji toleransi glukosa. EDS mengurangi kadar hemoglobin glikosilasi, laktat dehidrogenase, dan kreatinin kinase pada mencit yang diberi aloksan. EDS juga memperbaiki parameter stres oksidatif TBARS, aktivitas katalase dan superoksida dismutase dan

kadar glutathione. Ekstrak etanol daun *Syzygium cumini (EDS)* menunjukkan aktivitas antidiabetik melalui peningkatan sekresi insulin dan efek ini dapat dikaitkan dengan kandungan flavonoid dan senyawa fenolik yang ada dalam ekstrak daun.
Kata kunci : Ekstrak Etanol; *Syzygium cumin*, Antidiabetes, Fitokimia

Introduction

Diabetes Mellitus (DM) is a metabolic disorder characterized by loss of glucose homeostasis, as well as carbohydrate, fat, and protein metabolic disorders caused by the effects of insulin secretion, insulin action, or caused by both of these (Chevrier et al., 2016). DM is one of the metabolic disorders with micro and macrovascular complications that can produce morbidity and mortality. DM is considered as one of the five main causes of death in the world (Domingueti et al., 2016; Fan, 2017). The World Health Organization (WHO) estimates that DM is suffered by around 171 million people worldwide and the number is expected to reach 366 million by 2030 Huang et al., 2018; Zimmet, 2017).

In diabetics, hyperglycemia produces Reactive Oxygen Species (ROS) which cause lipid peroxidation and membrane damage which play an important role in producing secondary complications in DM such as disorders of the kidneys, eyes, blood vessels, and nerve damage. Antioxidants have been proven to prevent the destruction of β-cells by inhibiting peroxidation chain reactions so that they can provide protection against the development of diabetes (Volpe et al., 2018; Panigrahy et al., 2017). Studies on the DM treatment have made great progress with the presence of oral hypoglycemic agents, but research on the hunt for new drugs continues because existing synthetic drugs have harmful side effects (Labay et al., 2016). Natural herbal medicines which are claimed as anti-diabetic agents, their availability in commercial formulations are still very limited (Srivastava et al., 2019; Diningrat et al., 2020).

Syzygium cumini is known as Jamblang Aceh. It has been cultivated as a fruit plant (Hardiana et al., 2019; Sari, 2017). *Syzygium cumini* has been reported to contain phytochemicals such as polyphenols, flavonoids, ascorbic acid, tannins, alkaloids, saponins, phytosterols, diterpene, thiamine, and carotene which have bioactivity as anti-diabetic, antioxidant and anthelmintic (Munir and Qureshi 2018; Sari et al., 2018). Although the antioxidant content of *S. cumini* leaf extract is relatively high and has been used traditionally, systematic and scientific studies are still lacking in explaining the ability of its bioactivity as an anti-diabetic and its effect on oxidative stress induced by hyperglycemia.

Materials and Methods

Plant Material and Extractions

S. cumini leaves were collected during May 2019 from Aceh Besar and the surrounding areas. Jamblang leaves are dried at room temperature for 10 days, then pulverized and filtered with sieve No. 60, the collected powder is used for

extraction. The leaf powder was extracted separately using 90% ethanol by maceration method. Macerated extracts were concentrated using a rotary vacuum evaporator. The extracted paste is stored in an airtight container and its placed in a refrigerator (Sari et al., 2018; Nasution and Diningrat, 2020). The percent yield of *S. cumini* ethanol extract was 10.65% (w/w) with blackish colour.

Preliminary Phytochemical Screening: Testing of constituents of phytochemical compounds contained in *S. cumini* ethanol extract was carried out using a method known as gas chromatography-mass spectrometer analysis (GCMS) (Diningrat et al., 2019; Diningrat et al., 2020).

Chemicals

The alloxan used is produced by Sigma, St. Louis, Mo., USA. All chemicals used in this study whether for extraction, phytochemical tests, as well as toxicity tests and glucose tolerance tests, were chemicals with analytical quality (PA).

Animal test

The mice used in this study were those who had a bodyweight of 150-200 g. The mice used for this study were treated in the Unsyiah Animal House. Mice are randomly distributed into various groups and placed in propylene cages under standard laboratory conditions at a temperature of 25 ± 2ºC, the relative humidity of 50 ± 15% and a 12-hour light-dark cycle and fed on a standard commercial pellet diet in ad libitum (Istiak et al., 2018; Moghadasian et al., 2019).

