International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Phytochemical screening, Toxicity and Antidiabetic Effect of Extract of Gotu Kola Leaves (Centella Asiatica Urban)

Ida Duma Riris[1], Tita Juwitaningsih[2], Ramlan Silaban[3], Marini Damanik[4], Nora Susanti[5]

[1,2,3,4,5]Department of Chemistry, Faculty of Mathematics and Natural Sciences, State University of Medan, Medan, Indonesia

Abstract

Gotu gotu leaves (Centella Asiatica (L.) Urban) are wild plants that grow widely as grasses used by the community as traditional medicinal plants. This study aims to determine the antidiabetic potential of gotu gotu leaf extract originating from Indonesian, Toba Samosir Regency, North Sumatra Province. Results of the secondary metabolite phytochemical test contained in n-hexane, ethyl acetate and ethanol of gotu leaf extract. The results of phytochemical screening showed that n-hexane flavonoid extracts, saponins and steroids; ethyl acetate; saponins and steroids; Ethanol contains flavonoids, saponins, steroids, and tannins. The toxicity test used the BSLT (Brine Shrimp Lethality Test) method. The results of the toxicity test of Gotu Gotu leaf extract (Centella Asiatica (L.) Urban) against Artemia salina Leach larvae showed that the n-hexane extract LC50 was 199.53; ethyl acetate: 133.43 ethanol has toxic properties with an LC50 value of 110.03µg/ml. Antidiabetic in vitro with amylase enzyme inhibition test, as a comparison is acarbose obtained IC50 = 20.267 ppm; ethilasetate: 121.909 ppm and ethanol: 108.797 ppm.

Keywords: Centella Asiatica (L); phytochemicals; Toxicity; antidiabetic

Introduction

Diabetes is a chronic metabolic disorder characterized by elevated blood glucose levels, and the incidence of this condition has been steadily rising worldwide. Also known only as diabetes, DM and its complications affect people in developing and developed countries, leading to a significant socioeconomic challenge. It is estimated that 25% of the world population is affected by this disease. In recent years, there has been a growing interest in using herbal medicines and natural products as potential therapeutic agents for managing diabetes[1] Diabetes causes long-term damage, dysfunction, and failure of various organ systems (heart, blood vessels, eyes, kidneys, and nerves), leading to disability and premature death[2,3]. The severity of damage triggered by hyperglycemia on the respective organ systems may be related to how long the disease has been present and how well it has been controlled. Several symptoms such as thirst, polyuria, blurring of vision, and weight loss also accompany diabetes[4,5,6]. The antidiabetic effect of plant materials have been attributed to the mixture of phytochemicals or a single component of plant extracts. Medicinal plants produce a wide variety of phytochemicals, including alkaloids, phenolic acids, flavonoids, glycosides, saponins, polysaccharides, stilbenes, and tannins, which are intensively investigated for their

antidiabetic effect[7]. One such plant that has garnered attention for its antidiabetic properties is Gotu Kola (Centella asiatica Urban) from Dolok Nagodang Village, Uluan District, Toba Regency, North Sumatra Province, a medicinal herb native to various parts of Asia. in fitoscreening extract of found flavonoid, phenolic, terpenoid, quercetin, which could inhibit pancreatic lipase and α-amylase activity, thereby reducing glucose absorption and improving insulin sensitivity and toxixity with BSLT reseach on aqueous extract of Gotu Kola showed no significant toxicity effects in animal models[8]. Gotu Kola, scientifically known as Centella asiatica Urban, is a small, creeping herbaceous plant used in traditional medicine for centuries. In addition, the study results show that Gotu Kola has antioxidant properties and the potential to reduce symptoms and complications associated with diabetes mellitus[9]. Gotu Kola (Centella asiatica Urban), is a perennial herbaceous plant native to Asia, has long been recognized for its diverse medicinal properties, including its potential in managing diabetes mellitus, a chronic metabolic disorder characterized by dysregulation of blood glucose levels[10,11]. The leaves of Gotu Kola, also known as Centella asiatica Urban, have garnered particular attention for their antidiabetic capabilities, which have been the subject of extensive research and investigation. The antidiabetic potential of Gotu Kola leaves has been attributed to the presence of bioactive phytochemicals, particularly triterpenoid saponins, which have been reported to exhibit a range of beneficial effects on the various mechanisms underlying the pathogenesis of diabetes, such as inhibiting glucose absorption from the intestines, enhancing insulin secretion from the pancreas, improving glucose uptake by adipose and muscle tissues, and suppressing glucose production by the liver.

