RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v56.3496
Majalah Kedokteran Bandung. 2024;56(3):190–196

Majalah Kedokteran Bandung (MKB)

Received: August 2, 2023
Accepted: January 17, 2024
Available online: September 30, 2024

Nephroprotective Effects of Sunkist Peel Ethanol Extract on Diabetic Nephropathy

Maya Sari Mutia, Chrismis Novalinda Ginting, OK Yulizal, Suhartomi, Widya Yanti Sihotang
Faculty of Medicine, Dentistry and Health Science, Universitas Prima Indonesia, Medan, Indonesia

Abstract

The most common long-term diabetic complication is diabetic nephropathy which, in most cases, falls into the End-Stage Kidney Disease. Numerous studies on the nephroprotective effects of citrus fruit have been conducted recently. This study was performed to investigate the phytochemical properties of Sunkist peel ethanol extract and their possible effects on improving diabetic nephropathy. To get the extract, the Sunkist peels were macerated and the extract was analyzed by GC-MS. An in vivo assay of five different groups of 25 male Wistar rats was then performed using the extracts. The groups included control, standard, 500 mg/kg extract, 750 mg/kg extract, and 1,500 mg/kgBW extract. All rats were sacrificed after 28 days of treatment and VEGF level serum and kidney histology analysis were performed. The VEGF data were first analyzed descriptively, followed by one-way ANOVA or Kruskal-Wallis test based on the data distribution. Meanwhile, the kidney histology was observed and narratively described. Results showed that the Sunkist peel ethanol extract has a potential effect to suppress the VEGF level (p-value<0.05). The extract at the highest dose revealed the mildest pathology damage in the form of tubular structure degeneration without any glomerular damage. This study indicates that Sunkist peel extract has nephroprotective effects from its various phytochemical compounds that work as antioxidants and anti-inflammation.

Keywords: Diabetic, nephropathy, Sunkist peel, vascular endothelial growth factor

Introduction

Indonesia was reported as the 7th highest diabetic patient in 2019 by the International Diabetes Federation (IDF) after China, India, the United States, Brazil, Russia, and Mexico. Moreover, Saeedi et al. also estimated the prevalence of diabetes for 2030 and 2045 from IDF Atlas 9th edition. The estimated global population with diabetes mellitus is 9.3% aged 20–79 years in 2019, and it was predicted to rise to 10.2% by 2030 and 10.9% by 2045. Hence, it is evident that diabetic Mellitus is a chronic disease in some countries all over the world.[1,2]

Diabetes mellitus is a chronic metabolic disease that involves insulin dysfunction and impacts the blood glucose level. Prolonged high blood glucose levels may cause either microvascular or macrovascular complications. One of these microvascular is nephropathy.[3] The most common long-term diabetic complication was diabetic nephropathy, and most of these cases fall into End-Stage Kidney Disease (ESKD).[4] Ironically, around 50% of ESKD was found in some developing countries. Due to this reason, diabetic nephropathy is an essential health problem, so it has become important to develop various treatment modalities to prevent the complications of diabetes, especially diabetic nephropathy.[5]

Some studies have been performed to investigate various herbs for diabetic treatment. However, a limited number of studies looked for the nephroprotective effect of herbs against diabetic nephropathy. One of these herb that have been investigated for antidiabetic was Sunkist peels. Sunkist from the *Citrus* family reported various phytochemical contents like ascorbic acid, flavonoid, phenolic compounds, and pectin. Moreover, Suhartomi et al reported that *Citrus sinensis,* either as the crude extract or fraction, had numerous flavonoid contents that had either DPPH or Hydrogen peroxide scavenging activity as antioxidant properties.[6] Total Flavonoids in *Citrus sinensis* Ethanol and Ethyl acetate extract was 205.66±49.63 mg QE/gr. DW and 242.48±13.83 mg QE/gr. DW,

Corresponding Author:
Maya Sari Mutia
Faculty of Medicine, Dentistry and Health Science, Universitas Prima Indonesia, Medan, Indonesia
Email: mayasarimutia@unprimdn.ac.id

respectively. Depari et al reported that the Sunkist peel extract have antidiabetic activity and improve the hypercholesterolemia state in streptozotocin-induced diabetic rats. [7] Moreover, Mutia et al reported an advanced effect of Sunkist ethanol peels to extract other than antidiabetic which is the nephroprotective effect of the Sunkist peels ethanol extract against diabetic nephropathy by decreasing Blood Urea Nitrogen (BUN) and creatinine levels of Streptozotocin-Induced diabetic rats. [8,9]