Evaluation of toxicity

The toxicity study conducted in this study was an acute oral test. Tests are carried out according to acute toxic class procedures. Ethanol leaves extract of *S. cumini* at a single oral dose of 2000 mg/kg body weight was given to three mice. The toxicity effect on mice was observed continuously for 2 weeks for death and general behaviour. The test was repeated on three other mice to confirm the acute toxic class of LD50 determination (Ng'uni et al., 2018).

Oral glucose tolerance test (OGTT)

Oral glucose tolerance test is performed on normal fasting mice overnight (18 hours). Mice were divided into four groups (n= 6). Group I was considered a normal control group was receiving 1% w / v of the Tween 80 solution and Group II and Group III received ethanol extract of Syzygium cumini leaves (EDS) orally at doses of 200 and 400 mg/kg, whereas Group IV received glibenclamide (5 mg/kg). Determination of blood glucose level was determined in the following pattern: 0 minutes and 30 minutes to assess the effect of the test sample on mice with normal blood glucose. Mice were then given glucose 2g / kg orally, and then glucose levels were measured at 60, 90, 120 and 180 minutes after glucose administration. Measurement of blood glucose levels and fasting blood glucose

levels is done by taking blood samples from the tail end vein, and measurements are measured using a glucometer (Accu Check Active) (Giles-Rivas et al., 2020).

Experimental design

Diabetes induction

Induction of diabetes is done by giving alloxan. Alloxan was dissolved in a buffer (pH 4.5) which was just prepared immediately before use and given intra-peritoneally at a dose of 50 mg/kg body weight for each mouse and its blood glucose level was examined after 72 hours. Mice whose blood glucose levels are more than 250 mg / dL are considered diabetes and they are divided into five groups with each group consisting of six mice (Istiak et al., 2018).

Experimental Procedure

In the experiment, a total of 30 mice used (24 mice with diabetes, 6 normal control mice) were divided into five groups as follows:
Group I: normal control mice (treated 0.9% NaCl).
Group II: diabetes control (1% Tween 80 solution)
Group III: EDS treatment 200 mg/kg body weight.
Group IV: EDS treatment of 400 mg/kg body weight.
Group V: glibenclamide treatment 5 mg/kg body weight.

Blood samples were taken by the tail vein piercing method and liver samples were taken for biological incisions. Blood samples were taken a right before inducing diabetes and after drug administration on the 3rd, 7th, 14th and 21st days. Blood glucose levels were determined using a glucometer (Giles-Rivas et al., 2020).

Estimation of biochemical parameters: On the 22nd day, other biochemical parameters were assessed in blood/ serum. Glycosylated hemoglobin (HbA1c) was tested from blood samples by the Drabkin method (Nowak et al., 2020). The serum creatinine kinase was tested by the Tomas method. Lactate dehydrogenase (LDH) in serum is determined by the method of Wroblewski (Srivastava et al., 2019).

Pancreatic isolation is done by surgery on mice by cervical dislocation. The pancreas is collected and washed with saline phosphate solution (pH 7.4). Homogenate tissue of the pancreas (10%) was prepared in 0.15M cold ice KCl. Analysis of TBARS (thiobarbituric reactive acid) which is a marker for lipid peroxidase, catalase activity, superoxide dismutase (SOD) and glutathione content was assessed by standard methods. Protein levels in tissues were analyzed using conventional Biuret test (Picazo et al., 2017).

Statistical analysis: All data are presented as mean ± standard deviation, n=6. Differences between groups were evaluated by one-way analysis of variance (ANOVA) followed by the Dunnet test to determine statistical significance. The significance level of this study was $p < 0.05$ (Diningrat et al., 2020).

Results

Toxicity evaluation

Acute oral toxicity shows that the leaves ethanol extract of *S. cumini* did not produce significant changes in behavioral or neurological responses at a dose of 2000 mg/kg body weight until the observation period of 14 days. Acute oral toxicity studies reveal no stage of death or near death due to the leaves ethanol extract of *S. cumini*.