Therefore, exploring the antidiabetic properties of Gotu Kola leaves and their bioactive constituents holds significant promise for developing novel, plant-based therapeutic strategies for managing diabetes an. Compound identification can be done by Fitoscreening, The existence of activity in plants is characterized by toxicity in plants[12]. The higher the toxicity in the plant, the more likely it is to have a variety of bioactivity against various diseases. The toxicity test that is often carried out is the BSLT test considering that this test is quite accurate, easy and inexpensive

Research problem : The present study aims to investigate the antidiabetic potentials of the extract derived from Gotu Kola leaves from Indonesian Kabupaten Toba Samosir, Provinsi Sumatera Utara and to elucidate the potential mechanisms underlying its antidiabetic effects.

Material and Methods:

The Gotu Kola leaves were collected form from Dolok Nagodang Village, Uluan District, Toba Regency, North Sumatra Province, dried, and extracted using hexane, ethylacetate and ethanol solvent. In vitro assays were conducted to evaluate the extract's ability to inhibit α-amylase and α-glucosidase enzymes, which play crucial roles in carbohydrate digestion and absorption. Acarbose is The results of this study demonstrated that the Gotu Kola leaf extract exhibited potent inhibitory activities against both α-amylase and α-glucosidase enzymes, with IC_{50} values comparable to that of the positive control, acarbose.

FeCl3 1%, HCL 2N, aquades, dragendroff reagent, anhydrous acetate, concentrated H2SO4, Na2SO4, 2% DMSO liquid, Whattman filter paper, NaCl and Artemia salina Leach shrimp larvae. Potassium Na tartarate, aquadest, α-amylase, phosphate buffer pH 7, Dimethyl Sulfoxide (DMSO), 3,5 dinitro salicylate(DNS), NaOH 2M, starch.

Extraction

Extraction is carried out by maceration. Samples of gotu gotu leaf plants (Centella asiatica (L.) Urban) in

the form of powder were macerated as much as 500 g in a glass jar with n-hexane solvent for 3 x 24 hours, every day stirred; Then, it is filtered using a Buchner so that filtrates and residues are obtained. The residue from the n-hexane filtration is re-macerated with ethyl acetate solvent for 3 x 24 hours, filtered back using a buncher to obtain filtrates and residues. The residue is macerated again with ethanol solvent for 3 x 24 hours, then filtered using a buncher to obtain filtrates and residues. Then it is filtered using buncher so that filtrates and residues are obtained. The filtrate obtained from the three solvents is each concentrated with a vacuum rotary evaporator so that the extract is obtained and then poured and placed in a chemical glass. Furthermore, it is weighed and stored before testing[13].

Phytochemical Tests

Phytochemical screening was carried out, referring to the method that was used by Harborne (2013) using specific reagents[14]. These tests include the alkaloid, flavonoids, terpenoids, steroids, saponins, and tannins test.