Based on these previous studies, none of these studies looked for the nephroprotective effect and exact mechanism of action from ethanol extract as a nephroprotective effect against the nephropathy diabetic. It is hypothesized that the peel of Sunkist suppresses inflammation and oxidative stress associated with diabetic neuropathy through its antidiabetic, anti-inflammatory, and antioxidant effects. This study was conducted to analyze the phytochemical properties of an ethanol extract of Sunkist peels and to assess its potential for improving diabetic nephropathy in male Wistar rats through an animal trial.

Methods

The research utilized an experimental study with a post-test control group design, conducted in the Pharmacology Laboratory of Universitas Prima Indonesia in June 2021. The study procedures were approved by the Health Research Ethics Committee under letter no. 020/KEPK/UNPRI/II/2021. Materials used in the study included Sunkist peel, ethanol, sodium carboxymethyl cellulose, distilled water, streptozotocin, rat pellets, phytochemical screening reagents, metformin tablets, chloroform, 10% buffered formalin solution (BFS), normal saline, xylol, concentrated alcohol, hematoxylin and eosin stain powder, blood lancets, and a VEGF ELISA Kit.

The fruit of Sunkist were collected from the fruit market in Medan and the selection was based on the colour and quality of the peel. The identified Sunkist fruit was peeled to obtain the fruit peels extracted by maceration methods. 500 g Sunkist peel washed and dried to form a dry simplicial. After that, it was soaked in ethanol in a ratio of 1: 10 (simplicial: solvent) for five days and regularly stirred, then it was filtrated, and the residue was re-macerated two times for five days and evaporated at 70°C. Hence, it formed a concentrated form of extract.[8]

The evaluation of phytochemical compounds such as alkaloid, tannin, saponin, triterpenoid, steroid, flavonoid, glycone, aglycone, anthraquinone, and polyphenol was done by GC-MS spectroscopic detection with 70 eV ionization energy.[10] The amount of 0.5-gram sodium carboxymethyl cellulose was separated into the surface of warm distilled water (50 ml). After this mixture formed a clear semi-solid preparation, it was ground until it became homogenous. Then, the remaining distilled water (50 ml) was poured and stirred into this mixture, forming 0.5% Sodium carboxymethyl cellulose. Additionally, 1 gram of the concentrated extract was suspended in 0.5% sodium carboxymethyl cellulose to create an oral extract suspension with a concentration of 10 mg/mL. On the other hand, 0.5% Sodium carboxymethyl cellulose was used to make an oral metformin suspension by suspending 100 mg meshed metformin tablet into 5 ml 0.5% Sodium carboxymethyl cellulose, and it formed an oral suspension with a concentration of 20 mg/mL. All rats were induced by a single intraperitoneal injection of Streptozotocin solution at a dose of 50 mg/ kg BW. The streptozotocin powder was dissolved into 0.1 M citrate buffer (pH=4.5). However, before the injection, all rats were measured fasting blood glucose levels by a glucometer (10-12 hours fasting) that used a blood sample from a vein in the tail, and the fasting blood glucose level was also measured three days after the injection.[11] All rats with blood glucose levels higher than 200 mg/dL were selected as diabetic rats.[12] These diabetic rats were grouped into five groups: control, standard, intervention of Sunkist peels ethanol extract I, II, and III. The Control group received 1 ml 0.5% sodium carboxymethyl cellulose, and the standard group received metformin as a standard drug. Meanwhile, Sunkist peels ethanol extract I, II, and III groups received 500 mg/kgBW (0.5 mL/kgBW), 750 mg/kgBW (0.75 mL/ kgBW), and 1,500 mg/kg BW (1.5 mL/ kgBW) of Sunkist peels ethanol extract, respectively. It was given once a day in every morning for 28 days.[9,13]After 28 days, all rats were sacrificed by chloroform inhalation in a closed room. After that, all rats were fixed into paraffin blocks, and the abdomen wall was incised vertically to expose the organ in the abdomen cavity. Then, the kidney was dissected and washed into normal saline. Moreover, the washed kidney organ was kept in 10% buffer formalin solution (BFS) until it was processed to stain. The incision was also performed in the thorax of the rat for blood withdrawal. The blood

