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Original Research Article

Antidiabetic Potentials of Ethanol Extract of *Timonius flavescens* (Jacq.) Baker Leaf

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ABSTRACT

Diabetes Mellitus (DM) is a chronic disease caused by heredity or deficiency in insulin secretion. This disease has occupied the second position as an epidemic in Indonesia. The World Health Organization (WHO) states that nearly 70% of diabetic patients use plants as the main source of antidiabetic agents. One of the plants used by the community for a long time to lower blood sugar is *Timonius flavescens* (family Rubiaceae). This study aims to determine the content of antidiabetic active compounds in the ethanol extract of *T. flavescens* leaves by using mass spectrometry gas chromatography (GC-MS). The results of GC-MS showed that there were more than 40 compounds, then 10 of them had the highest value detected as having antidiabetic agent properties. These compounds include (3*β*)- stigmast-5-en-3-ol, 3*β*.-(acetyloxy)-15*α*.-hydroxy-5.*α*.-cholesta-8(14),9(11)-dien-7-one, alpha-tocopherol, hexade-canoic acid, nonanoic acid, phytol, 2,3-dihydrobenzofuran, heptanoic acid, neophytadiene, and campesterol which have been shown to have antidiabetic properties. The results of this study are expected to provide critical information for researchers and the public regarding the use of *T. flavescens* leaves as medicine.

Keywords: *Timonius flavescens*, antidiabetic activity, GC-MS analysis, ethanol extract

Introduction

Diabetes Mellitus (DM) is a chronic disease caused by heredity or deficiency in insulin secretion, with decreased organ response to secreted insulin.[1] In such cases, elevated blood glucose levels can damage a large part of the body's system, including blood vessels and nerves.[2] Diabetes mellitus is one of the most severe diseases and metabolic disorders that are currently incurable, indicated by the increase in blood glucose levels as a result of absolute or relative insulin deficiency and insulin failure to act on the target tissues.[3]

The World Health Organization (WHO) predicts that by 2030, the number of people with diabetes mellitus in the world will reach 21.3 million people.[4] Based on the latest epidemiological studies, type 2 DM has become an epidemic in Indonesia. Nearly 80% of DM is caused by the patient's lifestyle, especially in urban communities of Indonesia.[5] The World Health Organization (WHO) states that nearly 70% diabetic patients use plants as the main source of antidiabetic agents.[3,6] Approximately 800 types of plants are reported to have antidiabetic or immunostimulating potential, but only a few of them are supported by scientific research results.[7,8] One of the plants that have been used by the community for a long time to lower blood sugar level is *Timonius flavescens* (*Rubiaceae* genus).[8–10]

Part of the plant organ *Timonius flavescens* has lipoxygenase-inhibiting properties, anti-inflammatory, and suppresse muscarinic receptors and central nervous system.[11–13] Although phytochemical investigations of *T. flavescens* are not widely reported, many chemical compounds have been isolated from other *Timonius* species such as triterpenes and alkaloids. [13–16] Various species from the *Timonius* genus have long been used as medicine for various diseases, including malaria,[17] lung disease and gonorrhea,[18] as well as hypertension.[19]

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*Timonius flavescens (*of the *Rubiaceae* genus*)* is a plant that has long been used by the Batak people in Sumatra to lower blood sugar level or diabetes.[9,10] Ethanol extract from *T. flavescens* has been shown to have antidiabetic properties and potentially as an immunostimulant agent.[8] *T. flavescens* contains various secondary metabolites such as terpenoids, saponins, phenolics and flavonoids.[20] This research was conducted to determine the content of compounds in the ethanol extract of *T. flavescens* leaves using mass spectrometry gas chromatography (GC-MS), which has antidiabetic properties.

Materials and Methods

Plant material

The samples used in this study were *Timonius flavescens* leaves obtained in Sipahutar Village, North Sumatra, Indonesia which was collected in August 2021. The plant was identified by professor Tri Harsono from Universitas Negeri Medan with a reference number of No.0087/UN33.4.8.3/LB/2021. The leaves were cleaned with running water, then air-dried in a room protected from sunlight for approx. 5 days. The dried samples were then blended and filtered through a 60-mesh sieve.