Toxicity test

Brine Shrimp Lethality Test (BSLT) is a method for testing toxic substances and is used as the first bioassay for natural product research. The BSLT method uses Artemia salina Leach larvae as experimental animals**.** The toxicity test of gotu gotu extract (Centella asiatica L.) was carried out using the Brine Shrimp Lethality Test (BSLT) method [15]. The experimental animal used was Artemia salina shrimp larvae. This test was carried out by making a stock solution of 2000 ppm. The variation in the concentration of the extract used in the toxicity test was 1000, 500, 100, and 50 ppm and control 0 ppm (negative control). The creation of concentration variations aims to determine the influence of several concentration variations on the death of A. Salina. Furthermore, the test began with the hatching of A. salina eggs in a small aquarium filled with 500 mL of artificial seawater and artemia eggs as many as 2 spatula spoons were hatched for 48 hours. The hatching process is equipped with a 25-watt incandescent lamp that keeps the seawater warm, speeding up the hatching and dialysis process by using an aerator to provide enough oxygen for Artemia's survival. In each concentration of each extract, 10 shrimp larvae are used

Activity Antidiabetic

Testing of antidiabetic activity of gotu gotu leaves (Centella asiatica (L.) Urban) in vitro by the method of measuring the inhibition of α-amylase enzyme activity, which is the measurement of reducing sugars formed by the hydrolysis of starch by α-amylase enzymes[16]. The reagent used in this test uses 3,5-dinitrosalicylate (DNS), which is a reagent that can react with the formed reducing sugars and produce a color complex that can be quantified by absorbance measurement using a spectrophotometer. The inhibition of α-amylase enzyme activity can be observed based on the difference in absorbance values produced. Before the test, a α-amylase enzyme solution was made, where 20 mg of α-amylase enzyme dissolved with 10 ml of phosphate buffer pH 7. So that it produces an enzyme solution that has dissolved and produces a white color that is slightly cloudy. For the manufacture of 0.5% starch solution, it is carried out by dissolving 0.05 grams of starch with 10 ml of phosphate buffer pH 7

Result and Discussion

Randemen

Table1. Randemen of extract Gotu Kola Leaves (CentellaAsiatica Urban)

Solvent	Rendemen %
N-Hexsane	2,365
Ethil Asetat	3,174
Ethanol	5,908

Of the three extracts, the highest randement sample in ethanol extracts.

The table shows the toxicity test results of n-hexane, ethyl acetate, and ethanol extracts from gotu gotu leaves, as shown by LC50.

Table2. Effect of Variation in Extract Concentration *Gotu Kola Leaves (CentellaAsiatica Urban)* Extract on Artemia Salina Leach

Sample Extract	Concentration	Log Concentration	Probit	% Death	Dead	Total	LC50
N-hexane	50	1.698970004	4.16	20	6	30	199.43
	100	2	4.56	33	10	30	
	500	2.698970004	5.44	67	20	30	
	1000	3	6.13	87	26	30	
Ethyl acetate	50	1.698970004	2.39	27	8	30	12 12122 130.43
	100	2	4.82	43	13	30	
	500	2.698970004	5.44	67	20	30	
	1000	3	7.37	100	30	30	
Ethanol	50	1.698970004	4.48	30	9	30	110.55
	100	2	4.92	47	14	30	
	500	2.698970004	5.61	73	22	30	
	1000	3	7.37	100	30	30	

Based on the BSLT method, compounds that are said to be highly toxic if the LC50 value < 250 ppm, toxic LC50<1000 ppm, and non-toxic LC50 above 1000 ppm[17,18] Toxic properties indicate the presence of bioactivity in the plant. LC50 for the highest extract is in n-hexane extract, then ethyl acetate extract and the smallest is ethanol. The table below displays the results of phytochemical tests conducted on leaf extracts from various plants.