was collected by cardiac puncture using a 5 ml syringe and 25-G Needle. The blood was saved in red-coloured blood tube and centrifuged at 2,500 RPM for 10 minutes. After the centrifugation, the blood was separated, and the upper layer was collected into the microtube to undergo VEGF Serum measurement.[7,14] The obtained kidney tissue was sliced with a thick of 4–6 mm, and then it was dehydrated by alcohol in various concentrations (70%, 80%, 90%, and 95%) for 24 hours and in concentrated alcohol (100%) for an hour with three repetitions. The dehydrated tissue was washed with xylol for an hour with three repetitions. Moreover, it was washed with xylol for an hour with three repetitions. Then, it was infiltrated into a paraffin block to be dissected with a thickness of 4–5 µm. Finally, it was put into the object glass and stained with Haematoxylin-Eosin (HE). The damage to the kidneys was assessed based on changes in the histopathological structure, including tubular degeneration, albuminous degeneration, and tubular cell necrosis. In addition to kidney analysis, serum was also examined. The serum was analyzed to measure VEGF levels using the Enzyme-Linked Immunoassay (ELISA) method with an R&D Systems (Minneapolis, MN, USA) kit, and results were expressed in pg/ml.

The VEGF serum levels were analyzed using both descriptive and inferential statistics. Data distribution was first assessed using the Shapiro-Wilk test. If the data were normally distributed, one-way ANOVA was used for analysis; otherwise, Kruskal-Walli's analysis was performed. Data are presented as mean ± standard deviation, with statistical significance set at $p < 0.05$.

Results

The study identified that Sunkist as Kingdom Plantae, Division Spermatophyta, Class Dicotyledoneae, Ordo Sapindales, Family Rutaceae, Genus Citrus Species, Citrus sinensis (L.) Osbeck. Based on the result of identification, this study used Sunkist Peels with the scientific name of Citrus sinensis (L.) Based on the identification results, this study used Sunkist peels, scientifically known as Citrus sinensis (L.) Osbeck, which belongs to the Rutaceae family. Maceration was employed to extract the peels. The resulting extract contained various phytochemicals, including tannins, saponins, flavonoids, triterpenoids, steroids, and polyphenols.

Table 1 showed that there were ten main compounds, that were identified from GC-MS analysis of Sunkist peel Ethanol extract. These compounds were 2-Bromoethanol (1.82%) 4H-Pyran-4-One,2,3-Dihydro-3,5-Dihyroxyl-6-Methyl (4.43%), 2-Furancarboxaldehyde-5-(Hydromethyl)-(15.58%),2-Methoxy-4-Vinylphenol (1.84%), Alpha-Aminooxy-Propionic acid, ethyl ester (1.72%), Ethyl-α-d-glucopyranoside (20.61%), 3-Deoxy-d-Mannonic Acid (25.10%), Allo-Inositol (23.84%), Methyl [Methyl 5-Acetamido-7-O-Acetyl- 3,4,5-Trideocy-4-C-Methyl-8,9-O-(Methylethylidene)-

Table 1 GC-MS Analysis of the Extract

RT	Quality	Compounds	Concentration (%)
5.393	38	2-Bromoethanol	1.82
7.221	94	4H-Pyran-4-One, 2, 3-Dihydro-3,5-Dihyroxyl-6-Methyl	4.43
10.972	87	2-Furancarboxaldehyde-5-(Hydromethyl)-	15.58
11.758	49	2-Methoxy-4-Vinylphenol	1.84
22.804	46	Alpha-Aminooxy-Propionic acid, ethyl ester	1.72
27.838	76	Ethyl-α-d-glucopyranoside	20.61
28.927	64	3-Deoxy-d-Mannonic Acid	25.10
31.051	47	Allo-Inositol	23.84
39.794	47	Methyl [Methyl 5-Acetamido-7-O-Acetyl-3,4,5-Trideocy-4-C-Methyl-8,9-O-(Methylethylidene)-β-D-Glycerol-D-Glycerol-D-Galacto-2-Nonulopyranosid] Onate	1.47
40.284	78	2-(3,4-Dimethoxyphenyl)-5,6,7,8-Tetramethoxy-4H-1-Benzopyran-4-One	2.46

