Trop J Nat Prod Res, October 2025; 9(10): 5016 - 5024 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

In-vitro and *In-silico* Assessment of the Antidiabetic and Antioxidant Potential of *Urena lobata* Leaf Fractions

Yudi Purnomo[1]*, Andri Tilaqza[1], Denis M Mirza[1], Apon Z Mustopa[2]

[1]*Pharmacy Department, Faculty of Medicine, University of Islam Malang, MT. Haryono Street 193, Malang, 65144, Indonesia.*
[2]*Research Center for Genetic Engineering, Research Organization for Life Science and Environment, National Research and Innovation Agency (BRIN), KST Soekarno, Cibinong 16911, Indonesia.*

ARTICLE INFO

Article history:
Received 13 August 2025
Revised 25 September 2025
Accepted 04 October 2025
Published online 01 November 2025

ABSTRACT

Urena lobata (*U. lobata*), an ethnomedicinal plant, has long been utilized to treat diabetes, and animal studies have demonstrated its hypoglycemic activity. However, the mechanism underlying its anti-hyperglycemic effect and active compounds of herbs remains unclear. The study aim to investigate the antidiabetic and antioxidant potential of *U. lobata* leaf fractions using *in vitro* *α*-glucosidase inhibition and antioxidant assays, complemented by *in silico* molecular docking. Simplicia of *Urena lobata* leaves were extracted by the digestion method using methanol solvent. The extract was fractionated by n-hexane, ethyl acetate, n-butanol, and water, and designated as Fractions A, B, C and D, respectively. The *α*-glucosidase inhibition assay was conducted using p-nitrophenyl-*α*-D-glucopyranoside (p-NPG), and antioxidant potential was measured utilizing the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The most active fraction was analyzed via gas chromatography-mass spectrometry (GC-MS), and major phytoconstituents were docked *in silico* against *α*-glucosidase and aldose reductase using PyRx and Discovery Studio. Fraction C exhibited the strongest inhibitory activity against both *α*-glucosidase (IC_{50} = 66.87 ± 2.73 ppm) while Fraction B demonstrated stronger inhibitory activity against free radicals (IC_{50} = 50.68 ppm), compared to other fractions, though the fractions are lower than reference standards acarbose and ascorbic acid, respectively. Phytochemical analysis using GC-MS identified bioactive compounds, which were further confirmed by molecular docking. Pentatriacontane demonstrated optimal interactions with *α*-glucosidase (3TOP), and heneicosane showed optimal interactions with aldose reductase (1IEI). *Urena lobata* leaf extract and its phytoconstituents could be a promising source for novel antidiabetic and antioxidant agents.

***Keywords*:** Diabetes mellitus, Antidiabetic, Antioxidant, Alpha-glucosidase, Fraction, Urena lobata.

Introduction

The metabolic disease known as diabetes mellitus (DM) is typified by hyperglycemia brought on by anomalies in carbohydrate, fat, and protein metabolism due to insulin resistance, insulin deficiency, or its combination.[1] According to the *Diabetes Atlas* (2021), approximately 537 million people are currently living with DM, a number that is expected to increase by almost 46% to 643 million by 2030 and 783 million by 2045.[2,3] In 2017, DM was a leading cause of death globally, accountable for about 5 million deaths, and the disease is thought to cost the world's healthcare system USD 850 billion.[4] Diabetes is associated with a range of complications, including both micro- and macrovascular conditions due to oxidative damage.[5]

*Corresponding author Email: yudi.purnomo@unisma.ac.id
Tel: +62-341578920

Citation: Purnomo Y, Tilaqza A, Mirza DM, Mustopa AZ. *In Vitro* and *in Silico* Assessment of the Antidiabetic and Antioxidant Potential of *Urena Lobata* Leaf Fractions. Trop J Nat Prod Res. 2025; 9(10): 5016 – 5024 https://doi.org/10.26538/tjnpr/v9i10.43

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Individuals with DM typically experience elevated oxidative stress brought on by chronic, ongoing hyperglycemia, which impairs the antioxidant defense system and increases free radical production.[6] In diabetic patients, free radicals are a contributing factor to both micro- and macroangiopathy.[7] Excessive reactive oxygen species (ROS) production disrupts the body's antioxidant defenses, leading to oxidative stress that damages cell membranes and vital macromolecules, like lipid, proteins, and DNA.[8] Excess free radicals are produced in diabetes by non-enzymatic protein glycation and glucose oxidation, causing cellular damage that weakens the antioxidant defense system and contributes to complications, such as nephropathy, retinopathy, and neuropathy.[9,10] Recently, attention has shifted toward exploring natural sources of medicine to mitigate the effects of diabetes.

Indonesia is an archipelago state having the highest number of traditional communities and biodiversity in the world. Each community has local knowledge and practices in utilizing plants for primary health care. It is a part of local culture that is transmitted from one generation to the next and known by local wisdom.[11] Herbal remedies are commonly used in the management of DM due to their medicinal properties, ease of accessibility, affordability, and minimal processing requirements.[12] Plants rich in natural antioxidants have demonstrated antidiabetic effects in various studies, with bioactive compounds, such as phenolics and flavonoids, shown to lower blood glucose levels.[2] Antioxidants play a crucial role in protecting against diabetes by preserving beta-cell function and reducing complications.[13] Secondary metabolites include polyphenols, such as phenolic acids, stilbenes, flavonoids, and curcuminoids, which have antioxidant properties by scavenging ROS.