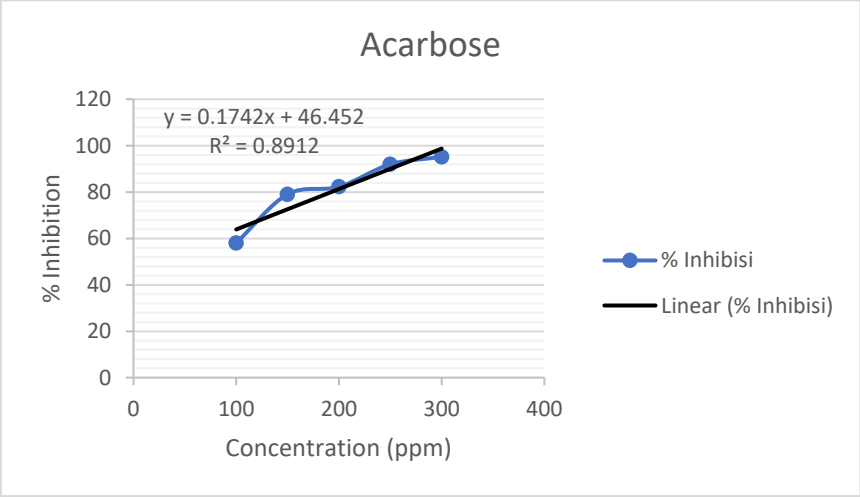
Table3. The Phytochemical Results of Extract from Gotu Gotu (Centella asiatica (L.).

Compound	Fraksi		
	N-Hexsane	Ethyl acetate	Ethanol
Alkaloid	-	-	-
Flavonoid	+	-	+++
Saponin	++	+	+++
Tanin	-	-	+
Terpenoid	-	-	-
Steroid	+++	+++	+++

Phytochemical studies reveal the existence of various phytochemicals as seen in the table
The inhibitory resistance of the extract to the amylase enzyme can be seen in this table

Table 4. of results for the α-amylase enzyme inhibition test using acarbose as a comparator.

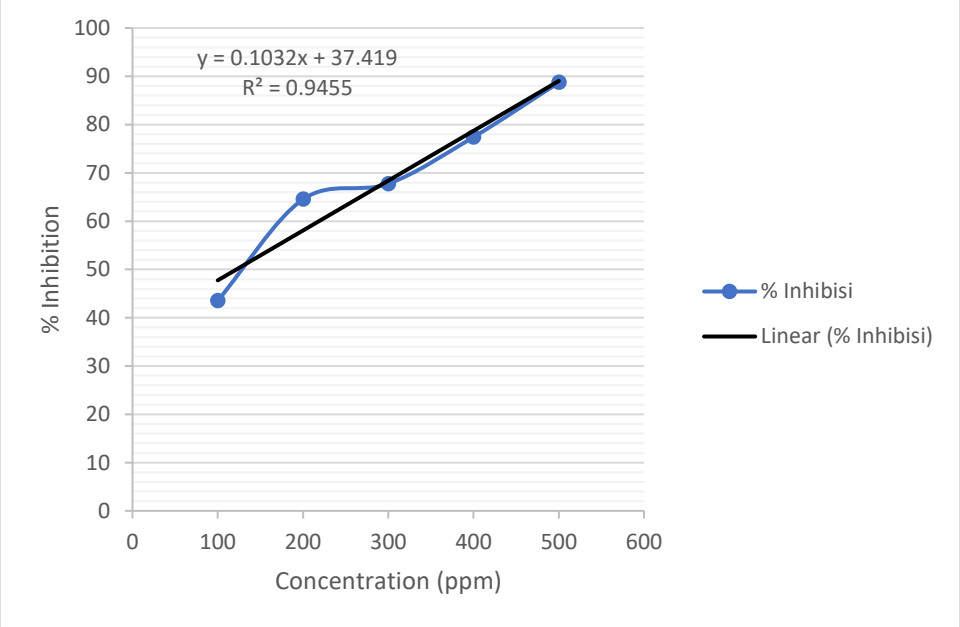
Sample (ppm)	α-amylase enzyme (S_1)	Without enzyme (S_0)	S_1-S_0	%Inhibition
Control	0,065			
Blank		0,003		
100	0,050	0,024	0,026	58,06451612
150	0,028	0,015	0,013	79,03225806
200	0,020	0,009	0,011	82,25806451
250	0,012	0,007	0,005	91,93548387
300	0,007	0,004	0,003	95,16129032