*RT: Retention Time

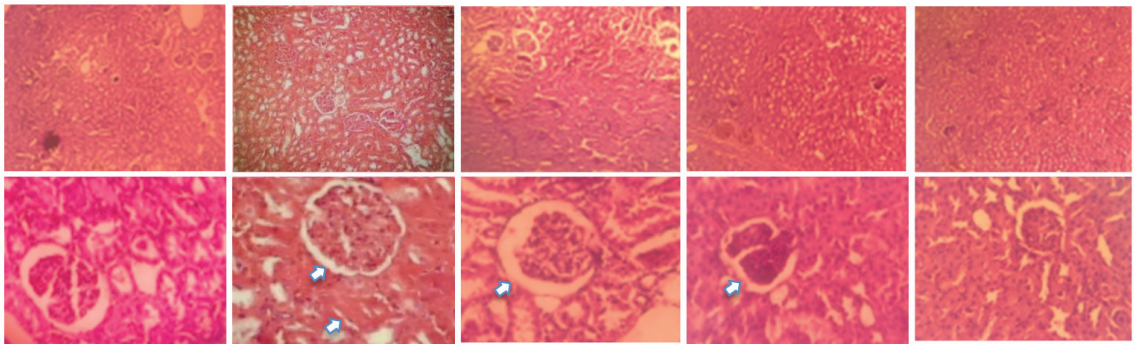

Figure 1 Histology of Kidney. Stain: Hematoxylin & Eosin (HE). Magnification: 10x (Upper) and 40x (Lower)

D-Glycerol-D-Glycerol-D-Galacto-2-Nonulopyranoside] Onate (1.47%), and 2-(3,4-Dimethoxyphenyl) -5,6,7,8- Tetramethoxy-4H-1-Benzopyran-4- One (2.46%).

The Sunkist peel extract was utilized for in vivo assays to assess its nephroprotective effects in a diabetic nephropathy rat model. The in vivo assays included histological examination of kidney tissues and measurement of VEGF levels to evaluate the nephroprotective impact of the Sunkist peel ethanol extract. The results of the histological analysis of kidney tissue are presented in Figure 1.

Based on Figure 1, it is obvious that the normal structure of the kidney was observed in the standard group, without any damage in pathologic. Serious damage was found in the control group. It showed a wide necrosis in the tubular structure with the foci of hemorrhage. Meanwhile, the other groups that received various doses of Sunkist peel extract also revealed various degrees of kidney tissue damage. At the lowest dose (Sunkist Peel Ethanol Extract-I), the kidney showed a glomerulus shrinkage and vacuolated tubular tissue. At the higher dose (Sunkist Peel Ethanol Extract-II), the kidney tissue revealed no vacuolated tubular structure like in the lowest dose group. However, the shrinkage of the glomerular structure was still found in the lowest dose group. At last, the highest dose group revealed less kidney pathology damage, which only revealed degeneration of tubular structure without any glomerular damage. Furthermore, this study not only performed a histology study against the kidney tissue but also evaluated VEGF level as the inflammatory marker to support the histology study against the kidney tissue.

The inflammatory marker VEGF level was assessed to evaluate the severity of microvascular damage, including diabetic nephropathy, and to complement the histological study. Initially, the data distribution of VEGF levels was analyzed using the Shapiro-Wilk test, which revealed that the VEGF levels in the control and Sunkist peel ethanol extract-III groups were not normally distributed (p-value<0.05). Consequently, data analysis was continued with the Kruskal-Wallis test.

The VEGF levels revealed that there was a significant difference between the groups (p-value<0.05. Moreover, it can be seen that the increased dose of Sunkist peels extracts significantly followed by the increase of VEGF level. The highest VEGF level found in the control group was 275.57 mg/kg, and the lowest was

Table 2 VEGF Levels of All Treatment Groups

Groups	VEGF Level (mg/kg)	p-value
Control	275.57 (274.56–299.10)	
Standard	265.50 (235.55–291.03)	
Extract-I	245.62 (235.21–246.99)	<0.05
Extract-II	187.90 (159.17–195.75)	
Extract-III	127.58 (111.34–185.60)	

found in the Sunkist Peel Ethanol Extract-III, that was 127.58 mg/kg.