Around the world, the medicinal plant known as pulutan (*Urena lobata*) is found in tropical and subtropical areas. This plant can be found in Africa, Asia, South America, and Central America. In Indonesia, *Urena lobata (U. lobata)* grows as a wild plant, and it is used as a disinfectant and to cure wounds and fever. Nigerians use the plant's roots as a medicinal herb to treat DM.[14] The concept that this plant might be able to treat diabetes stems from its bitter flavor. According to earlier research, rats given streptozotocin have hypoglycemic effects when *U. lobata* leaf extracts are administered.[14,15] Extracts from *U. lobata* leaves have demonstrated anti-diabetic qualities, particularly in relation to their dipeptidyl peptidase-4 (DPP-4) and *α*-glucosidase inhibitory activity.[16,17] Additionally, several studies have demonstrated that *U. lobata* possesses antioxidant activity. However, the specific phytoconstituents responsible for these activities remain poorly characterized, primarily due to the complexity of compounds present in crude extracts. Fractionation is one of the methods employed to separate active compound groups based on the polarity using some organic solvents. The use of a range of solvents from non-polar to polar in fractionation allows for the evaluation of the pharmacological effects of individual extract components. Until now, the bioactivity test of *U.lobata* leaf fractions have not been done completely and this is the initial stage of discovering compounds that have medicinal properties. Therefore, this study was conducted to evaluate the antidiabetic potential of *U. lobata* leaf fractions through *α*-glucosidase inhibitory and antioxidant assays, supported by *in silico* analysis.

Materials and Methods

Sample preparation

Simplicia of *Urena lobata* leaf was obtained in January 2021 with certificate number 074/027/101.8/2021 from UPT Laboratorium Herbal Materia Medika Batu, Indonesia (-7.86754, 112.51924). The simplicia powder (1000 g) was subjected to extraction in methanol (E.Merck, pro analysis) at a 1:5 ratio using a shaker for 6 hours and repeated three times using fresh solvent. After evaporating the solvent, the concentrated extract (30 g) was fractionated by partitioning employing n-hexane (E.Merck, pro analysis) solvent, ethyl acetate (E.Merck, pro analysis), *n*-butanol (E.Merck, pro analysis) and water. The fractions were designated as Fractions A, B, C, and D, respectively. Each fraction was evaporated to produce a paste form.

Antidiabetic assay using α-glucosidase inhibitory activity

To evaluate *U. lobata* leaf extract and its fractions' *α*-glucosidase inhibitory activity, a 0.1 M potassium phosphate buffer (p-NPG) substrate solution was used. The reagen for *α*-glucosidase inhibitory activity from Sigma aldrich (pro analysis grade). Similarly, potassium phosphate buffer was employed to dissolve 0.1 unit/mL *α*-glucosidase. All samples were dissolved in methanol (E.Merck, pro analysis), and 30 µL of each sample or control was mixed with 30 µL of *α*-glucosidase solution and 60 µL of p-nitrophenyl-*α*-D-glucopyranoside (p-NPG) as the substrate. The reaction was halted by adding 120 µL of 0.2 M sodium carbonate in phosphate buffer (pH 6.8) after 40 minutes of incubation at 37°C. Acarbose was utilized as the reference medication, and the amount of released p-nitrophenol (p-NP) was then measured at 405 nm using a microplate reader (Thermo Scientific). The % inhibition was calculated using equation 1

$$\%\ \ inhibition = \frac{(Absorbance_{control} - Absorbance_{sampel})}{Absorbance_{control}}\ x\ 100\%$$

…..(equation 1)

Antioxidant assay using DPPH

The antioxidant activity of *U. lobata* leaf extract and its fractions was determined by employing the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. The reagen for antioxidant activity from Sigma aldrich (pro analysis grade). All samples were dissolved in methanol, and 2.0 mL of each sample or control was added to 2.0 mL of 0.1 mM DPPH solution (prepared in methanol) in a microplate. The mixture was incubated in the dark for 30 minutes. A spectrophotometer (Shimadzu) was used to measure the solution's absorbance at 517 nm, and ascorbic acid (50 mg/mL) was used as the reference standard. The % inhibition of DPPH was calculated using equation 1. The antioxidant activity index (AAI) of each extract or fraction was computed by dividing the final concentration of DPPH by the IC_{50} value of the respective extract or fraction, as described by Scherer and Godoy.[18]

Identification of phytoconstituents

The most potent antidiabetic and antioxidant fraction of *U. lobata* leaf was dissolved in methanol, and a Shimadzu QP2010 Ultra gas chromatography-mass spectrometry (GC-MS) system was filled with 1 µL of the solution. The GC analysis was performed using an Rtx®-5MS capillary column (30 m Å~ 0.25 mm Å~ 0.25 µm; Crossbond® 5% diphenyl / 95% dimethylpolysiloxane). The oven temperature was conditioned to rise from 60 to 325 °C at a rate of 15 °C per minute, holding at 60 °C for two minutes and at 325 °C for six minutes. The injector and detector temperatures were both set at 230°C. Splitless mode and a carrier pressure of 68.4 kPa were employed. In electron impact mode (eV) on a Shimadzu ISQ single quadrupole mass spectrometer, phytoconstituents were identified and their structures verified. The MS data was recorded using the full scan mode, which has a mass range of m/z 50-550 amu.

Molecular docking study

An MSI operating system, a 12th Gen Intel(R) Core (TM) i7-12650H running at 2.70 GHz, 16 GB of RAM, and an NVIDIA GeForce RTX 3050 were used to perform the computational analysis. PubChem (https://pubchem.ncbi.nlm.nih.gov) provided the phytoconstituents' chemical structures, which were then further optimized to produce geometrically stable conformations appropriate for *in silico* analysis. The Protein Data Bank (https://www.rcsb.org/) provided the macromolecular protein targets aldose reductase (PDB ID: 1IEI) and *α*-glucosidase (PDB ID: 3TOP). The PyRx 0.8 program (Sarkis, USA) was used to carry out the molecular docking. Next, Discovery Studio Visualizer V21.1.0.20298 (Dassault Systèmes Biovia Corp., France) was utilized to visualize the results of the molecular docking simulations. To validate a docking protocol, the Root Mean Square Deviation (RMSD) of the native ligand's pose after docking is compared to its original crystallographic pose. An RMSD value below 2 Å generally indicates a successful and reliable docking.[19]

Statistical analysis

The data are shown as mean ± standard deviation (SD), and the IC_{50} value was calculated by employing linear regression analysis in the Statistical Package for the Social Sciences (SPSS; version 16.0).