Leaf extract

A total of 250 grams *T. flavescens* leaf powder was macerated with 96% ethanol as a solvent of 1000 mL in a vessel for 5 days. Then the marinade was filtered with Whatman No 1 filter paper, then the pulp was macerated again with 96% ethanol and the same ratio (1:4) for up to 3 repetitions.[21] All the filtered macerate were mixed and evaporated with a vacuum rotary evaporator until it turned into viscous extract. It was then stored in a refrigerator at 4^0C.[22]

Phytochemical analysis

Phytochemical analysis of the ethanol extract of the leaves of *T. flavescens* on secondary metabolites was carried out qualitatively, including testing for alkaloids, flavonoids, phenols, tannins, terpenoid saponins and steroids. The qualitative test for the content of secondary metabolites was carried out using the standard method.[23]

Gas Chromatography-Mass Spectroscopy (GC-MS)

Gas Chromatography-Mass Spectroscopy (Shimadzu QP 5000) was conducted using a non-polar DB-5 crosslinked column with a length of 30 m x ID 0.25 mm x 0.25 m film thickness, consisting of 5% phenyl

methyl polysiloxane. The initial temperature was programmed at 50ºC for two minutes, then increased to 300ºC at a speed of 6.5ºC/minute for 10 minutes at final temperature. The injector is set at 280^{0}C and the detector at 300ºC. Helium gas was used as the carrying agent. 1 µl ethanol extract of *T. flavescens* leaves was diluted in 200 µl of hexane, then injected into the GC-MS.[24–26] Interpretation of the mass spectrum was carried out using the National Institute Standard and Technology (NIST) database. Testing of *T. flavescens* leaf ethanol extract samples was repeated 3 times. Ten compounds with the highest retention index was analyzed using PubChem National Library of Medicine, National Center for Biotechnology Information.

Result and Discussion

The results of the phytochemical test of the ethanol extract of *T. flavescens* are presented in Table 1. The results of *T. flavescens* leaf extract screening by GC-MS was the first study conducted. The chemical components present in the leaf extract of *T. flavescens* were identified using GC-MS analysis with the active principle and retention time (RT), molecular formula, and area concentration shown in Table 2.

The phytochemical components of the ethanol extract of *T. flavescens* from the GC-MS test are presented in Figure 1. The compounds detected in 3 replications with GC-MS amounted to more than 40, 15 of which were detected as having the best antidiabetic agent properties. The test results of ethanol extract from *T. flavescens* leaves of showed different results in each test replication. However, compounds such as (3β)-stigmast-5-en-3-ol, 3β.-(acetyloxy)-15α.-hydroxy-5.α.-cholesta-8(14),9(11) -dien-7-one, alpha-tocopherol, nonanoic acid, phytol, dan 2,3-dihydrobenzofuran appeared in each replicate. (3β)-stigmast-5-en-3-ol is compounds with the highest retention time in each replicate, and therefore became the main components of the ethanol extract of *T. flavescens* leaves. In addition, based on the high value of retention time, several other compounds were also successfully identified, such as hexadecanoic acid, heptanoic acid, neophytadiene, dan campesterol.

Ethnobotanically, the Batak people in North Sumatra Province, Indonesia have consumed boiled water from the leaves of *T. flavescens* to lower blood sugar. The results of the analysis of the antidiabetic activity of phytochemical compounds contained in the ethanol extract of the leaves of *T. flavescens* are presented in Table 3.

(3β)-stigmast-5-en-3-ol is a compound with the highest percentage of area that has antidiabetic and antioxidant properties. (3β)-stigmast-5-en-3-ol is a plant phytosterol that is commonly found in many plants. Other common names for (3β)-stigmast-5-en-3-ol yaitu Betasitosterol, (3β)-stigmast-5-en-3-ol, 22:23-dihydrostig-masterol, alpha-dihydrofucosterol, cinchol, cupreol, rhamnol, quebrachol and (3β)-stigmast-5-en-3-ol.[27] This compound has been proven effective in treating type 2 diabetes mellitus by means of increased insulin production, both through antioxidant [28,29] and β-cell regeneration properties.[28] In addition, (3β)-stigmast-5-en-3-ol was also reported to be able to reduce cholesterol levels.[30] (3β)-stigmast-5-en-3-ol can also increase glucose transport in rat skeletal muscle.[31]

Table 1. Phytochemical analysis of *T. flavescens* leaf ethanol extract

No	Test	Result
1	Alkaloids	+
2	Flavonoids	-
3	Phenol	+
4	Tannins	+
5	Saponins	-
6	Terpenoids	-
7	Steroids	+

- = Absence; + = Presence

Table 2: Phytochemical screening of *T. flavescens* leaf ethanol extract with GC-MS