Discussion

This study answered the aims of investigating the nephroprotective effect of Sunkist peel extract against the diabetic nephropathy rat model. The Sunkist peels may protect the macroscopic structure of the kidney from diabetic nephropathy. The improvement histology view from kidney tissue among the rats that received the Sunkist peels extract can be seen from the improvement histology view. This improvement was also followed by decreases in VEGF levels, which act as inflammatory markers in this study. Furthermore, it showed the increase of Sunkist Peels Ethanol Extract also increased the nephroprotective effect against the diabetic nephropathy that Streptozotocin induced.

Streptozotocin is a cytotoxic glucose analog compound that may inhibit DNA synthesis in bacterial and mammalian cells. A prolonged hyperglycaemic state or uncontrolled diabetes may cause significant kidney damage. This damage can trigger abnormalities in kidney function and histological structure, including accumulation of extracellular matrix, thickening of the glomerular membrane, and glomerular sclerosis.[16,17] Kidney damage was also assessed based on the degeneration of tubular structures. Streptozotocin induces kidney damage, leading to degeneration and necrosis of both proximal and distal tubular epithelium.[18] Diabetic nephropathy can cause several pathology and clinical changes. These changes include thickening of the glomerular basement membrane (GBM), diffuse or nodular glomerulosclerosis, arteriolar hyalinosis, tubulointerstitial fibrosis, and increased albumin excretion in the urine (or albuminuria). Albuminuria can occur due to the increased permeability of the glomerular filtration barrier to albumin and various protein plasma.[5,19] This change in permeability may be characterized by VEGF, a potent inducer of microvascular permeability. In the diabetic state, upregulation of VEGF and/or its signaling activity in the glomerulus can lead to albumin leakage from the glomerular filtration barrier.Moreover, VEGF also causes nitric oxide-mediated vasodilation and contributes to albuminuria in diabetic settings. Thus, it was obvious that a decrease in VEGF Level followed the increase of the Sunkist Peels ethanol extract, which means the increase of Sunkist peel ethanol extract may reduce the severity of albuminuria and improve the kidney function in the nephropathy diabetic.[18,20]. The previous study with the same methods, the previous study evaluated different parameters that were BUN and Creatinine levels. The previous study demonstrates that the Sunkist peels extract may protect rat kidneys from diabetic nephropathy.[9]

The nephroprotective effect of Sunkist peel ethanol extract was associated with the presence of the various phenolic compounds including phenol, flavonoid, and tannin. The previous study found the total phenolic, tannin, and flavonoid content were 9.89±0.19 mg GAE/ g extract, 44.27±0.29 mg TAE/ g extract, and 3.83±0.00 mg QE/ g extract. Another study performed by Gulo et al. with two different solvents also reported that the total flavonoid content from sweet orange peel ethanol and ethyl acetate was 205.66±49.63 and 242.48±13.83 mg QE/ gr DW, respectively. However, the previous study only reported the total phenolic compounds contents but did not report the obvious content of these compounds. Thus, the recent study continued to analysed these compounds by GC-MS analysis and the GC-MS detected some phenolic compounds like 2-Bromoethanol (1.82%) 4H-Pyran-4-One, 2,3-Dihydro-3,5-Dihyroxyl-6-Methyl (4.43%), 2-Furancarboxaldehyde-5-(Hydromethyl)-(15.58%), 2-Methoxy-4-Vinylphenol (1.84%), Alpha-Aminooxy-Propionic acid, ethyl ester (1.72%), Ethyl-α-d-glucopyranoside (20.61%), 3-Deoxy-d-Mannonic Acid (25.10%), Allo-Inositol (23.84%), Methyl [Methyl 5- Acetamido- 7- O-Acetyl- 3, 4, 5-Trideocy- 4-C-Methyl-8,9-O-(Methylethylidene)-D-Glycerol-D- Glycerol-D-Galacto-2-Nonulopyranoside] Onate (1.47%), and 2-(3,4-Dimethoxyphenyl)-5,6,7,8-Tetramethoxy- 4H-1-Benzopyran-4-One (2.46%). [6,21]