Results and Discussion

Antidiabetic activity of Urena lobata leaf fractions and its molecular docking analysis

Using an *α*-glucosidase inhibition assay, the antidiabetic properties of *U. lobata* leaf extract and its fractions were assessed *in vitro* (Table 1). Based on these results, the inhibitory activity of the *n*-butanol fraction (Fraction C) from *U. lobata* on *α*-glucosidase was higher than the extract and other fractions. Moreover, compared to acarbose as a reference medication, the inhibitory activities were lower.

Phytoconstituents that have been identified in the Fraction C of *U. lobata* leaves (Fig. 1 and Table 2). Benzoic acid is the most prevalent phytoconstituent in fraction C of the leaf extract of *U. lobata*, according to the qualitative analysis conducted using GC-MS. Fraction C of the *U. lobata* leaf extract also contained other compounds, including n-hexadecanoic acid, tetracosane, and heneicosane eventhough the concentration was low.

The findings of the *in silico* examination of the preferred secondary metabolites in Fraction C of *U. lobata* leaf (Table 3). Molecular docking studies indicated that n-hexadecanoic acid has a low free binding energy. Meanwhile, heneicosane, tetracosane, 1-propene-1,2,3-tricarboxylic acid, and tributyl ester were higher than n-hexadecanoic. The amino acid residue interactions of n-hexadecanoic acid and 1-propene-1,2,3-tricarboxylic acid tributyl ester were found to be similar to those of acarbose, with approximately three shared residues.

Table 1: Inhibition percentage of *Urena lobata* leaf fractions on *α*-glucosidase

Sample	Concentration (ppm)	% inhibition	IC_{50} (ppm)
Extract	50	24.47 ± 0.85	72.64 ± 0.78
	100	85.80 ± 0.56	
	200	90.49 ± 0.21	
Fraction A	100	31.52 ± 1.59	202.15 ± 1.71
	200	53.76 ± 1.48	
	400	79.60 ± 0.97	
Fraction B	100	35.03 ± 3.17	152.29 ± 1.03
	200	68.01 ± 0.99	
	400	80.84 ± 0.71	
Fraction C	50	36.87 ± 1.19	66.87 ± 11.03
	100	73,17 ± 2,14	
	200	85.06 ± 0.52	
Fraction D	200	11.77 ± 0.68	885.72 ± 13.54
	400	26.58 ± 3.53	
	800	44.58 ± 0.26	
Acarbose*	6,25	15.53 ± 3.92	14.26 ± 0.47
	12,5	50.36 ± 1.51	
	25,0	87.43 ± 0.45	

A: n-hexane; B: ethyl acetate; C: *n*-butanol; D: water; *: reference drug

The study demonstrated that the *α*-glucosidase inhibitory activities of *U. lobata* leaf fractions varied, likely due to differences in their phytochemical compositions, which influence their interactions with the enzyme. Fraction C of *U. lobata* is more potent than other fractions. They contain compounds, such as benzoic acid, *n*-hexadecanoic acid, tetracosane, and heneicosane, based on GC-MS analysis. Benzoic acid can interact with *α*-glucosidase, potentially increasing their effectiveness as inhibitors,[20] whereas n-hexadecanoic acid not only has the potential to inhibit *α*-glucosidase enzyme but also *α*-amylase enzyme.[21] The *U. lobata* leaf extract exhibited the second-highest *α*-glucosidase inhibitory activity, which may be attributed to its rich content of quercetin and flavone derivatives, such as gossypetin and chrysoeriol. Notably, its total phenolic content reached 25%.[14]

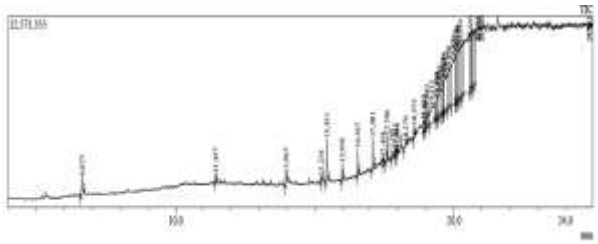

Figure 1: GC-MS chromatogram from n-butanol fractions (Fraction C) of *Urena lobata* leaf

Flavonoids, including quercetin, have been widely recognized as effective *α*-glucosidase inhibitors.[22] Gossypetin and chrysoeriol also have potential in inhibiting the enzyme *α*-glucosidase.[23,24] Quercetin, gossypetin, and chrysoeriol can lower blood glucose levels by preventing the activity of *α*-glucosidase, thereby reducing glucose absorption in the intestine.[22,23,24] The inhibitory activity against the *α*-glucosidase enzyme is not only influenced by the type of compound present but also by other factors, such as the levels of isoflavones contained in the herbs, molecular size, and structural variations of tannins. Isoflavones, for example, have stronger inhibitory activity compared to other flavone compounds. Research shows that isoflavones can bind to the active site of *α*-glucosidase and inhibit the activity of this enzyme significantly.[24] The activity of *α*-glucosidase is also influenced by the molecular structure on its binding side. *α*-glucosidase is generally more effectively inhibited by tannin structures that are bulkier and more complex. This is supported by studies showing that tannins with larger polymer structures can interact better with the enzyme, thus increasing their effectiveness as inhibitors.[25,26] In this context, Fraction C of *U. lobata*, which is rich in complex compounds, has greater potential than other fractions.

