Research Article

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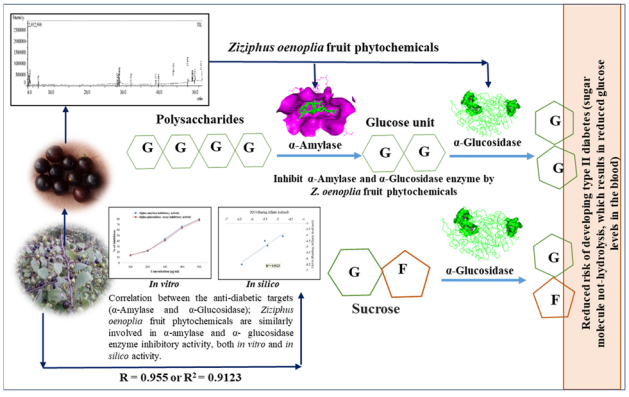
Assessment of anti-diabetic properties of *Ziziphus oenopolia* (L.) wild edible fruit extract: *In vitro* and *in silico* investigations through molecular docking analysis

https://doi.org/10.1515/chem-2024-0032
received February 27, 2024; accepted April 16, 2024

Abstract: Globally, healthcare is concerned about the rising prevalence of type 2 diabetes. Phytochemicals from medicinal plants have shown great promise in improving human health. The present study aimed to determine the secondary metabolites of *Ziziphus oenopolia* (L.) fruit extract that contribute to its anti-diabetic activity. The anti-diabetic properties were assessed by *in vitro* and *in silico* approaches using α-amylase and α-glucosidase inhibitory assays. Gas chromatography and mass spectroscopy analyses were used to profile *Z. oenopolia* fruit contents, and a total of four bioactive chemicals and eight phytocompounds were tentatively identified, including flavonoids, terpenoids, phenols, steroids, tannins, and saponins. The *Z. oenopolia* fruit hydroalcoholic extract inhibits α-amylase and α-glucosidase enzymes in a dose-dependent manner (IC_{50} = 328.76 and 337.28 µg/mL, R^2 = 0.979 and 0.981). Additionally, phytochemicals found in *Z. oenopolia* fruit exhibit the ability to inhibit anti-diabetic targets, specifically α-amylase and α-glucosidase (2QV4 vs 3A4A; correlation coefficient, r = 0.955), as demonstrated by computational analysis. This establishes the fruit as a promising and environmentally friendly option for treating hyperglycemia, highlighting the positive correlation between anti-diabetic objectives.



Graphical abstract: The evaluation of *in vitro* and *in silico* anti-diabetic activity of wild edible fruit *Ziziphus oenophilia* (L.) extract: molecular docking and statistical analysis.

Keywords: *Ziziphus oenopolia*, phytochemicals, anti-diabetic activity, α-amylase, α-glucosidase, molecular docking

1 Introduction

Diabetes mellitus (DM) is a life-threatening condition characterized by high blood sugar levels over time, reflecting

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one of the multifactorial problems associated with the disease [1]. Insulin resistance is a prevalent feature of type 2 diabetes and often affects adults. DM affects 422 million people globally, most of whom reside in low- and middle-income nations, and is directly responsible for 1.5 million deaths annually [2]. Figure 1 illustrates how the enzymes α-amylase and α-glucosidase hydrolyze carbohydrates and increase postprandial glucose levels. Additionally, the aim of controlling postprandial hyperglycemia was prevented by these enzymes. Because increased hyperglycemia damages the kidneys, heart, blood vessels, and nerves, it has become a serious health concern [3,4]. Many synthetic hypoglycemic medications are used to treat DM, but all have side effects [5]. Therefore, it is critical to discover new bioactive compounds with potent anti-diabetic actions and minimal adverse effects. The search for novel medications made of natural resources to treat DM is still ongoing. Around 60% of people worldwide utilize traditional medicines made from healing herbs, particularly in India, where diabetes is treated using herbal medications and plants [6,7]. Natural therapies have prevented many diseases and are also less harmful. Plant secondary metabolites play a role in the success and cost-efficiency of herbal therapies for diabetes [8].