No	Library	Area			RT			CAS	Molecular Formula
		1	2	3	1	2	3		
1.	(3β)- Stigmast-5-en-3-ol	17.808	17.791	17.799	12.6	12.97	11.93	000083-46-5	$C_{29}H_{50}O$
2.	3β.-(Acetyloxy)-15α.-hydroxy-5.α.-cholesta-8(14),9(11)-dien-7-one	5.43	8.43	7.42	18.928	18.928	18.928	071076-40-9	$C_{29}H_{44}O_4$
3.	Alpha-Tocopherol	5.91	5.96	6.55	16.218	16.218	16.218	000059-02-9	$C_{29}H_{50}O_2$
4.	Hexadecanoic acid	6.2	-	-	9.601	-	-	000112-39-0	$C_{16}H_{32}O_2$
5.	Nonanoic Acid	5.19	3.4	3.81	14.012	14.004	14.012	055268-58-1	$C_9H_{18}O_2$
6.	Phytol	3.63	3.7	4.9	10.789	10.781	10.789	000150-86-7	$C_{20}H_{40}O$
7.	2,3-dihydrobenzofuran	3.3	3.68	4.36	4.592	4.6	4.592	000496-16-2	C_8H_8O
8.	Heptanoic acid	-	-	3.26	-	-	8.105	000111-14-8	$C_7H_{14}O_2$
9.	Neophytadiene	-	2.9	-	-	8.994	-	000504-96-1	$C_{20}H_{38}$
10.	Campesterol	-	2.78	-	-	14.047	-	000474-62-4	$C_{28}H_{48}O$

Compound 3β.-(Acetyloxy)-15α.-hydroxy-5.α.-cholesta-8(14),9(11)-dien-7-one belongs to the class of Cholestane steroids. This compound has not been widely studied regarding its effect on the body's metabolism. However, it has been reported that Cholestane steroids derived from the decoction of the roots of *Peniocereus greggii* can be used for the treatment of diabetes in traditional medicine in Mexico.[32]

Alpha-Tocopherol has been reported to be an effective antidiabetic with its antioxidant properties.[33] Treatment of Diabetes using tocopherol fraction significantly increased the glucagon peptide-1 (GLP-1) hormone in the cecum of diabetic mice.[34] GLP-1 is known to have a number of important biological activities, including insulin release, glucagon inhibition, and maintenance of pancreatic β-cell mass.[35] In addition, alpha-Tocopherol can also increase blood pressure significantly higher in people with type 2 diabetes mellitus.[36,37] The administration of alpha-tocopherol supplementation in diabetic patients did not significantly affect the lipid profile,[38] glucose levels, glycated hemoglobin, triacylglycerides, lipoprotein levels, and serum malondialdehyde.[39]

Heptanoic acid has been identified in various plants that have potential as antidiabetic, including *Holothuria thomasi*,[40] Sanbai melon seed oil[41] and even seaweed.[42] However, there is no literature that specifically examines this compound. The mechanism of antidiabetic by *Holothuria*

thomasi plant occurs by lowering blood glucose levels which is carried out by regenerating insulin through increasing plasma insulin levels and insulin release from the pancreas,[40,43] and inhibits the formation of glucose in the bloodstream.[40,44] Sanbai melon seed oil is able to prevent hyperlipidemia associated with diabetes by regulating impaired glucose and lipid metabolism in diabetic rats caused by reactive oxygen species (ROS).[41] Antidiabetic test conducted by Unnikrishnan[42] on seaweed showed that seaweed extracts of *S. polycystum* and *S. wightii* had a significant effect in inhibiting the main carbohydrate hydrolyzing enzymes such as α-glucosidase, and inhibits incretin-degrading enzymes such as DPP-IV, which can delay carbohydrate digestion and glucose absorption and prevent postprandial hyperglycemia. Based on the three studies, it was concluded that heptanoic acid has antidiabetic potential.