n-Hexadecanoic acid, identified in Fraction C, exhibited the strongest binding affinity to *α*-glucosidase among the tested compounds, with a binding energy of –6.0 kcal/mol, although still lower than the standard inhibitor acarbose (–8.9 kcal/mol). It was able to interact with the active site of the *α*-glucosidase enzyme through hydrophobic interactions involving Trp1523, Phe1559, and His1584, as well as hydrogen bonding with Asp1157 and Arg1510. Since it has a long carbon chain that is hydrophobic, this molecule can mimic the structure of the hydrophobic part of the carbohydrate substrate normally catalyzed by *α*-glucosidase. By occupying the active site, n-hexadecanoic acid blocks the entry of the actual substrate, thereby competitively inhibiting the enzyme activity.[27] The second and third orders after n-hexadecanoic acid are heneicosane and tetracosane, with binding energies of -3.5 kcal/mol and -2.0 kcal/mol, respectively. Heneicosane and tetracosane have similarities with acarbose in binding to one of the active sites of the *α*-glucosidase enzyme, Phe1560 and Phe1559, through hydrophobic bonds. Hydrophobic interactions with phenylalanine residues in the enzyme's active site can interfere with the binding of natural substrates (carbohydrates) to the *α*-glucosidase enzyme. This binding may lead to conformational changes in the enzyme, which may block substrate access to the catalytic site, thereby reducing enzymatic activity.[28]

Antioxidant activity of Urena lobata leaf fractions and its molecular docking analysis

Antioxidant potential of *U. lobata* leaf extract and fractions was examined *in vitro*, and the results (Table 4) indicated that the inhibition activity of the *ethyl acetate* fraction (Fraction B) of *U. lobata* on free radicals is higher than the extract and other fractions. As a reference medication, ascorbic acid exhibited higher inhibitory activities.

Phytoconstituents of Fraction B of *U. lobata* leaf extract that were identified (Fig. 2 and Table 5). Caffeine is the most prevalent secondary metabolite in Fraction B of *U. lobata* leaf extract, according to the qualitative analysis conducted by GC-MS. Fraction B of *U. lobata* was also found to contain additional substances, including loliolide, neophytadiene, caffeine, n-hexadecanoic acid, and 6,10,14-trimethyl-2-pentadecanone. Even though the concentration of the compounds was low.

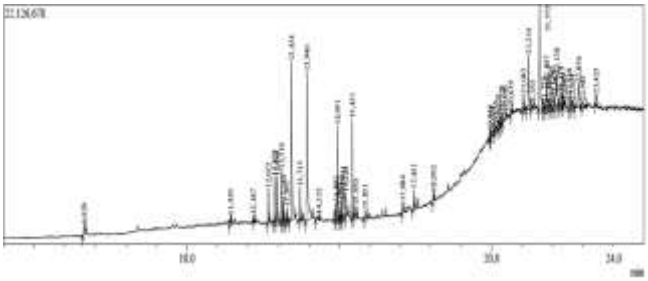

Figure 2: GC-MS chromatogram of ethyl acetate fraction (Fraction B) from *Urena lobata* leaf

Table 2: Identified compounds in n-butanol fraction (Fraction C) of *Urena lobata* leaf by GC-MS analysis

Peak	R. Time	Area (%)	Similarity	Compound
1.	6.625	1.34	90	3-Hydroxy-3-phenylbutan-2-one
2.	11.437	0.44	92	Diethyl Phthalate
3.	13.967	0.75	90	n-Hexadecanoic acid
4.	15.431	2.09	94	Butyl citrate
5.	17.081	1.36	95	Tetracosane
6.	17.458	0.34	76	Bis(2-ethylhexyl) phthalate
7.	17.596	1.23	94	Heneicosane
8.	17.765	0.52	77	Tetratriacontyl pentafluoropropionate
9.	17.907	0.52	78	Octacosane, 2-methyl-
10.	17.966	0.26	73	3-Methylhexacosane
11.	18.270	0.47	69	Eicosyl nonyl ether
12.	18.572	0.63	90	Pentatriacontane
13.	18.910	0.72	61	Octadecane, 1,1'-[(1-methyl-1,2-ethanediyl)bis(oxy)]bis-
14.	18.960	0.90	43	2-Propenoic acid, 3-(2,4-dimethoxyphenyl)-, E)-
15.	19.040	1.09	68	Heptacosane
16.	19.215	3.97	66	17-Pentatriacontene
17.	19.380	1.69	57	2,6-Lutidine 3,5-dichloro-4-dodecylthio-
18.	19.405	2.18	59	Cyclotrisiloxane, hexamethyl-
19.	19.480	4.78	59	Heptadecane, 8,8-dipentyl-
20.	19.580	2.58	56	Cyclohexane, tetradecyl-
21.	19.645	2.56	54	2-Hydroxy-4-methoxynicotinonitrile, TMS
22.	19.675	6.01	55	4-tert-Butylphenol, TMS derivative
23.	19.800	6.56	53	Propanenitrile,3-(5-diethylamino-1-methyl-3pentynyloxy)-
24.	19.960	13.48	40	Benzoic acid, 2-hydroxy-, 2-ethoxy-2-oxoethyl ester, TMS
25.	20.071	2.54	47	Ethyl homovanillate, TMS derivative
26.	20.110	2.60	70	4-Methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene, 2TMS derivative
27.	20.140	7.51	61	4-Methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene, 2TMS derivative
28.	20.230	8.30	54	[1,2,4] triazolo[1,5-a] pyrimidine-6-carboxylic acid, 7-amino-, ethyl ester
29.	20.320	6.37	56	1,4-Bis(trimethylsilyl) benzene
30.	20.585	6.62	69	4-Methyl-2,4-bis(p-hydroxyphenyl) pent-1-ene, 2TMS derivative
31.	20.636	4.71	46	Gliricidol
32.	20.740	2.47	64	1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-
33.	24.847	0.45	55	3-Phenyl-2H-chromene

R. Time: Retention time

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 3: Molecular docking of selected compounds from n-butanol fractions (Fraction C) of *Urena lobata* leaf with *α*-glucosidase