Graph of the relationship between increase in acarbose concentration and % inhibition From the data of table a linear regression equation is obtained, namely y = 0.1742x + 46.452 so that the result of IC50 = 20.267 ppm is obtained

Table 5. Results of α-amylase enzyme inhibition test by n-hexane extract of gotu gotu leaf (Centella asiatica (L.) Urban)

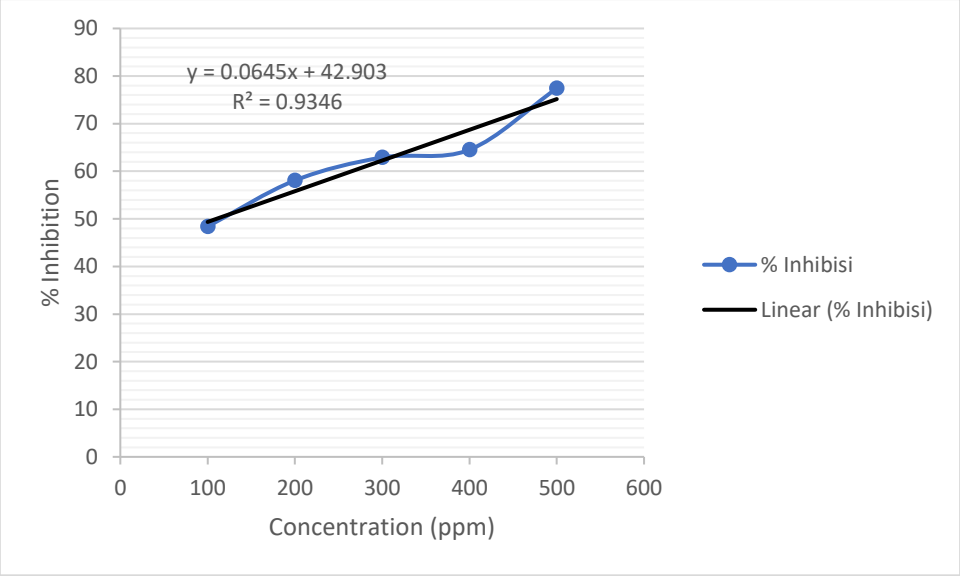
Sample(ppm)	α-amylase enzyme (S_1)	Without enzym (S_0)	S_1-S_0	%Inhibition
Control	0,065			
Blank		0,003		
100	0,070	0,035	0,035	43,54838870
200	0,055	0,033	0,022	64,51612903
300	0,048	0,028	0,020	67,74193548
400	0,039	0,025	0,014	77,41935483
500	0,025	0,018	0,007	88,70967741



Graph of the relationship between the increase in the concentration of n-hexane extract of gotu gotu leaf (Centella asiatica (L) Urban) to % of inhibition From the graph data, a linear regression equation was obtained, namely y = 0.1032x + 37.419 so that the IC50 = 121.909 ppm result was obtained

Table 6. Results of α-amylase enzyme inhibition test by ethyl acetate extract of gotu gotu leaves (Centella asiatica (L.) Urban)

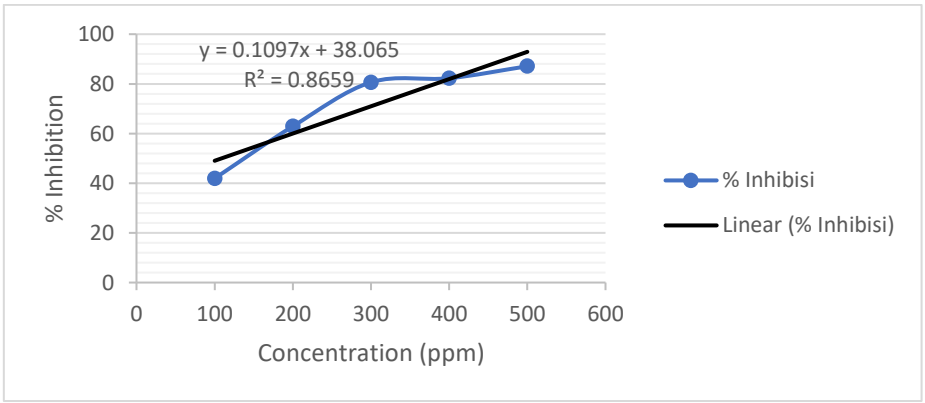
Sample(ppm)	α-amylase enzyme (S_1)	Without enzym (S_0)	S_1-S_0	%Inhibition
Control	0,065			
Blank		0,003		
100	0,068	0,036	0,032	48,38709677
200	0,054	0,028	0,026	58,06451612
300	0,046	0,023	0,023	62,90322580
400	0,038	0,016	0,022	64,51612903
500	0,025	0,011	0,014	77,41935483