Effects of *S. cumini* leaves ethanol extract on oral glucose tolerance tests: The extract treatment (200 & 400 mg/kg) caused a significant decrease in normal blood glucose within 30 minutes of treatment (Table 1). Glucose level (2 g/ kg) results in a significant increase in blood glucose in normal mice. Treatment with *S. cumini* leaves ethanol extract 200 mg/kg, 400 mg/kg and Glibenclamide (5 mg/kg) showed a significant decrease in blood glucose levels over a 120 minute period compared to the normal control group as shown in Table 1. Treatment of *S. cumini* leaves ethanol extract showed a significant decrease in blood glucose levels at 120 minutes compared to controls (Table 1).

Table 1. Effect of *S. cumini* leaves ethanol extract on oral glucose tolerance at normal levels

Group	Treatment	Blood glucose levels (mg/dL)					
		0 minute	30 minutes	60 minutes	90 minutes	120 minutes	180 minutes
I	Normal Control	71.43±3.70	71.80±1.31	71.38±2.96	174.10±4.12	144.05±3.49	128.26±3.60
II	Ethanol Standards	72.54±3.17	59.21±3.90**	57.69±1.14***	143.28±5.20**	107.28±6.20**	57.38±2.12***
III	200 mg/kg	70.23±2.70	62.20±2.11**	59.48±1.96***	152.30±4.32	122.04±2.49**	80.56±2.60***
IV	400 mg/kg	71.33±5.70	59.30±1.61**	57,68±2.52***	145.10±4.12	112.05±3.49***	71.56±6.50***

Mean values ± standard deviation (n=6), * p <0.05, ** p <0.01, *** p <0.001 compared to the normal control group.

Effects of *S. cumini* leaves ethanol extract on alloxan-induced hyperglycemia

In alloxan-induced mice (50 mg/kg), blood glucose levels increased significantly from 72.2 to 288.1 mg/dl at the diabetes control level. The ethanol extract treatment which was given until the 21st day at a dose of 200 mg/kg bw and 400 mg/kg bw had reduced blood glucose levels respectively from 233.4 to 186.2 (19%) and 231.6 to 165.3 (28%) mg/dL. Meanwhile, the glibenclamide treatment which is a standard drug for diabetes, also reduced blood glucose levels from 230.8 to 151.8 (33%) mg/dl (Table 2).

Table 2. Effect of *S.Cumini* leaves ethanol extract on glucose level at diabetic level of induction of alloxan

Group	Treatment	Blood glucose levels (mg/dL) Observation Day			
		3	7	14	21
I	Normal Control	72.2±0.17	71.4±1.7	71.9±0.57	70.11±0.18
II	Ethanol Standards	232.7±1.79	263.8±1.53***	258.3±4.04***	288.1±0.22***
III	200 mg/kg	233.4±4.05	206.2±2.39***	190.8±2.08***	186.2±0.29***
IV	400 mg/kg	231.6±4.09	195.2±4.75***	183.6±5.02***	165.3± 0.82***
V	Glibenclamide (5 mg/kg)	230.8±2.54	182.4±3.32**	156.3±1.47***	151.8± 0.34***

Mean values ± standard deviation (n=6), * p <0.05, ** p <0.01, *** p <0.001 compared to the diabetes control group.

Effects of *S. cumini* leaves ethanol extract on glycosylated hemoglobin (HbA1C), Creatinine Kinase (CK) and Lactate dehydrogenase (LDH)

Alloxan has increased glycosylated hemoglobin levels along with elevated serum creatinine kinase and lactate dehydrogenase (LDH) levels in the blood. The ethanol extract treatment of Syzygium leaves on alloxan-induced mice in both doses of 200 and 400 mg/kg reduced glycosylated hemoglobin levels and decreased CK and LDH activity. Likewise, the glibenclamide treatment showed a significant decrease in blood HbA1C levels, CK and LDH activity when compared with group II (control) (Table 3).

Table 3. Effect of *S. cumini* leaves ethanol extract on HbA1C, CK serum, LDH serum

Group	Treatment	*Whole blood HbA1C (%)*	*Serum Creatinine Kinase (CK),(IU/L)*	*Serum LDH (IU/L)*
I	Normal Control	4.12 ± 0.22	59.14 ± 2.88	180.12 ± 5.40
II	Ethanol Standards	13.33 ± 0.32***	141.32 ± 2.91**	305.44± 7.11***
III	200 mg/kg	8.20 ± 0.34**	120.18 ± 0.65**	251.31 ± 8.23**
IV	400 mg/kg	6.54 ± 0.27***	110.18± 0.64***	243.21± 8.30***
V	Glibenclamide (5 mg/kg)	4.17 ± 0.43***	78.62 ± 2.67***	221.76± 9.34***

Mean values ± standard deviation (n = 6), * p <0.05, ** p <0.01, *** p <0.001 compared to the diabetes control group.