Compound	3TOP (*α*-glucosidase)	
	Binding affinity	Amino acid residue
Heneicosane	-3.5	Trp1523; **Phe1560**; His1584 (Alkyl) Phe1559 (Pi-Sigma) Asp1526 (Unfavorable)
Pentatriacontane	12.2	Trp1418; **Phe1559** (Alkyl) Tyr1251 (Pi-Sigma) Met1421; Arg1510; Trp1523; Asp1526; His1584 (Unfavorable)
Tertracosane	-2.0	Trp1523; **Phe1559**; His1584 (Alkyl) Trp1355; Met1421; Trp1523; Asp1526; (Unfavorable) Tyr 1251 (Pi-Sigma)
n-Hexadecanoic	-6.0	**Asp1157; Arg1510** (Hydrogen) Trp1523; **Phe1559**; His1584 (Alkyl)
Acarbose*	-8.9	Asp1157; Asp1279; Met1421; Arg1510; Asp1526; His1584 (Hydrogen) Asp1420 (Carbon-Hydro) Tyr1251; Phe1559; Phe1560 (Alkyl)

*: reference drug

Table 4: Antioxidant activity of *Urena lobata* leaf fractions

Sample	Concentration (ppm)	% inhibition	IC_{50} (ppm)	Antioxidant activity index
Extract	50	43.13 ± 2.33	173.03	0.231
	100	46.32 ± 1.47		
	200	48.80 ± 1.98		
	400	68.13 ±1.30		
Fraction A	50	9.76 ± 0.28	1434.79	0.027
	100	14.87 ± 1.60		
	200	20.85 ± 1.44		
	400	28.31 ± 0.83		
Fraction B	50	48.62 ± 0.96	50.68	0.789
	100	52.49 ± 2.45		
	200	56.16 ± 1.15		
	400	61.15 ± 1.47		
Fraction C	50	45.43 ± 3.48	104.05	0.388
	100	51.49 ± 0.62		
	200	55.31 ± 1.94		
	400	64.17 ± 1.00		
Fraction D	50	25.02 ± 2.21	682.16	0.058
	100	27.96 ± 1.52		
	200	33.32 ± 1.63		
	400	38.54 ± 3.03		
Ascorbic acid*	20	20.46 ± 4.12	43.31	0.923
	40	43.03 ± 3.22		
	60	79.51 ± 1.86		
	80	89.48 ± 0.37		

A: n-hexane; B: ethyl acetate; C: *n*-butanol; D: water; *: reference drug

Table 5: Identified compounds in ethyl acetate fraction (Fraction B) of *Urena lobata* leaf by GC-MS

Peak	R. Time	Area (%)	Similarity	Compound
1.	6.626	0.86	91	3-Hydroxy-3-phenylbutan-2-one
2.	11.430	0.45	92	Diethyl Phthalate
3.	12.187	0.50	86	3-Buten-2-one, 4-(4-hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-
4.	12.672	1.90	91	Loliolide
5.	12.878	2.65	96	Loliolide
6.	12.966	2.51	87	(S,E)-4-Hydroxy-3,5,5-trimethyl-4-(3-oxobut-1-en-1-yl)cyclohex-2-enone
7.	13.119	2.33	97	Neophytadiene
8.	13.180	1.06	95	2-Pentadecanone, 6,10,14-trimethyl-
9.	13.291	0.77	90	Neophytadiene
10.	13.434	11.67	97	Caffeine
11.	13.715	1.59	97	Hexadecanoic acid, methyl ester
12.	13.946	10.57	95	n-Hexadecanoic acid
13.	14.332	0.77	65	3-Phenylcyclopentanecarboxylic acid
14.	14.892	1.07	93	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-
15.	14.951	4.53	98	Phytol
16.	15.000	0.45	89	Methyl stearate
17.	15.093	1.48	83	1,13-Tetradecadiene
18.	15.131	2.65	93	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
19.	15.220	2.09	92	Octadecanoic acid
20.	15.431	4.54	96	Butyl citrate
21.	15.550	0.63	63	4-Heptadecyne, 1-chloro-
22.	15.851	0.55	87	Nonacosanal
23.	17.084	0.81	94	Tetracontane
24.	17.451	0.92	94	Bis(2-ethylhexyl) phthalate
25.	18.092	0.45	92	Tetracontane
26.	19.954	0.55	58	Uvaol
27.	20.025	0.85	62	Cyclotrisiloxane, hexamethyl-
28.	20.125	0.81	45	Labd-8-en-15-ol
29.	20.225	0.77	64	Cholesta-4,6-dien-3-ol, (3.beta.)-
30.	20.328	1.25	73	alpha.-Tocopheryl acetate
31.	20.450	1.19	59	(3R,3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-dimethyloctahydro-1H-cyclopenta[1,3]cyclopropa
32.	20.639	0.46	76	Lup-20(29)-en-3-ol, acetate, (3.beta.)-
33.	21.043	1.31	77	Campesterol
34.	21.214	4.83	88	Stigmasterol
35.	21.355	0.55	72	Cyclotrisiloxane, hexamethyl-
36.	21.572	8.95	91	gamma.-Sitosterol
37.	21.725	0.83	76	Cyclotrisiloxane, hexamethyl-
38.	21.807	2.33	86	beta.-Amyrin
39.	21.880	0.79	65	Ethoxy(phenyl)silanediol, 2TMS
40.	21.965	1.45	80	beta.-Amyrin
41.	22.065	1.47	61	Ginsenol
42.	22.138	3.85	91	alpha.-Amyrin
43.	22.271	1.18	57	Stigmasta-3,5-dien-7-one
44.	22.385	0.52	59	Glutinol
45.	22.548	1.50	83	Stigmast-4-en-3-one
46.	22.654	0.88	55	Ginsenol
47.	22.856	3.08	74	Lanosterol
48.	22.985	0.74	68	4,4'-bi-4H-pyran, 2,2',6,6'-tetrakis(1,1-dimethylethyl)-4,4'-dimethyl-
49.	23.423	0.96	84	Friedelan-3-one

R. Time: Retention time

The findings of the *in silico* examination of a few chosen secondary metabolites in Fraction B of the leaf extract of *U. lobata* are based *on* ≥ 90% similarity with the GC-MS internal compound database (Table 6). Molecular docking showed that 6,10,14-trimethyl-2-pentadecanone has a low free energy of binding. Meanwhile, neophytadiene, phytol, and n-hexadecanoic acid exhibited lower binding affinities compared to 6,10,14-trimethyl-2-pentadecanone. The latter compound shared approximately three amino acid residue interactions with acarbose, indicating a potential similarity in binding behavior.