The graph shows the relationship between the increasing concentration of ethyl acetate extract from pegagan leaves (Centella asiatica (L) Urb.) and its percentage of inhibition.
The graph data showed a linear regression equation is obtained, namely y = 0.0645x + 42.903 so that the result of IC50 = 110.03100775 ppm is obtained

Table 7. Results of α-amylase enzyme inhibition test by ethanol extract of gotu gotu leaves (Centella asiatica (L.) Urban)

Sample(ppm)	α-amylase enzyme (S_1)	Without enzym (S_0)	S_1-S_0	%Inhibition
Kontrol	0,065			
Blanko		0,003		
100 ppm	0,060	0,024	0,036	41,93548387
200 ppm	0,039	0,016	0,023	62,90322580
300 ppm	0,025	0,013	0,012	80,64516129
400 ppm	0,018	0,007	0,011	82,25806451
500 ppm	0,011	0,003	0,008	87,09677419



Graph of the relationship between the increase in ethanol concentration of Gotu leaf extract (Centella asiatica (L) Urban) and % of inhibition.

The graph showed *a linear regression equation is obtained, namely y = 0.1097x + 38.065 so that the result of IC50 = 108.797 ppm is obtained*

As the concentration increases, it is shown to affect the inhibition power of the α-amylase enzyme produced. From the data from the results of the above study, the % inhibition of extract from gotu gotu leaves (Centella asiatica (L.) Urban) was obtained, the higher the ppm concentration of the extract and acarbose, the higher the % of inhibition obtained.

Inhibition of α-amylase enzyme activity from n-hexane, ethyl acetate, and ethanol gotu leaf extracts (Centella asiatica (L.) Urban) or acarbose may increase as at higher concentrations In testing samples with UV that the more active the extract used, the less starch is hydrolyzed. This happens because the extract can stop the activity of the α-amylase enzyme to interact with the amylase substrate, so the intensity of the resulting color will decrease. The lowest percentage of inhibition was found in the concentrations of n-hexane extract, ethyl acetate extract, and 100 ppm ethanol extract, which were 43.54%, 48.38%, and 41.39%, while the highest percentage of inhibition was found in the samples of n-hexane extract, ethyl acetate extract, and 500 ppm ethanol extract, which were 88.70%, 77.41%, and 87.09%. Therefore, it can be concluded that the n-hexane fraction with a concentration of 500 ppm has the best ability to inhibit the enzyme α-amylase than other concentrations. In this regard, medicinal plants are considered the best source of new chemical entities with fewer side effects. This is in accordance with the principle of enzyme inhibition activity, namely the greater the concentration of the extract, the greater the value of the inhibition obtained[19,20]

From the results of this study, it was obtained that the % inhibition of n-hexane extract, ethyl acetate extract, and ethanol extract of gotu gotu leaves was smaller compared to acarbose, so the IC50 value in the extract would be greater than that of acarbose. The test compound has a small IC50 value, indicating high inhibitory activity against the α-amylase enzyme[21]. So, if a compound or plant extract has an IC50 value close to or below 100 ppm, it can be considered a potential antidiabetic agent. Based on the strength level of % inhibition of the α-amylase enzyme, the samples of n-hexane extract, ethyl acetate extract, and ethanol extract were included in the moderate category while the positive control of acarbose was included in the very strong category. Percent Criteria for α-Amylase Enzyme Inhibition: Very Strong: ≥ 90%, Strong: 50–89%, Moderate: 31–49%, Weak: ≤ 30%[22,23]. Acarbose is used as a positive control for comparison with the extract in inhibition of the enzyme α-amylase. From the data above, it can be seen that the % of acarbose inhibition is higher than the gotu gotu leaf extract sample, because acarbose is a drug that has been used as an antidiabetic drug.