The results of the GC-MS analysis in the current study are consistent with those reported by Ahmed et al.,[22] who found that the hydroethanolic extract of navel orange peels contains various phytochemicals with potent biological activities. These compounds included 4H-Pyran-4-One, Thymine, 5-Hydroxymethylfurfural and 4-hexen-3-one,4,5-dimethyl-, Dodecane, 2-Methoxy-4-vinylphenol, 3-methoxyacetophenone, 9,12-Octadecadienoic acid, 3-methoxyacetophenone, Oleic acid, 9-octadecenoic acid, Lupanine, Stigmasterol, and 9-Octadecenamide. Moreover, this study also described the potential biological activities of these compounds. Other compounds that

also found in the Sunkist peels extract in the recent study were 2-Methoxy-4-Vinylphenol and 4H-Pyran-4-One. 4H-Pyran-4-One, detected at a retention time of 7.221 and a concentration of 4.43%, it has various biological activities, including hepatoprotective effect, anti-oxidant, anti-bacterial, antiviral, anti-cancer, and anti-inflammatory effects. In the recent study, 4H-Pyran-4-One was found as the 4H-Pyran-4-One, 2, 3-Dihydro-3,5-Dihyroxyl-6-Methyl formed a complex compound with the 2,3-Dihydro-3,5- Dihyroxyl-6-Methyl and this compound was indicated have a potent free radical scavenging activity. Another compound found in the extract was 2-Methoxy-4-vinylphenol, which was detected at a retention time of 11.758 and a concentration of 1.84%. This compound is known to exhibit several biological activities, including antioxidant, antimicrobial, and anti-inflammatory effects. Additionally, a study conducted by Suhartomi et al. demonstrated that various fractions of orange peels, including methanol, n-hexane, and dichloromethane, possess antioxidant activity, specifically hydrogen peroxide and DPPH scavenging activities. Therefore, these compounds contribute to the nephroprotective effects of Sunkist peel ethanol extract through their antioxidant and anti-inflammatory properties. [6,22]

Overall, the findings indicate that Sunkist peel extract has potential nephroprotective effects against diabetic nephropathy, primarily through its antioxidant and anti-inflammatory properties. This results in reduced injury to kidney tubular cells, as evidenced by a decrease in the average severity of renal tubular degeneration and necrosis. However, the study has limitations; specifically, it did not measure the Glomerular Filtration Rate (GFR). Future research should include GFR assessment to better evaluate the impact of Sunkist peel extract on the physiological functions of the glomeruli.

References

1. Zhou B, Carrillo-Larco RM, Danaei G, et al. Worldwide trends in hypertension prevalence and progress in treatment and control from 1990 to 2019: a pooled analysis of 1201 population-representative studies with 104 million participants. Lancet. 2021;398(10304):957–80. doi:10.1016/S0140-6736(21)01330-1
2. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res Clin Pract. 2019;157:107843. doi:10.1016/j.diabres.2019.107843
3. Syafril S. Pathophysiology diabetic foot ulcer. IOP Conf Ser Earth Environ Sci. 2018;125(1):012161. doi:10.1088/1755-1315/125/1/012161
4. Selby NM, Taal MW. An updated overview of diabetic nephropathy: diagnosis, prognosis, treatment goals and latest guidelines. Diabetes, Obes Metab. 2020;22(S1):3–15. doi:10.1111/dom.14007
5. Sagoo MK, Gnudi L. Diabetic nephropathy: an overview. Diabet Nephrop Methods Protoc. Published online 2020:3-7.
6. Suhartomi, Gulo KN, Saragih AD, Martinus AR, Ikhtiari R. Antioxidant properties of sweet orange peels in several fractions of methanolic extract. ICHIMAT. Scitepress Digital Library; 2020:371–78. doi:10.5220/0009515503710378
7. Depari SAF, Rambe DJA, Meilando R, Lisya C, Mutia MS, Lubis YEP. Uji efektivitas ekstrak etanol kulit jeruk sunkist (citrus sinensis (L.) Osbeck) terhadap kadar gula darah tikus wistar (rattus norvegicus) dengan hiperkolesterolemiai yang diinduksi Streptozotocin. Biospecies. 2021;14(1):1–9.
8. Mutia MS, Chiuman L. Hepatoprotective Effect of citrus sinensis (L.) osbeck ethanol extract in paracetamol-induced rats. Majalah Kedokteran Bandung. 2019;51(4):189–93. doi:10.15395/mkb.v51n4.1794
9. Sari M, Ginting CN, Yulizal O. Sunkist peel ethanol extract ameliorates diabetic nephropathy in streptozotocin-induced diabetic wistar rats. Open Access Maced J Med Sci. 2021;9(A):1086–91. doi:10.3889/oamjms.2021.7362
10. Sabina E, Zaidul ISM, Ghafoor K, Jaffri JM, Sahena F, Babiker EE, et al. Screening of Various Parts of Phaleria macrocarpa Plant for α-Glucosidase Inhibitory Activity. J Food Biochem. 2016;40(2):201–10. doi:10.1111/jfbc.12212
11. Bayir MH, Yıldızhan K, Altındağ F. Effect of hesperidin on sciatic nerve damage in stz-induced diabetic neuropathy: modulation of TRPM2 channel. Neurotox Res. 2023;41(6):638–47. doi:10.1007/s12640-023-00657-0
12. Wardana AP, Tukiran. Skrining fitokimia