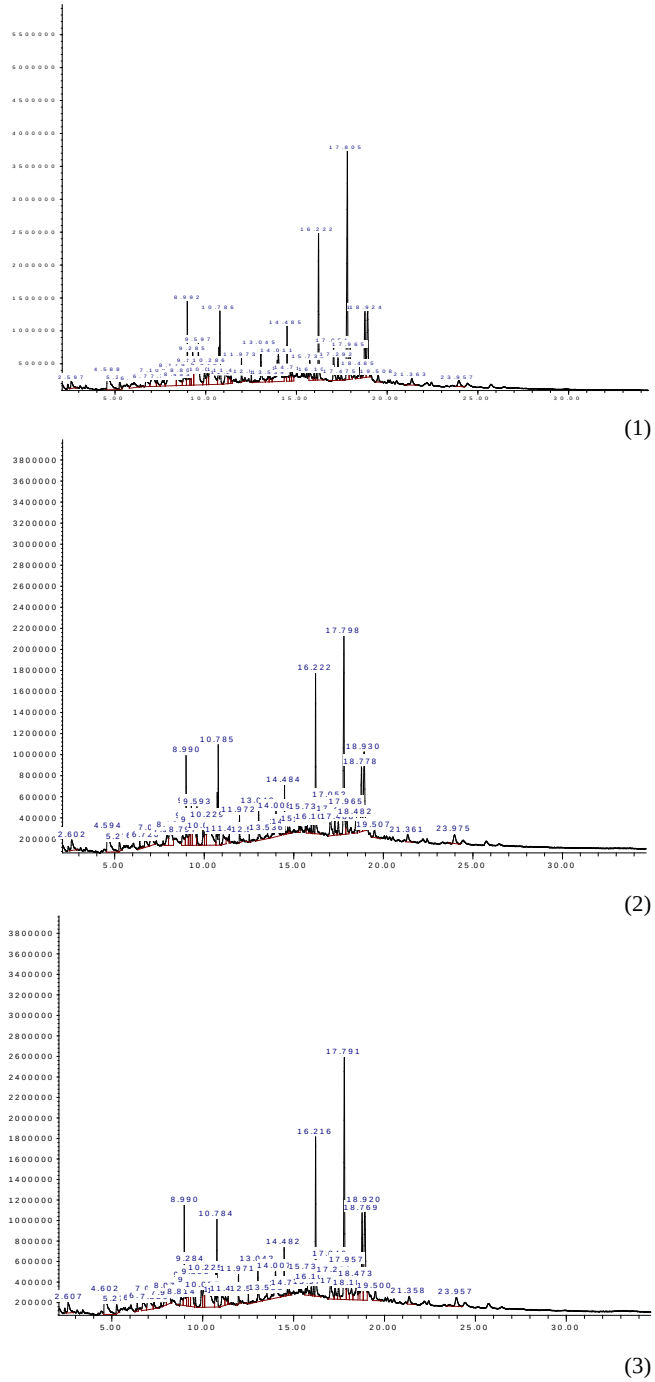

Figure 1. GC-MS analysis ethanolic extract of leaf *T. flavescens*. Testing with 3 repetitions

Phytol is known to improve insulin resistance in diabetics.[45] Phytol derivatives, namely phytanic acid, can trigger RXR (retinoid X receptor) and activate peroxisome proliferator-activated receptor (PPAR).[45,46] RXRs, commonly referred to as 'rexinoids', function as thiazolidinediones (TZDs) which improve insulin resistance, reduce hyperglycemia in type 2 diabetes and obesity, and increase pre-adipocyte differentiation.[45,47]

he 2,3-dihydrobenzofuran compounds have synonyms p-vinylphenol, benzofuran, coumaran, and dihydrocou-marone are known to act as antidiabetics by inhibiting the activity of α-glucosidase enzyme.[48] The way 2,3-dihydrobenzofuran works, which is by inhibiting the performance of this enzyme, can reduce blood glucose levels in diabetics by reducing the absorption of glucose from the intestines.[49] The 2,3-dihydrobenzofuran derivative is known to inhibit the sodium-dependent glucose transporter (SGLT) present in the intestines and kidneys so that it can be used as a therapeutic agent for diabetes including insulin-dependent diabetes mellitus (type I diabetes mellitus), non-insulin-dependent diabetes, mellitus (type II diabetes mellitus), diabetic complications, diseases caused by hyperglycemia such as obesity and the like.[50]

Nonanoic acid stimulates GLP-1 and PYY via OR51E1 signaling in L cells, thus having a potential role in olfactory receptor-mediated events in GLP-1 and PYY secretion and thus potentially being used in therapeutic approaches to treat diabetes.[51] GLP-1 secretion has insulinotropic activity whose upregulation is considered a potential pharmacological target in treating type 2 diabetes.[52]

The ethanol extract of Allium saralicum with the main compounds linolenic acid-methyl ester, phytol, and neophytadiene can improve hyperglycemia due to diabetes and prevent anemia after diabetes by controlling hematological parameters.[53] While neophytadiene is a terpene that is found in many plants that have the potential as antiradical and antidiabetic.[54,55] Neophytadiene and various other active compounds in the leaf extract of the plant *Eryngium caeruleum* act as free radical scavengers and prevent the development of diabetes mellitus in experimental animals.[54]

Plasma levels of campesterol are associated with dyslipidemia in patients with type 2 diabetes.[56,57] In people with the metabolic syndrome and type 2 diabetes, campesterol levels are significantly lower.[58,59] Lower levels of absorption of campesterol can be used as an indicator of an increased risk of developing type 2 diabetes caused by insulin sensitivity.[60]

Conclusion

The ethanol extract of the leaves of *T. flavescens* with GC-MS contains various metabolites that act as antidiabetic. These compounds have been proven to act as antidiabetic for type 1 diabetes mellitus and type 2 diabetes mellitus.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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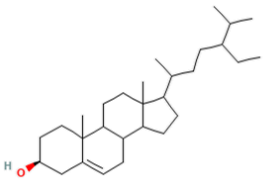
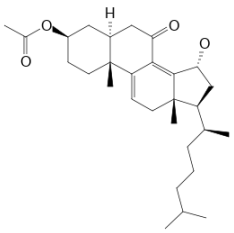
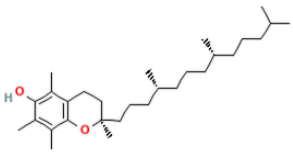
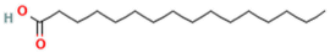
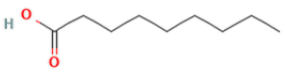
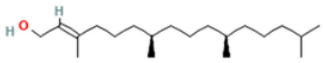
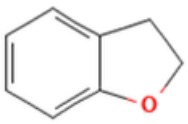
Table 3: Antidiabetic activity of phytochemical compounds contained in ethanol extract of leaf *T. flavescens*