Table 6: Molecular docking of selected compounds in ethyl acetate fraction (Fraction B) of *Urena lobata* leaf with aldose reductase

Compound	1IEI (aldose reductase)	
	Binding affinity	**Amino acid residue**
3-Hydroxy-3-phenylbutan-2-one	-6.9	**Cys298** (Hydrogen) **Cys303** (Alkyl) Leu300 (Pi-sigma) Trp111 (Pi-stacked)
Loliolide	-6.6	**Tyr48** (Hydrogen) Trp20; Val47; Phe122; Trp219 (Alkyl)
Neophytadiene	-7.2	Val47; Trp79; Cys80; His110; Phe115 Phe122; Tyr209; Trp219; Cys298; **Cys303**; **Tyr309** (Alkyl) Trp20; Trp111 (Pi-Sigma)
6,10,14-trimethyl-2-Pentadecanone	-7.4	**Trp111** (Hydrogen) Val47; Trp79; Cys80; Phe122; Trp219; **Leu300**; **Cys303** (Alkyl) Trp20 (Pi-sigma)
Caffeine	-5.3	**Tyr48; Cys298** (hydrogen) Trp111 (Alkyl) Trp20 (Pi Sigma) Tyr209 (Carbon Hydro)
n-Hexadecanoic	-6.6	**His110** (Hydrogen) Cys80; Trp111; **Cys303** (Alkyl)
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	-7.1	Trp20 (Hydrogen) Trp79; Cys80; Phe115; Phe122; Leu300; Cys303 (Alkyl)
Phytol	-7.1	**Cys298** (Hydrogen) Trp20; Val 47; Trp79; Cys80; Phe122; Trp219; **Tyr309** (Alkyl) His110; Trp111 (Pi-sigma)
9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	-7.2	Tyr309 (Hydrogen) Phe122; Trp219; **Leu300** (Alkyl) Trp20 (PI-sigma)
Zenarestat*	-9.5	Tyr48; His110; Trp111; Cys289 (Hydrogen) Val47; Ala299 (Halogen) Leu300; Cys303; Tyr309 (Alkyl) Trp20 (Pi Stacked)

* : reference drug

The findings of the *in vitro* assays showed that *U. lobata* leaf extract exhibited not only *α*-glucosidase inhibitory activity but also significant antioxidant activity, suggesting its potential dual role in managing DM. The description of the antioxidant assay in this study was observed in Fraction B of *U. lobata* leaf extract (Table 4) with an IC_{50} value of 50.68 ppm. This value is comparable to that of ascorbic acid, the standard antioxidant, which exhibited an IC_{50} value of 43.31 ppm, whereas other fractions showed IC_{50} values exceeding 100 ppm. These results indicated that Fraction B of *U. lobata* leaf extract has a relatively strong antioxidant potential.[29] The antioxidant activity observed in Fraction B of *Urena lobata* leaf extract is likely attributed to the existence of neophytadiene, 6,10,14-trimethyl-2-pentadecanone, and 9,12,15-octadecatrienoic acid (Z,Z,Z)-, which were qualitatively identified using GC-MS analysis.[30,31] Functional groups such as alkenes, ketones, and carboxylates present in each compound affect the antioxidant activity of Fraction B of *U. lobata* leaf extract. The antioxidant potential of compounds containing these functional groups has not yet been fully elucidated.

Therefore, the activity of the three identified compounds was further investigated using molecular docking analysis targeting the aldose reductase enzyme.[32]

Molecular docking analysis revealed that the compounds present in Fraction B of *U. lobata* leaf extract exhibited good binding affinity toward the aldose reductase enzyme, indicating their potential antioxidant activity. This favorable affinity is supported by the binding energy values of each compound (Table 6). Compound 6,10,14-trimethyl-2-pentadecanone has the highest binding energy of -7.4 kcal/mol, so it could demonstrate its stability in binding to aldose reductase.[33] Based on the results, there are interesting phenomena observed in 6,10,14-trimethyl-2-pentadecanone with a carboxylate group that produced the highest energy compared to neophytadiene (-7.2 kcal/mol) and 9,12,15-octadecatrienoic acid, (Z,Z,Z)- (-7.2 kcal/mol). This phenomenon is likely attributed to the presence of the carboxylate group at the terminal end of 6,10,14-trimethyl-2-

pentadecanone, which may enhance its interaction with the target enzyme.[34] The carboxylate group has potential as an antioxidant compound by donating hydrogen to neutralize free radicals.[35] The potential of this compound as both an antioxidant and an aldose reductase inhibitor is further supported by its higher amino acid residue similarity, showing a 30% match with the native ligand, compared to the other compounds. The 6,10,14-trimethyl-2-pentadecanone has hydrogen bond interaction through Trp111 and also alkyl bond interaction through Leu300 and Cys303. This interaction may lead to reduced aldose reductase activity, similar to the inhibitory effects observed with Zenarestat or the native ligand in this study.[32] Based on these findings, 6,10,14-trimethyl-2-pentadecanone is predicted to have pivotal activity as an aldose reductase inhibitor in Fraction B of *U. lobata* leaf extract, even though it has a lower binding affinity than the native ligand. Moreover, the safety of the Fraction B of *U. lobata* has to be evaluated through toxicity tests. A previous study on *U. lobata* leaf extract showed a mild to moderate toxicity level in zebrafish (*Danio rerio*). [36] Additionally, a molecular docking study predicted that stigmasterol and *β*-sitosterol could be toxic in *U. lobata*.[36]

Conclusion

The *n*-butanol fraction (Fraction C) of *U. lobata* demonstrated strong antidiabetic potential through *α*-glucosidase inhibition, with *n*-hexadecanoic acid predicted as the active compound based on *in silico* analysis. Meanwhile, the ethyl acetate fraction (Fraction B) exhibited notable antioxidant activity, with 6,10,14-trimethyl-2-pentadecanone identified as a potential lead compound. Sub-fractionation process need to be done to know the phytoconstituent which is regulated the activity.