Effects of *S. cumini* leaves ethanol extract on oxidative stress parameters

Alloxan has also increased TBARS formation along with decreased levels of CAT, SOD and glutathione. The treatment of *S. cumini* leaves ethanol extract in both doses reduced TBARS levels but increased CAT, SOD activity and glutathione content. The glibenclamide treatment showed a significant decrease (p

<0.01) which was similar in TBARS levels with an increase in CAT, SOD activity and glutathione content when compared with group II (Table 4).

Table 4. Effect of *S. cumini* leaves ethanol extract on oxidative stress parameters

Group	Treatment	TBARS (nmol MDA/mg protein)	CAT (nmol H_2O_2-consumed/ min/mg protein)	SOD (IU/mg protein)	GSH (level of phosphorous liberated/ min/mg protein)
I	Normal Control	0.389±0.037	3.33±0.089	2.59±0.069	42.4±0.945
II	Ethanol Standards	3.521±0.562***	0.59±0.020***	0.132±0.025***	9.58±1.034***
III	200 mg/kg	1.813±0.451*	1.19±0.132**	2.34±0.272**	31.24±1.116***
IV	400 mg/kg	0.623±0.011***	1.71±0.070**	2.62±0.091***	31.12±1.129***
V	Glibenclamide (5 mg/kg)	0.123±0.011***	2.19±0.156***	2.48±0.087***	32.65±1.651***

Mean values ± standard deviation (n = 6), * p <0.05, ** p <0.01, *** p <0.001 compared to the diabetes control group.

Discussion

This research has shown anti-diabetic activity of *S. cumini* leaves ethanol extract in alloxan-induced diabetes and oxidative stress. The alloxan treatment has resulted in the destruction of β cells after three days and reaches a peak at three to four weeks in mice (II). B cells are very sensitive to damage caused by nitric oxide and free radicals because of their low levels of free radical scavenging enzymes (Picazo et al., 2017; Sari, 2017). Improved glycemic control in oral glucose tolerance tests by Syzygium leaf ethanol extract showed that the extract also reduced blood glucose levels even in normal mice. The effect of reducing blood glucose levels in normal mice may be due to an increase in the efficiency of peripheral tissue for the absorption of glucose from the blood. Thus the extract can also be beneficial in patients with type II diabetes (Giles-Rivas et al., 2020; Nowak et al., 2020).

In this study, the experimental results showed significant anti-diabetic and antioxidant activity of *S. cumini* leaves ethanol extract at concentrations of 200 & 400 mg/kg bw. This study has been able to explore the potential of *S. cumini* leaves ethanol extract for the treatment of diabetes and related complications such as oxidant stress to prove the traditional claim that Jamblang leaves can be used for the diabetes treatment (Diningrat et al., 2020; Panigrahy et al., 2017). This study shows that an increase in glucose levels was successfully controlled by *S. cumini* leaves ethanol extract, this is indicated by a decrease in glycosylated hemoglobin levels commonly used as a control indicator of diabetes where glycohemoglobin levels approach normal values in diabetics in metabolic control (Volpe et al., 2018; Zimmet et al., 2017).

The *S. cumini* leaves ethanol extract treatment which was treated in mice at a dose of 200 mg/kg and 400 mg/kg showed a decrease in LDH and creatinine levels in serum when compared with the diabetes control group. Alloxan-induced oxidative stress in diabetics is also a predictor of heart damage. Because LDH and CK are specific markers of cardiac damage, elevated serum LDH and CK levels are considered markers of heart damage due to oxidative stress (Volpe et al., 201; Fan et al., 2017). Alloxan-induced diabetes mice are associated with hyperlipidemia and elevated serum creatinine levels (Chevrier et al., 2016; Domingueti et al., 2016). Reduction of LDH and creatinine in the serum of mice given *S. cumini* leaves ethanol extract is considered important as a manifestation of decreased blood glucose levels.