Beneficial phytochemicals, also known as bioactive substances present in fruits, vegetables, and grains, are present in medicinal plants and aid in the prevention of illnesses and infections [9,10]. Numerous bioactive substances with particular biological characteristics and no negative consequences, such as polyphenols, alkaloids, terpenoids, and saponins, are abundant in many plants and have potentially synergistic effects [11]. This study uses the fruit of the *Ziziphus oenopolia* (L.) Mill medicinal plant, which is a member of the Rhamnaceae family and is utilized in traditional South Indian cuisine (Tamil name: Suraimullu, Surai ilanthai). In rural areas, *Z. oenopolia* has been used for its gastrointestinal, hypotensive, diuretic, wound healing, antibacterial, anti-inflammatory, antioxidant, antimicrobial, anti-inflammatory, and hepatoprotective effects [12–14]. Pancreatic α-amylase (AM2A), intestinal maltase-glucoamylase, dipeptidyl peptidase-4, liver receptor homolog-1 (NR5A2), retinol-binding protein-4, peroxisome proliferator-activated receptor alpha, and protein tyrosine phosphatase non-receptor type 9 were the main anti-diabetic targets identified.

The current study aimed to identify phytochemicals and test the anti-diabetic effects of the hydroalcoholic extract of *Z. oenopolia* in the laboratory and on a computer by inhibiting the activity of α-glucosidase and α-amylase enzymes. In the therapy for type 2 DM, α-glucosidase stands as a crucial target enzyme. Inhibition of this enzyme effectively reduces blood glucose levels. Similarly, the inhibition of α-amylase, an essential regulatory enzyme in diabetes, plays a significant role [15,16]. The inhibition of both of these enzymes constitutes a response to the regulation of hyperglycemia.

2 Materials and methods

2.1 Extraction and phytochemical screening of *Z. oenopolia* fruits

In January 2023, the fruits of *Z. oenopolia* were gathered from local villagers in the Thanjavur area. *Z. oenopolia* (Voucher ID: R.K.001) was collected from Thanjavur and deposited in St. Joseph College. Trichy, Tamil Nadu, India. To eliminate any traces of contaminants, the fruits of *Z. oenopolia* were first repeatedly rinsed with purified water. The fruits (seeds included) were roughly ground and dried at room

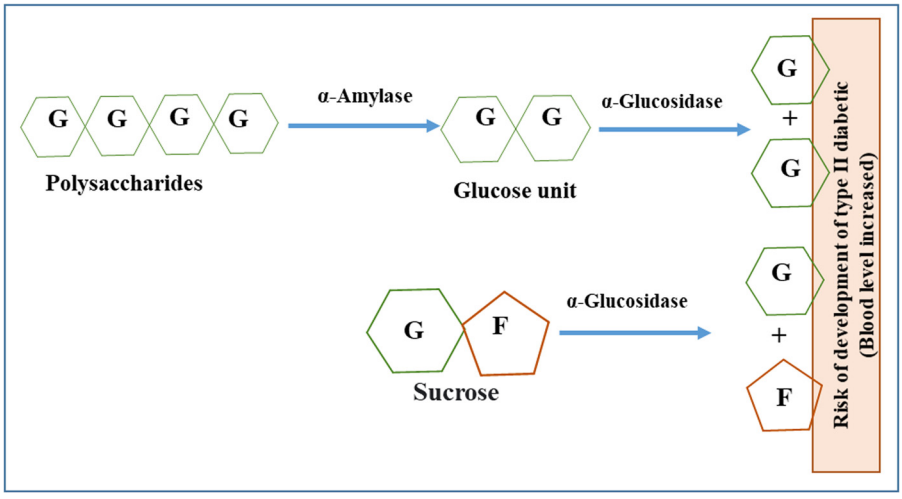


Figure 1: The α-amylase and alpha-glucosidase enzymes hydrolyze carbohydrates and increase postprandial glucose levels.

temperature to eliminate any remaining moisture. For the entire day, the powder was extracted using ethanol and an aqueous extract. This was done in the same way as Sofowara [17], Trease and Evans [18], and Harborne [19]. After 24 h of filtering and concentrating, the extract was tested for preliminary phytochemicals using standard methods. *Z. oenopolia* fruit powder was extracted by hydroalcoholic extraction using gas chromatography and mass spectrometry (GC-MS), and its anti-diabetic effects were tested *in vitro*. GC-MS analysis was carried out using a JEOL-GC MATE II. The sample was run in its entirety within the 50–650 *m/z* range, and the results were compared using the National Institute of Standards and Technology 14 Mass Spectral Library search.

2.2 Quantitative methodology

McDonald et al. employed Folin–Ciocalteu's reagent for the estimation of total phenol content by adding diluted extract with Folin–Ciocalteu's reagent and aqueous Na_2CO_3, heating at 45°C for 15 min, undergoing further investigation colorimetrically, calibrating and expressing in terms of standard gallic acid [20].