Name of the compound	Nature of Compound	Structure	Mol.Wt (g/mol)	Antidiabetic activity
(3*β*)- Stigmast-5-en-3-ol	Phytosterols		414.7 g/mol	• Increased insulin production[29] • β-cell regeneration[28] • Increase glucose transport in skeletal muscle[31]
3β.-(Acetyloxy)-15*α*.-hydroxy-5.*α*.-cholesta-8(14),9(11)-dien-7-one	Cholestane steroids		456.7 g/mol	• It has not been reported but Cholestane steroids are known to have antidiabetic properties[32]
Alpha-Tocopherol	Vitamin E		430.7 g/mol	• Increased glucagon peptide-1 (GLP-1) hormone[34]
Hexadecanoic acid	Palmitic Acid		256.42 g/mol	• Regeneration of insulin levels and insulin release from pancreas[43] • Inhibits major carbohydrate hydrolyzing enzymes and incretin-degrading enzymes[42]
Nonanoic Acid	Pelargonic acid		158.24 g/mol	• Stimulates GLP-1 and PYY via OR51E1 signaling in L cells[51]
Phytol	Diterpenoid		296.5 g/mol	• Improve insulin resistance[45] • Reduce hyperglycemia in type 2 diabetes[47]
2,3-dihydrobenzofuran	Organic Heteropolycyclic		120.15 g/mol	• Inhibition the activity of α-glucosidase enzyme[48] • Reduce absorption of gluocse from the intestines[49]

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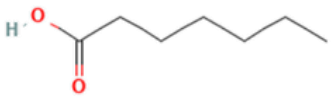
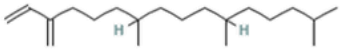
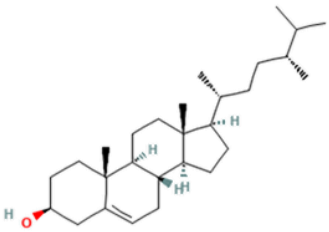
Name of the compound	Nature of Compound	Structure	Mol.Wt (g/mol)	Antidiabetic activity
Heptanoic acid	Carboxylic Acid		130.18 g/mol	• Inhibition the sodium-dependent glucose transporter (SGLT) present in the intestines and kidneys[50] • Lowering blood glucose levels[40] • Increase plasma insulin levels and insulin release from the pancreas[43] • Inhibit the formation of glucose in the bloodstream[44]
Neophytadiene	Alkene & Diterpene		278.5 g/mol	• Prevent anemia after diabetes by controlling hematological parameters[53] • Prevent the development of diabetes melitus[54]
Campesterol	Phytosterols		400.7 g/mol	• Indicator incerased risk of developing type 2 diabetes caused insulin sensitivity[60]

References

1. Javadi N, Abas F, Hamid AA, Simoh S, Shaari K, Ismail IS, Mediani A, Khatib A. GC-MS-Based Metabolite Profiling of *Cosmos caudatus* Leaves Possessing Alpha-Glucosidase Inhibitory Activity. J Food Sci. 2014; 79(6):C1130-C1136.
2. Matsui T, Tanaka T, Tamura S, Toshima A, Tamaya K, Miyata Y, Tanaka K, Matsumoto K. α-Glucosidase Inhibitory Profile of Catechins and Theaflavins. J Agric Food Chem. 2007; 55(1):99-105.
3. Achi NK, Ohaeri OC. GC-MS determination of bioactive constituents of the methanolic fractions of *Cnidoscolus aconitifolius*. J Pharm Res Int. 2015; 5(3):163-72.
4. Fard MH, Naseh G, Lotfi N, Hosseini SM, Hosseini M. Effects of aqueous extract of turnip leaf (*Brassica rapa*) in alloxan-induced diabetic rats. Avicenna J phytomedicine. 2015; 5(2):148-156.
5. Wahjuni S, Laksmiwati AAIAM, Bogoriani IW. Administration of ethanol extract of mustard greens (*Brassica* rapa L.) leaves increased Superoxide Dismutase levels in Hyperglycemic rat. J Phys Conf Ser. 2019; 1341(3):032025.
6. Bailey CJ, Day C. Traditional Plant Medicines as Treatments for Diabetes. Diabetes Care. 1989; 12(8):553-564.
7. Alarcon-Aguilara F, Roman-Ramos R, Perez-Gutierrez S, Aguilar-Contreras A, Contreras-Weber C, Flores-Saenz J. Study of the anti-hyperglycemic effect of plants used as antidiabetics. J Ethnopharmacol. 1998; 61(2):101-110.
8. Lbn Gaol AYD, Ilyas S, Hutahaean S, Sipahutar H. Antidiabetic Activity and Immunostimulant Potential of Bosibosi (*Timonius flavescens* (Jacq) Baker) Leaves Ethanol Extract in Alloxan-Induced Diabetic Rats. J Phys Conf Ser. 2021; 1819(1):012071.
9. Darwin SP. New species of the Timonius flavescens alliance (Rubiaceae: Guettardeae) in Papuasia. Syst Bot. 1997; 22(1):85-98.
10. Davis AP, Govaerts R, Bridson DM, Ruhsam M, Moat J, Brummitt NA. A Global Assessment of Distribution, Diversity, Endemism, and Taxonomic Effort in the Rubiaceae 1. Ann Missouri Bot Gard. 2009; 96(1):68-78.
11. Abu Bakar FI, Abu Bakar MF, Abdullah N, Endrini S, Rahmat A. A Review of Malaysian Medicinal Plants with Potential Anti-Inflammatory Activity. Adv Pharmacol Sci. 2018; 2018:1-13.