Increased lipid peroxidase levels are indicative of a decrease in the enzymatic antioxidant defense mechanism (Sasongko, 2015). Several studies have shown that oxygen free radicals are produced under conditions of diabetes, and a reduction in the activity of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) also contribute to the development of oxidative stress in Diabetes Mellitus (Sari et al. 2017). In this study, it was observed that the ethanol extract of Syzygium leaves increased SOD, CAT activity and GSH content in pancreatic tissue of diabetic mice. This shows the mechanism of repairing oxidative damage carried out by the *S. cumini* leaves ethanol extract in diabetic mice. The increased SOD and CAT activity in diabetic mice can be attributed to the strong antioxidant properties of the *S. cumini* leaves ethanol extract.

The anti-diabetic potential of *S. cumini* leaves ethanol extract is caused by the presence of secondary metabolites (flavonoids, alkaloids, phenolics, glycosides, and terpenes) contained in *Syzygium cumini* (Sari et al., 2018; Diningrat et al., 2020). These secondary metabolites are reported to have different anti-diabetic potential and are responsible for the observed anti-diabetic effects (Sari et al., 2018).

Conclusions

Ethanol extract of *Syzygium cumini* leaves ethanol extract has shown an anti-diabetic effect on diabetes. The anti-diabetic effect is demonstrated by the ability of *Syzygium cumini* leaves ethanol extract to repair oxidative damage at pancreas as indicated by a decrease in blood glucose levels and levels of oxidative stress. This effect can be attributed to the presence of antioxidant compounds contained in *Syzygium cumini* leaves. Further mechanistic studies will be needed to look at the mechanism of each antioxidant compound suitable for the anti-diabetic effect of *Syzygium cumini*.

Acknowledgement

We would like to thank the Biology Study Program Faculty of Science and Technology of UIN Ar-Raniry, LPPM UIN Ar-Raniry, LPPM Medan State University, and DRPM Kemenristekdikti.

Conflicts of interest

The authors of this article stated there were no conflicts of interest in this study.

References

Chevrier, G., Mitchell, P., Beaudoin, M. S., & Marette, A. (2016). Impact of Dietary Proteins on Energy Balance, Insulin Sensitivity and Glucose Homeostasis: From Proteins to Peptides to Amino Acids. In *The Molecular Nutrition of Amino Acids and Proteins* (pp. 241-264). Academic Press. https://doi.org/10.1016/B978-0-12-802167-5.00018-9

Diningrat, D. S., Risfandi, M., Harahap, N. S., Sari, A. N., & Siregar, H. K. (2020). Phytochemical Screening and Antibacterial Activity Coix lacryma-jobi Oil. *Journal of Plant Biotechnology*, *47*(1), 100-106. https://doi.org/10.5010/JPB.2020.47.1.100

Domingueti, C. P., Dusse, L. M. S. A., das Graças Carvalho, M., de Sousa, L. P., Gomes, K. B., & Fernandes, A. P. (2016). Diabetes mellitus: the linkage between oxidative stress, inflammation, hypercoagulability and vascular complications. *Journal of Diabetes and its Complications*, *30*(4), 738-745. https://doi.org/10.1016/j.jdiacomp.2015.12.018

Fan, W. (2017). Epidemiology in diabetes mellitus and cardiovascular disease. *Cardiovascular endocrinology*, *6*(1), 8. https://dx.doi.org/10.1097%2FXCE.0000000000000116

Giles-Rivas, D., Estrada-Soto, S., Aguilar-Guadarrama, A. B., Almanza-Pérez, J., García-Jiménez, S., Colín-Lozano, B., ... & Villalobos-Molina, R. (2020). Antidiabetic effect of Cordia morelosana, chemical and pharmacological studies. *Journal of Ethnopharmacology*, 112543. https://doi.org/10.1016/j.jep.2020.112543

Hardiana, H., Rasnovi, S., & Zumaidar, Z. (2019). Use of plants as traditional medicine in swamedication in Pidie communities. *Jurnal Natural*, *19*(2), 35-41. https://doi.org/10.24815/jn.v19i2.13192

Huang, H., Ma, H., van Triest, H. J., Wei, Y., & Qian, W. (2018). Automatic detection of neovascularization in retinal images using extreme learning machine. *Neurocomputing*, *277*, 218-227. https://doi.org/10.1016/j.neucom.2018.04.065