Olajire and Azeez estimated the total flavonoid content using the aluminum chloride method, which involves adding the methanolic extract to 5 mL double distilled water (ddH_2O) and 0.3 mL 5% $NaNO_2$. Then, 1.5 mL of 2% methanolic $AlCl_3$ was added to $NaNO_2$ at 5 min intervals. After 5 min, 2 mL of 1 mol dm^{-3} NaOH was added, making up the solution to 100 mL, and vigorously shaken for 5 min at 200 rpm. The solution was incubated for 10 min, and the absorbance was read. Flavonoid content was calculated using a standard calibration curve [21].

2.3 *In vitro* anti-diabetic activity of hydroalcoholic *Z. oenopolia* fruit extract

The Apostolidis et al.'s method was used to perform *in vitro* α-amylase and α-glucosidase inhibition assays. Different concentrations (100–500 µg/mL) of hydroalcoholic *Z. oenopolia* fruit extract have been used as natural inhibitors [22]. The IC_{50} and between-target protein relationships were calculated using statistical methods of regression and correlation using MS Excel.

2.4 Molecular docking study

Using GC-MS, the ligands were identified as phytochemicals in *Z. oenopolia* fruits. Selected phytochemicals were collected from the PubChem database, while anti-diabetic targets were retrieved from the Protein Data Bank (PDB). The ligands were changed to the PDB format using Open Bable software, and the target proteins (2QV4 and 3A4A) were removed. Prior to docking, all ligands and water molecules were eliminated. The produced protein was then stored as PDB and generated using the PyMOL program. PyRx 0.8, a virtual screening tool (Autodock Vina program) for grid dimensions (2QV4 = center_x = 16.73, center_y = 62.73, and center_z = 17.20), employed molecular docking software. The docked complexes (3A4A = center_x = 21.78, center_y = −0.1,5 and center_z = 18.46) were created by Trott and Olson (2010), and PyMOL and BIOVIA Discovery Studio Visualizer were used as visualization tools [23].

3 Results and discussion

3.1 Phytochemical profile of *Z. oenopolia* (L.) fruit extract

Recent research has revealed that biologically produced secondary metabolites from plants, microbes, and invertebrate animals have bioactive properties [24–26]. Several significant plant secondary metabolites, including terpenoids and flavonoids, are involved in the immunological responses of plants [24,27]. Plants produce secondary metabolites from a variety of plant components, including fruits, as part of a natural defense mechanism against environmental stressors. In this study, the researchers examined the phytochemicals found in the fruit of *Z. oenopolia* using both aqueous and ethanolic extracts. When *Z. oenopolia* fruit was extracted with water or alcohol, it exhibited a range of secondary moieties, which were confirmed through the screening process, as indicated in Table 1. There is a higher concentration of flavonoids, terpenoids, tannins, and quinones in ethanolic extracts than in aqueous extracts. Observations from Rathore et al. [28] were aligned with the presence of flavonoids, glycosides, phenolics, saponins, and sterols in *Z. mauritiana*, albeit with a notable absence of alkaloids, mirroring the outcomes of the current investigation. The lack of some phytochemicals, the various solvents employed, the extraction and analysis techniques, seasonal variations, and the collection location are only a few possible causes for this [29]. Kumar et al. [27] found that a greater number of compounds were soluble and present in aqueous ethanol compared to water or pure solvents alone. Numerous plant-derived phytochemical components have been linked to antioxidant, anti-inflammatory, anti-larvicidal, and antibacterial

Table 1: Phytochemical qualitative screening of *Z. oenopolia* fruit extract

S. No	Phytochemicals	Extract	
		Aqueous	Ethanolic
1	Flavonoids	+	++
2	Terpenoids	+	++
3	Glycosides	+	+
4	Polyphenol	++	++
5	Alkaloids	−	−
6	Coumarins	−	−
7	Steroids	+	+
8	Anthraquinones	+	+
9	Tannins	+	++
10	Quinones	+	+
11	Saponins	++	+

+; presence, −; absence and ++ higher concentration.

properties [9,30]. Fruits contain antioxidant and anti-inflammatory compounds like phenolics, alkaloids, and flavonoids, which can help treat diseases like cancer [31]. These compounds act as free-radical scavengers and reduce oxidative stress. However, this study did not explore how phytochemicals affect disease management, necessitating further research. Phenolic compounds found in fruit flesh and flavonoids in seeds contribute to their flavor and color, which aid in the reduction of many ailments [32,33]. The total phenol and flavonoid contents of *Z. oenopolia* fruit extract were estimated. The total phenolic content (151.21 ± 7.78 mg GAE/g) and flavonoid content (34.90 ± 3.67 mg QE/g) of *Z. oenopolia* is presented in Table 2. Phenolic compounds are potent antioxidants that increase the consumption of fruit.