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.

Aknowledgements

The authors would like to thank the management of Talenta of the National Research and Innovation Agency (BRIN) and Research Grant of Medical Faculty of University of Islam Malang which supported the research activity.

References

1. Nathan DM, Buse JB, Davidson MB, Heine RJ, Holman RR, Sherwin R, Zinman B. Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care. 2009; 32(1): 193–203. https://doi.org/10.2337/dc06-9912
2. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, Stein C, Basit A, Chan JCN, Mbanya JC, Pavkov ME, Ramachandaran A, Wild SH, James S, Herman WH, Zhang P, Bommer C, Kuo S, Boyko EJ, Magliano DJ. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Res Clin Pract. 2022; 183: 109119. https://doi.org/10.1016/j.diabres.2021.109119
3. Singh SK, Singh R, Singh SK, Iquebal MA, Jaiswal S, Rai PK. Prevalence of obesity in newly onset diabetes mellitus and its relationship with uric acid: An Indian cross-sectional study. Int J Gen Med. 2023; 16: 1217–26. https://doi.org/10.2147/IJGM.S402683
4. Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, Malanda B. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract. 2018; 138: 271–281. https://doi.org/10.1016/j.diabres.2018.02.023
5. Fowler MJ. Microvascular and macrovascular complications of diabetes. Clin Diabetes. 2008; 26(2): 77–82. https://doi.org/10.2337/diaclin.26.2.77
6. Scott JA, King GL. Oxidative stress and antioxidant treatment in diabetes. Ann N Y Acad Sci. 2004; 1031(1): 204–213. https://doi.org/10.1196/annals.1331.020
7. Salem RM, Todd JN, Sandholm N, Cole JB, Chen W-M, Andrews D, Pezzolesi MG, McKeigue PM, Hiraki LT, Qiu C, Nair V, di Liao C, Cao JJ, Valo E, Onengut-Gumuscu S, Smiles AM, McGurnaghan SJ, Haukka JK, Harjutsalo V, Florez JC. Genome-wide association study of diabetic kidney disease highlights biology involved in glomerular basement membrane collagen. J Am Soc Nephrol. 2019; 30(10): 2000–2016. https://doi.org/10.1681/ASN.2019030218
8. He Y, Ding Y, Liang B, Lin J, Kim T-K, Yu H, Hang H, Wang K. A systematic study of dysregulated microRNA in type 2 diabetes mellitus. Int J Mol Sci. 2017; 18(3): 456. https://doi.org/10.3390/ijms18030456
9. Aruoma OI, Neergheen VS, Bahorun T, Jen LS. Free radicals, antioxidants and diabetes: Embryopathy, retinopathy, neuropathy, nephropathy and cardiovascular complications. Neuroembryol Aging. 2007; 4(3): 117–137. https://doi.org/10.1159/000109344
10. Luna C, Estévez M. Formation of allysine in *β*-lactoglobulin and myofibrillar proteins by glyoxal and methylglyoxal: Impact on water-holding capacity and in vitro digestibility. Food Chem. 2018; 271: 87–93. https://doi.org/10.1016/j.foodchem.2018.07.167
11. Bhagawan WS, Suproborini A, Putri DLP, Nurfatma A, Putra RT. Ethnomedicinal study, phytochemical characterization, and pharmacological confirmation of selected medicinal plant on the northern slope of Mount Wilis, East Java, Indonesia. Biodiversitas J Biol Divers. 2022; 23(8): 4303–13. https://doi.org/10.13057/biodiv/d230855
12. World Health Organization (WHO). WHO traditional medicine strategy 2014–2023. Geneva: WHO; 2013. Available from: http://apps.who.int/iris/bitstream/10665/92455/1/9789241506090_eng.pdf?ua=1 (Accessed 23.05.2022)
13. Shafras M, Sabaragamuwa R, Suwair M. Role of dietary antioxidants in diabetes: An overview. Food Chem Adv. 2024; 4: 100666. https://doi.org/10.1016/j.focha.2024.100666
14. Purnomo Y, Soeatmadji DW, Sumitro SB, Widodo MA. Anti-hiperglycemic Effect of *Urena lobata* Leaf Extract by Inhibition of Dipeptidyl Peptidase IV (DPP-IV) on Diabetic Rats. Int J Pharmacogn Phytochem Res. 2015; 7(5): 1073–1079.
15. Purnomo Y, Soeatmadji DW, Sumitro SB, Widodo MA. Inhibitory activity of *Urena lobata* leaf extract on dipeptidyl peptidase-4 (DPP-4): is it different in vitro and in vivo ?. J Med Plants. 2018; 10(2): 99–105. https://doi.org/10.5958/0975-6892.2018.00016.3
16. Purnomo Y, Makdasari J, Fatahillah FI. J Basic Clin Physiol Pharmacol. Inhibitory Activity of *Urena lobata* Leaf Extract on Alpha-amylase and Alpha-glucosidase: In vitro and In silico Approach. J Basic Clin Physiol Pharmacol 32(4): 889-94. 2021; 32(4): 889–894. https://doi.org/10.1515/jbcpp-2020-0430
17. Purnomo Y, Soeatmadji DW, Sumitro SB, Widodo MA. Dipeptidyl peptidase-4 inhibitory activity of Indonesian anti-diabetic herbs : *Carica papaya*, *Tithonia diversifolia*, *Urena lobata*. Res J Pharm Technol. 2023; 16(1): 273–2482. https://doi.org/10.52711/0974-360X.2023.00050