Conclusion

Although the inhibition power of gotu gotu leaf extract (Centella asiatica (L.) Urban) is relatively moderate, the use of this plant has been very accustomed to being used as a medicinal plant by devouring its fresh leaves. For this reason, further research is needed to be considered as an inventory of herbal plants for antidiabetics. Ethical Clearance: The

Research Ethical Committee at scientific research by ethical approval of head of medan state university research institute with a contract. 0227/UN33.8/PPKM/PPT/2024

Conflict of Interest: NonFunding: State University of Medan

Reference:

1. Invitro Studies on Antidiabetic Effect of Aegle Marmelos Plant Aqueous Extract. (2016).Inter national

Journal of Science and Research (IJSR), 5(5), 252–255. https://doi.org/10.21275/v5i5.nov163042
2. Complications of diabetes: beyond the heart, kidneys, and eyes. (2019). Heart and Metabolism, 80, 23–27. https://doi.org/10.31887/hm.12019.80/jpkovalik
3. Chronic complications of diabetes. (2006). Atlas of Diabetes Mellitus, 80–119. https://doi.org/10.1201/b15304-11
4. 3. Hellgren, K.-J., Agardh, E., & Bengtsson, B. (2014). Progression of Early Retinal Dysfunction in Diabetes Over Time: Results of a Long-term Prospective Clinical Study. Diabetes, 63(9), 3104–3111. https://doi.org/10.2337/db13-1628
5. 4. Polyuria is the Cause of Diabetes Complications. (2018). International Journal of Diabetes & Metabolic Disorders, 3(1). https://doi.org/10.33140/ijdmd/03/01/00001
6. Basson, R., & Bayat, A. (2020). Fibrosis and diabetes: Chronic hyperglycemia triggers organ-specific fibrotic mechanisms. Wound Healing, Tissue Repair, and Regeneration in Diabetes, 121–147. https://doi.org/10.1016/b978-0-12-816413-6.00007-1
7. Noberta, S., Triratna, S., Aditiawati, A., & Husin, S. (2013). Association between hyperglycemia and organ dysfunction in shock patients. Paediatrica Indonesiana, 53(1), 26. https://doi.org/10.14238/pi53.1.2013.26-31
8. F, K. (2018). Wakouba an Antidiabetic Plant Extract with Protective Effect against Cardiovascular and Nephropathies Complications in Streptozotocin-Induced Diabetic Rats. Medical Journal of Clinical Trials & Case Studies, 2(6). https://doi.org/10.23880/mjccs-16000156
9. Yahya, M. A., & Nurrosyidah, I. H. (2020). Antioxidant activity ethanol extract of gotu kola (Centella asiatica (L.) Urban) with DPPH method (2,2-Diphenyl-1-Pikrilhidrazil). Journal of Halal Product and Research, 3(2), 106. https://doi.org/10.20473/j
10. Ayodeji B. Oyenihi., Blessing O. Ahiante ., Omolola R. Oyenihi ¨, Bubuya Masola (2020) Centella asiatica: its potential for treating diabetes. Diabetes (second edition) . 2020 pages 2013-2222. Academic Press
11. Damayanti, I. A. M., Antari, N. W. S., & Megayanti, S. D. (2022). Antioxidant potential of gotu kola leaf extract (Centella asiatica (L.) Urban) as an alternative to antihyperglycemic herbal drinks. Jurnal Pijar Mipa, 17(6), 782–786. https://doi.org/10.29303/jpm.v17i6.417
12. Duma Riris, I., Simorangkir, M., & Silalahi, A. (2018). ANTIOXIDANT, TOXICITY AND ANTIBACTERIAL PROPERTIES OF Ompu-ompu (Crinum asiaticum-L) ETHANOL EXTRACT. Rasayan Journal of Chemistry, 11(3), 1229–1235.
13. Duma Riris, I., Simorangkir, M., & Silalahi, A. (2018). ANTIOXIDANT, TOXICITY AND ANTIBACTERIAL PROPERTIES OF Ompu-ompu (Crinum asiaticum-L) ETHANOL EXTRACT. Rasayan Journal of Chemistry, 11(3), 1229–1235. https://doi.org/10.31788/rjc.2018.1133090
14. J. B. Harborne, Phytochemical Methods, Chapman & Hall London International Diabetes Federation, Atlas Sixth Ed., 2013 (www idf.org/diabetesatlas).
15. (Pohan, D. J., Marantuan, R. S., & Djojosaputro, M. (2023). Toxicity Test of Strong Drug Using the BSLT (Brine Shrimp Lethality Test) Method. International Journal of Health Sciences and Research, 13(2), 203–209. https://doi.org/10.52403/ijhsr.20230228
16. Antidiabetic activity (In vitro alpha amylase inhibitory) of ethanol extract of Carissa carandas Linn. roots. (2020). International Journal of Pharmaceutical Research, 12(03). https://doi.org/10.31838/ijpr/2020.12.03.002
17. Widyaningsih, S., Chasani, M., Rastuti, U., & Salamah, U. (2022). Phytochemicals and toxicity of