dan aktivitas antioksidan ekstrak kloroform tumbuhan gowok (syzygium polycephalum). Pros Semin Nas Kim dan Pembelajarannya. 2016;(September):1-6.
13. Nagaraja S, Ahmed SS, Bharathi DR, Goudanavar P, Rupesh KM, Fattepur S, et al. Green synthesis and characterization of silver nanoparticles of psidium guajava leaf extract and evaluation for its antidiabetic activity. molecules. 2022;27(14):4336. doi:10.3390/molecules27144336
14. Li B, Wang H, Zhou G, et al. VEGF-loaded biomimetic scaffolds: a promising approach to improve angiogenesis and osteogenesis in an ischemic environment. RSC Adv. 2017;7(8):4253–9. doi:10.1039/C6RA25294J
15. Guo L, Jiang F, Tang YT, Si MY, Jiao XY. The association of serum vascular endothelial growth factor and ferritin in diabetic microvascular disease. Diabetes Technol Ther. 2014;16(4):224–34. doi:10.1089/dia.2013.0181
16. Worotikan R V., Tuju EA, Kawuwung F. Analisa efektivitas antidiabetes ekstrak etanol buah andaliman (zanthoxylum acanthopodium DC) pada histopatologi ginjal tikus putih (rattus norvergicus) yang diinduksi alloksan [analysis of antidiabetic effectiveness of lemon pepper fruit (zanthoxylum aca. J Sains Mat Edukasi. 2017;5(1):29–37.
17. Alotaibi MR, Fatani AJ, Almnaizel AT, Ahmed MM, Abuohashish HM, Al-Rejaie SS. In vivo assessment of combined effects of glibenclamide and losartan in diabetic Rats. Med Princ Pract. 2019;28(2):178–85. doi:10.1159/000496104
18. Chen S, Ziyadeh FN. Vascular endothelial growth factor and diabetic nephropathy. Curr Diab Rep. 2008;8(6):470–6. doi:10.1007/s11892-008-0081-3
19. Gnudi L, Long DA. Diabetic nephropathy: methods and protocols. Springer; 2020.
20. Chyla G, Slaaga-Zaleska K, Dabkowski K, Kuchta A, Jankowski M. Suramin Enhances the Urinary Excretion of VEGF-A in Normoglycemic and Streptozotocin-Induced Diabetic Rats. Pharmacol Reportd. 2021;73:841–6.
21. Gulo KN, Suhartomi, Saragih AD, Raif MA, Ikhtiari R. Antioxidant activity of flavonoid compounds in ethanol and ethyl acetate extract from citrus sinensis. In: AIMS 2021-International Conference on Artificial Intelligence and Mechatronics Systems. IEEE; 2021:1–6. doi:10.1109/AIMS52415.2021.9466078
22. Ahmed OM, Fahim HI, Ahmed HY, et al. The preventive effects and the mechanisms of action of navel orange peel hydroethanolic extract, naringin, and naringenin in n-acetyl-p-aminophenol-induced liver injury in wistar rats. Oxid Med Cell Longev. 2019;2019:1–19. doi:10.1155/2019/2745352