12. Chung LY, Yap KF, Mustafa MR, Goh SH, Imiyabir Z. Muscarinic Receptor Activity of Some Malaysian Plant Species. Pharm Biol. 2005; 43(8):672-682.
13. Chung LY, Soo WK, Chan KY, Mustafa MR, Goh SH, Imiyabir Z. Lipoxygenase inhibiting activity of some Malaysian plants. Pharm Biol. 2009; 47(12):1142-1148.
14. Erdelmeier C, Hauer H, Sticher O, Rali T. 10-Deoxysecogalioside: A New Iridoid Glycoside from *Timonius timon*. Planta Med. 1994; 60(05):484-485.
15. Johns S, Lamberton J. The identification of a new alkaloid from *Timonius kaniensis* (Rubiaceae) as dihydrocupreine. Aust J Chem. 1970; 23(1):211-212.
16. Khan IA, Sticher O, Rali T. New triterpenes from the leaves of Timonius timon. J Nat Prod. 1993; 56(12):2163-2165.
17. Subeki. Potency of the Indonesian Medicinal Plants as Antimalarial Drugs. J Teknol dan Ind Has Pertan. 2008; 13(1):25-30.
18. Setzer MC, Setzer WN, Jackes BR, Gentry GA, Moriarity DM. The medicinal value of tropical rainforest plants from Paluma, North Queensland, Australia. Pharm Biol. 2001; 39(1):67-78.
19. Suharjito D, Darusman LK, Darusman D, Suwarno E. Comparing Medical Plants use for Traditional and Modern Herbal Medicine in Long Nah Village of East Kalimantan. Bionatura. 2014; 16(2):95-102.
20. Laely N, Sipahutar H. Potensi Aktivitas Biologis Senyawa Fenolik Ekstrak Daun Bosibosi (*Timonius flavescens* (Jacq.) Baker). J Biosains. 2017; 3(1):43-48.
21. Gultom ES, Hartanti T, Maritsa H, Prasetya E. Antibacterial activity test on ethanol extract fraction of Kirinyuh (*Chromolaena odorata* L.) leaves for multi-drug resistant organisms bacteria. Biog J Ilm Biol. 2021; 9(1):26-34.
22. Okigbo R, Mbajaka C, Njoku C. Antimicrobial potential of (Uda) *Xylopia aethopica* and *Ocimum gratissimum* on some pathogens of man. Int J Mol Med Adv Sci. 2005; 1(4):392-397.
23. Richardson PM, Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Second Edition. Brittonia. 1990; 42(2):115-115.
24. Zayed MZ, Samling B. Phytochemical constituents of the leaves of leucaena leucocephala from malaysia. Int J Pharm Pharm Sci. 2016; 8(12):174-179.
25. Alagammal M, Tresina PS, Mohan VR. GC-MS determination of bioactive components of *Polygala javana* DC. Int J Curr Pharm Res. 2012; 4(2):42-44.
26. Trabalon M, Niogret J, Legrand-Frossi C. Effect of 20-hydroxyecdysone on cannibalism, sexual behavior, and contact sex pheromone in the solitary female spider, Tegenaria atrica. Gen Comp Endocrinol. 2005; 144(1):60-66.
27. Vivancos M, Moreno JJ. *β*-Sitosterol modulates antioxidant enzyme response in RAW 264.7 macrophages. Free Radic Biol Med. 2005; 39(1):91-97.
28. Gupta R, Sharma AK, Dobhal MP, Sharma MC, Gupta RS. Antidiabetic and antioxidant potential of *β*-sitosterol in streptozotocin-induced experimental hyperglycemia. J Diabetes. 2011; 3(1):29-37.
29. Sujatha S, Anand S, Sangeetha KN, Shilpa K, Lakshmi J, Balakrishnan A, Lakshmi BS. Biological evaluation of (3*β*)-stigmast-5-en-3-ol as potent anti-diabetic agent in regulating glucose transport using in vitro model. Int J Diabetes Mellit. 2010; 2(2):101-109.
30. Muñoz-Gómez RJ, Rivero-Cruz I, Ovalle-Magallanes B, Linares E, Bye R, Tovar AR, Noriega LG, Tovar-Palacio C, Mata R. Antidiabetic Sterols from *Peniocereus greggii* Roots. ACS Omega. 2022; 7(15):13144-13154.
31. Bharti SK, Kumar A, Sharma NK, Prakash O, Jaiswal SK, Krishnan S, Gupta AK, Kumar A. Tocopherol from seeds of *Cucurbita pepo* against diabetes: Validation by invivo experiments supported by computational docking. J Formos Med Assoc. 2013; 112(11):676-690.
32. Metwally NS, Mohamed AM, El Sharabasy FS. Chemical constituents of the Egyptian plant *Anabasis articulata* (Forssk) moq and its antidiabetic effects on rats with streptozotocininduced diabetic hepatopathy. J Appl Pharm Sci. 2012; 2(4):54-65.