ketapang fruit flesh (Terminalia catappa. Linn) using the BSLT method. Pharmaciana, 12(3), 275.https://doi.org/10.12928/pharmaciana.v12i3.22219

18. Toxicity Test of the Brine Shrimp Lethality Test (BSLT) Method of the Kebiul Seed Fraction (Caesalpinia Bonduc (L). Roxb). (2021). International Journal of Pharmaceutical Research, 13(02). https://doi.org/10.31838/ijpr/2021.13.02.409
19. Anigboro, A. A., Avwioroko, O. J., Ohwokevwo, O. A., Pessu, B., & Tonukari, N. J. (2021). Phytochemical profile, antioxidant, α-amylase inhibition, binding interaction and docking studies of Justicia carnea bioactive compounds with α-amylase. Biophysical Chemistry, 269, 106529. https://doi.org/10.1016/j.bpc.2020.106529
20. In Vitro Evaluation of Alpha-amylase Inhibition, Antioxidant Potential and Phytochemical Analysis of Acacia Modesta. (2023). Journal of Hunan University Natural Sciences, 50(2). Internet Archive. https://doi.org/10.55463/issn.1674-2974.50.2.30.
21. Poovitha, S., & Parani, M. (2016). In vitro and in vivo α-amylase and α-glucosidase inhibiting activities of the protein extracts from two varieties of bitter gourd (Momordica charantia L.). BMC Complementary and Alternative Medicine, 16(S1). https://doi.org/10.1186/s12906-016-1085-1
22. J, N., A, R., Raju R, S., Rao GSN, K., DSNBK, P., & G, C. (2021). ANTIDIABETIC ACTIVITY, ALPHA-AMYLASE AND ALPHA- GLUCOSIDASE INHIBITORY EFFECT OF PASTINACA SATIVA EXTRACT. Bulletin of Pharmaceutical Sciences. Assiut, 44(2), 387–395. https://doi.org/10.21608/bfsa.2021.207157.
23. Jaber, S. A. (2023). In vitro alpha-amylase and alpha-glucosidase inhibitory activity and in vivo antidiabetic activity of Quercus coccifera (Oak tree) leaves extracts. Saudi Journal of Biological Sciences, 30(7), 103688. https://doi.org/10.1016/j.sjbs.2023.103688.