Istiak, A., Hazra, P., Hossain, M. I., Aminatu, A. S., & Rafiq, K. (2018). Hypoglycemic, hypolipidemic and kidney protective potential of combined formulation of Tribulus terrestris and Andrographis paniculata in alloxan

induced mice. *African Journal of Pharmacy and Pharmacology*, *12*(21), 269-277.DOI: 10.5897/AJPP2018.4928

Labay, L. M., Caruso, J. L., Gilson, T. P., Phipps, R. J., Knight, L. D., Lemos, N. P., ... & Williams, E. (2016). Synthetic cannabinoid drug use as a cause or contributory cause of death. *Forensic science international*, *260*, 31-39. https://doi.org/10.1016/j.forsciint.2015.12.046

Moghadasian, M. H., Masisi, K., Le, K., Beta, T., Shen, G. X., & Fischer, G. (2019). The Potential Anti-Diabetic Effects of Saskatoon Berry in Experimental Mouse Models. *Austin J Nutri Food Sci*, *7*(1), 1111. https://austinpublishinggroup.com/nutrition-food-sciences/fulltext/ajnfs-v7-id1111.php

Munir, M., & Qureshi, R. (2018). Anti-diabetic plants of Pakistan. In *Plant and Human Health, Volume 1* (pp. 463-545). Springer, Cham. https://doi.org/10.1007/978-3-319-93997-1_12

Ng'uni, T., Klaasen, J. A., & Fielding, B. C. (2018). Acute toxicity studies of the South African medicinal plant Galenia africana. *Toxicology reports*, *5*, 813-818. https://doi.org/10.1016/j.toxrep.2018.08.008

Nowak, K., Zawadzki, M., & Jurek, T. (2020). Postmortem determination of HbA1c and glycated albumin concentrations using the UHPLC-QqQ-MS/MS method for the purposes of medicolegal opinions. *Microchemical Journal*, *155*, 104733. https://doi.org/10.1016/j.microc.2020.104733

Panigrahy, S. K., Bhatt, R., & Kumar, A. (2017). Reactive oxygen species: sources, consequences and targeted therapy in type 2 diabetes. *Journal of drug targeting*, *25*(2), 93-101. https://doi.org/10.1080/1061186X.2016.1207650

Picazo, A., Jiménez-Osorio, A. S., Zúñiga-Mejía, P., Pedraza-Chaverri, J., Monroy, A., Rodriguez-Arellano, M. E., & Barrera-Oviedo, D. (2017). Hypoglycemic drugs induce antioxidant aldehyde dehydrogenase activity and remain high in patients with glycemic control in type 2 diabetes. *European journal of pharmacology*, *800*, 57-62. https://doi.org/10.1016/j.ejphar.2017.02.027

Sari, A. N. (2017). Potensi Antioksidan Alami Pada Ekstrak Daun Jamblang (*Syzigium Cumini (L.) Skeels*). *EKSAKTA: Berkala Ilmiah Bidang MIPA*, *18*(02), 107-112. https://doi.org/10.24036/eksakta/vol18-iss02/61

Sari, A. N., Kusdianti, K., & Diningrat, D. S. (2018). Analisis GC-MS Senyawa Bioaktif Pencegah Penyakit Degeneratif Dari Ekstrak Etanol Kulit Buah Jamblang (*Syzygium Cumini*). *Elkawnie*, *4*(2), 101-114. http://dx.doi.org/10.22373/ekw.v4i2.4143

Srivastava, A., Srivastava, P., Pandey, A., Khanna, V. K., & Pant, A. B. (2019). Phytomedicine: A Potential Alternative Medicine in Controlling Neurological Disorders. In *New Look to Phytomedicine* (pp. 625-655). Academic Press. https://doi.org/10.1016/B978-0-12-814619-4.00025-2

Volpe, C. M. O., Villar-Delfino, P. H., dos Anjos, P. M. F., & Nogueira-Machado, J. A. (2018). Cellular death, reactive oxygen species (ROS) and diabetic complications. *Cell death & disease*, *9*(2), 1-9. DOI 10.1038/s41419-017-0135-z

Zimmet, P. Z. (2017). Diabetes and its drivers: the largest epidemic in human history?. *Clinical diabetes and endocrinology*, *3*(1), 1. https://doi.org/10.1186/s40842-016-0039-3