33. Unnikrishnan PS, Suthindhiran K, Jayasri MA. Antidiabetic potential of marine algae by inhibiting key metabolic enzymes. Front Life Sci. 2015; 8(2):148-159.
34. Han YE, Kang CW, Oh JH, Park SH, Ku CR, Cho YH, Lee MK, Lee EJ. Olfactory Receptor OR51E1 Mediates GLP-1 Secretion in Human and Rodent Enteroendocrine L Cells. J Endocr Soc. 2018; 2(11):1251-1258.
35. Elmazar MM, El-Abhar HS, Schaalan MF, Farag NA. Elmazar, M. M., El-Abhar, H. S., Schaalan, M. F., & Farag, N. A. Phytol/Phytanic Acid and Insulin Resistance: Potential Role of Phytanic Acid Proven by Docking Simulation and Modulation of Biochemical Alterations. PLoS ONE. 2013; 8(1):1-10.
36. Villarroya F, Iglesias R, Giralt M. Retinoids and Retinoid Receptors in the Control of Energy Balance: Novel Pharmacological Strategies in Obesity and Diabetes. Curr Med Chem. 2005; 11(6):795-805.
37. Rayanil K, Sutassanawichanna W, Suntornwat O, Tuntiwachwuttikul P. A new dihydrobenzofuran lignan and potential α -glucosidase inhibitory activity of isolated compounds from *Mitrephora teysmannii*. Nat Prod Res. 2016; 30(23):2675-2681.
38. Ahmad S, Ullah F, Ayaz M, Ahmad A, Sadiq A, Mohani SN-U-H. Nutritional and medicinal aspects of *Rumex hastatus* D. Don along with in vitro anti-diabetic activity. Int J Food Prop. 2019; 22(1):1733-1748.
39. Won-jung K, Ji-sook K, Wook J, Song J-Y, Moon-seop L, Nam-doo K, Gwi-hyeon, S. 2,3-dihydrobenzofuran derivatives as an sglt inhibitor and pharmaceutical composition comprising same. South Korea; KR20150130177A, 2014:1-27.
40. El Barky AR, Hussein SA, Alm-Eldeen AA, Hafez YA, Mohamed TM. Anti-diabetic activity of Holothuria thomasi saponin. Biomed Pharmacother. 2016; 84:1472-1487.
41. Rajalakshmi K, Christian GJ, Shanmuga PP, Jeeva GR. Validation of Anti-diabetic Potential of Avirai kudineer a Siddha herbal formulation-A Review. IOSR J Dent Med Sci. 2015; 14(7):2279-2861.
42. Fazelipour S, Hadipour Jahromy M, Tootian Z, Goodarzi N. Antidiabetic effects of the ethanolic extract of *Allium saralicum* R.M. Fritsch on streptozotocin-induced diabetes in a mice model. Food Sci Nutr. 2021; 9(9):4815-26.
43. Sadiq A, Rashid U, Ahmad S, Zahoor M, AlAjmi A, Ullah R, Noman OM, Ullah F, Ayaz M, Khan I, Islam Z, Ali W. Treating Hyperglycemia from Eryngium caeruleum M. Bieb: In-vitro α-Glucosidase, Antioxidant, in-vivo Antidiabetic and Molecular Docking-Based Approaches. Front Chem. 2020; 8(558641):1-19.
44. de Mello VDF, Lindström J, Eriksson JG, Ilanne-Parikka P, Keinänen-Kiukaanniemi S, Pihlajamäki J, Tuomilehto J, Uusitupa M. Markers of cholesterol metabolism as biomarkers in predicting diabetes in the Finnish Diabetes Prevention Study. Nutr Metab Cardiovasc Dis. 2015; 25(7):635-642.
45. Fleisher LA. Phytosterols. In: Essence of *Anesthesia Practice*. Elsevier; 2011:682.
46. Wang HX, Ng TB. Natural products with hypoglycemic, hypotensive, hypocholesterolemic, antiatherosclerotic and antithrombotic activities. Life Sci. 1999; 65(25):2663-2677.
47. Payum T. GC-MS analysis of *Mussaenda roxburghii* Hk.f.: A folk food plant used among tribes of arunachal pradesh, India. Pharmacogn J. 2016; 8(4):395-398.
48. Crowe-McAuliffe C, Graf M, Huter P, Takada H, Abdelshahid M, Nováček J, Murina V, Atkinson GC, Hauryliuk, V, Wilson, DN. Structural basis for antibiotic resistance mediated by the *Bacillus subtilis* ABCF ATPase VmlR. Proc Natl Acad Sci U S A. 2018; 115(36):8978-8983.
49. Hodgson JM, Croft KD, Woodman RJ, Puddey IB, Bondonno CP, Wu JHY, Beilin LJ, Lukoshkova EV, Head GA, Ward NC. Effects of vitamin E, vitamin C and polyphenols on the rate of blood pressure variation: results of two randomised controlled trials. Br J Nutr. 2014; 112(9):1551-61.