18. Ramírez D, Caballero J. Is it reliable to take the molecular docking top scoring position as the best solution without considering available structural data?. Molecules. 2018; 23(5), 1038. https://doi.org/10.3390/molecules23051038
19. Scherer R, Godoy HT. Antioxidant activity index (AAI) by the 2, 2-diphenyl-1-picrylhydrazyl method. Food Chem. 2019; 112(3):654– 658. https://doi.org/10.1016/j.foodchem.2008.06.026
20. Megawati, Darmawan A, Fajriah S, Primahana G, Dewi RT, Minarti, Meiliawati L. Antioxidant and *α*-glucosidase activities of benzoic acid derivate from the bark of *Myristica fatua* Houtt. In: Proceedings of the symposium on AIP Conference (Vol. 1904, No. 1). AIP Conf Proc. 2017; 1904(1). https://doi.org/10.1063/1.5011884
21. Wechakorn K, Payaka A, Masoongnoen J, Wattanalaorsomboon S, Sansenya S. Inhibition potential of n-hexadecanoic and oleic acids from edible insects against *α*-glucosidase, *α*-amylase, tyrosinase, and acetylcholinesterase: in vitro and in silico studies. J Sci Food Agric. 2025; 105(7): 3701–371. https://doi.org/10.1002/jsfa.14121
22. Günal-Köroğlu D, Catalkaya G, Yusufoğlu B, Kezer G, Esatbeyoglu T, Abd El-Aty AM, Capanoglu E. Quercetin: Potential antidiabetic effects through enzyme inhibition and starch digestibility. Food Saf Health. 2025; 3(1): 9–22. https://doi.org/10.1002/fsh3.12066
23. Khalid H, Butt MH, ur Rehman Aziz A, Ahmad I, Iqbal F, Shamim A, Nishan U, Ullah R, Ibrahim MA, Moura AA, Shah M, Sun W. Phytobioinformatics screening of ayurvedic plants for potential *α*-glucosidase inhibitors in diabetes management. Curr Plant Biol. 2024; 40: 100404. https://doi.org/10.1016/j.cpb.2024.100404
24. Lam T-P, Tran N-VN, Pham L-HD, Lai NV-T, Dang B-TN, Truong N-LN, Truong N-LN, Nguyen-Vo S-K, Hoang T-L, Mai TT, Tran T-D. Flavonoids as dual-target inhibitors against *α*-glucosidase and *α*-amylase: a systematic review of in vitro studies. Nat Prod Bioprospect. 2024; 14(1): 4. https://doi.org/10.1007/s13659-023-00424-w
25. Zhong Y, Ni D, Yang Y, Li Y, Wang L, Tian J, Yang F, Ye X. Inhibitory effect and mechanism of tannic acid against two starch digestive enzymes. Food Qual Saf. 2024; 8(7): 1–9. https://doi.org/10.1093/fqsafe/fyad057
26. Barrett A, Ndou T, Hughey CA, Straut C, Howell A, Dai Z, Kaletunc G. Inhibition of *α*-amylase and glucoamylase by tannins extracted from cocoa, pomegranates, cranberries, and grapes. J Agric Food Chem. 2013; 61(7): 1477–1486. https://doi.org/10.1021/jf304876g
27. Lu H, Yang Z, Yu M, Ji N, Dai L, Dong X, Xiong L, Sun Q. Characterization of complexes formed between debranched starch and fatty acids having different carbon chain lengths. Int J Biol Macromol. 2021; 167: 595–604. https://doi.org/10.1016/j.ijbiomac.2020.11.198
28. Lu H, Xie T, Wu Q, Hu Z, Luo Y, Luo F. Alpha-Glucosidase inhibitory peptides: Sources, preparations, identifications, and action mechanisms. Nutrients. 2023; 15(19): 4267. https://doi.org/10.3390/nu15194267
29. Molyneux P. The Use of the Stable Free Radical Diphenylpicrylhydrazyl (DPPH) for Estimating Antioxidant Activity. J Sci Technol. 2004; 26(2): 211–219.
30. Kim K-B, Nam YA, Kim HS, Hayes AW, Lee B-M. *α*-Linolenic acid: Nutraceutical, pharmacological and toxicological evaluation. Food Chem Toxicol. 2021; 70: 163–178. https://doi.org/10.1016/j.fct.2014.05.009
31. Endris YA, Abdu KY, Abate SG. Endris YA, Abdu KY, Abate SG. 2024. Investigation of Bioactive Phytochemical Compounds of the Ethiopian Medicinal Plant using GC-MS and FTIR. Heliyon. 2024; 10(15): e34687. https://doi.org/10.1016/j.heliyon.2024.e34687
32. Yuan C, Hao X. Antibacterial mechanism of action and in silico molecular docking studies of Cupressus funebris essential oil against drug resistant bacterial strains. Heliyon. 2023; 9(8): e18742. https://doi.org/10.1016/j.heliyon.2023.e18742
33. Bikadi Z, Hazai E. Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of AutoDock. J Cheminform. 2009; 1(15). https://doi.org/10.1186/1758-2946-1-15
34. Deiab GIA, Saadah LM, Basheti IA. Using Drug Chemical Structure in the Education Pharmacology and Clinical Therapeutics Key Concepts. Braz J Pharm Sci. 2022; 58: e21070. https://doi.org/10.1590/s2175-97902022e21070
35. Biela M, Kleinová A, Klein E. Phenolic acids and their carboxylate anions: Thermodynamics of primary antioxidant action. Phytochemistry. 2022; 200: 113254. https://doi.org/10.1016/j.phytochem.2022.113254
36. Purnomo Y, Aini N, Noerhayati E. Acute Toxicity Level of Pulutan (*Urena lobata*) Leaf Extract on Zebrafish (Danio rerio) and its Analysis by In silico Study. Res J Pharm Technol. 2022; 15(6): 2477–2482. https://doi.org/10.52711/0974-360X.2022.00413