50. Ward NC, Wu JH, Clarke MW, Puddey IB, Burke V, Croft KD, Hodgson JM. The effect of vitamin E on blood pressure in individuals with type 2 diabetes: a randomized, double-blind, placebo-controlled trial. J Hypertens. 2007; 25(1):227-34.
51. Mohammad A, Falahi E, Barakatun-Nisak MY, Hanipah ZN, Redzwan SM, Yusof LM, Gheitasvand M, Rezaie F. Systematic review and meta-analyses of vitamin E (alpha-tocopherol) supplementation and blood lipid parameters in patients with diabetes mellitus. Diabetes Metab Syndr Clin Res Rev. 2021; 15(4):102158.
52. Ble-Castillo JL, Carmona-Díaz E, Méndez JD, Larios-Medina FJ, Medina-Santillán R, Cleva-Villanueva G, Díaz-Zagoyabe, JC. Effect of α-tocopherol on the metabolic control and oxidative stress in female type 2 diabetics. Biomed Pharmacother. 2005; 59(6):290-195.
53. Wang F, Li H, Zhao H, Zhang Y, Qiu P, Li J, Wang S. Antidiabetic Activity and Chemical Composition of Sanbai Melon Seed Oil. Evidence-based Complement Altern Med. 2018; 2018: 5434156.
54. Heim M, Johnson J, Boess F, Bendik I, Weber P, Hunziker W, Flühmann B. Phytanic acid, a natural peroxisome proliferator-activated receptor (PPAR) agonist, regulates glucose metabolism in rat primary hepatocytes. FASEB J. 2002; 16(7):718-720.
55. Nauck MA, Meier JJ. Incretin hormones: Their role in health and disease. Diabetes, Obes Metab. 2018; 20(8):5-21.
56. Conforti F, Loizzo MR, Statti GA, Menichini F. Comparative Radical Scavenging and Antidiabetic Activities of Methanolic Extract and Fractions from Achillea ligustica ALL. Biol Pharm Bull. 2005; 28(9):1791-1794.
57. Simonen P, Gylling H, Miettinen TA. The validity of serum squalene and non-cholesterol sterols as surrogate markers of cholesterol synthesis and absorption in type 2 diabetes. Atherosclerosis. 2008;197(2):883-888.
58. Ooi EMM, Ng TWK, Chan DC, Watts GF. Plasma markers of cholesterol homeostasis in metabolic syndrome subjects with or without type-2 diabetes. Diabetes Res Clin Pract. 2009; 85(3):310-316.
59. Gylling H, Plat J, Turley S, Ginsberg HN, Ellegård L, Jessup W, Jones PJ, Lütjohann D, Maerz W, Masana L, Silbernagel G, Staels B, Borén J, Catapano AL, Backer GD, Deadfield J, Descamps OS, Kovanen PT, Riccardi G, Tokgözoglu L, Chapman MJ. Plant sterols and plant stanols in the management of dyslipidaemia and prevention of cardiovascular disease. Atherosclerosis. 2014; 232(2):346-360.
60. Pihlajamäki J, Gylling H, Miettinen TA, Laakso M. Insulin resistance is associated with increased cholesterol synthesis and decreased cholesterol absorption in normoglycemic men. J Lipid Res. 2004; 45(3):507-512.