Table 2: Phytochemical quantitative analysis of *Z. oenopolia* fruit extract

S. No	Phytochemicals	*Z. oenopolia* fruit
1	Total phenol (mg GAE/g)	151.21 ± 7.78
2	Flavonoids (mg QE/g)	34.90 ± 3.67

Values expressed as mean ± SD (*N* = 3).

The 70% ethanolic (hydroalcoholic) *Z. oenopolia* fruit extract was found to contain 18 phytochemicals, including 4 bioactive substances: heneicosane, quinic acid, 9-octadecenoic acid, methyl ester, and linoleic acid ethyl ester (Figure 2, Tables 3 and 4). By comparing a query mass spectrum with the reference data in a spectrum-matching library, the NIST database was used to interpret the GC-MS data. GC-MS analysis of Hibiscus asper leaves revealed phytochemical profiles that included flavonoids, tannins, phenols, saponins, alkaloids, glycosides, terpenoids, and steroids, along with 23 bioactive compounds in the aqueous methanol fraction, including phytol, *n*-hexadecanoic acid, octadecatrienol acid, methyl palmitate, and octadecatrienol acid [37,38]. Ten chemicals were identified in the chloroform-methanol extract of *Rhazya stricta* by Baeshen et al. [39]. These chemicals include methyl stearate, methyl palmitate, methyl tetradecanoate, (–)−1,2-Didehydroaspidospermidine, and methyl laurate. Phytochemicals, including tannins, glycosides, saponins, steroids, terpenoids, alkaloids, and flavonoids, were identified in the methanolic extract of Garcinia kola. Phytochemicals contained in *Z. oenopolia* fruit extract have been linked to biological activity, including antidiabetic effects.

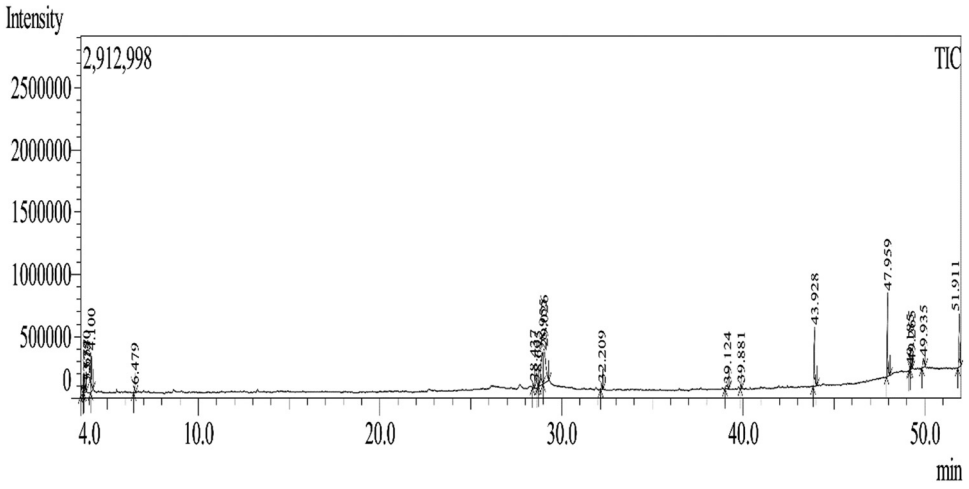
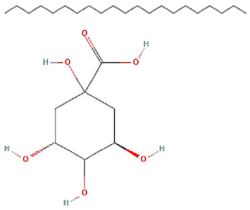


Figure 2: GC-MS chromatogram of the hydroalcoholic extract of *Z. oenopolia* fruit.

Table 3: Phytochemicals profile of the hydroalcoholic extract of *Z. oenopolia* fruit by GC-MS analysis

Peak #	R. time	Molecular weight (g/mol)	Molecular formula	Molecular name	Kovats index
1	3.575	89	$C_3H_7NO_2$	D-Alanine	855
2	3.657	252	$C_{12}H_{20}SSi_2$	Thiophene,2-(trimethylsilyl)-5-[(trimethylsilyl)ethynyl]-	1,318
3	3.740	68	C_4H_4O	Furan	553
4	4.100	92	$C_3H_8O_3$	Glycerin	967
5	6.479	148	$C_6H_{16}O_2Si$	1-[(Trimethylsilyl)oxy] propan-2-ol	766
6	28.437	296	$C_{21}H_{44}$	Heneicosane	2,109
7	28.695	202	$C_{12}H_{26}O_2$	4,5-Decanediol, 6-ethyl-	1,475
8	28.955	148	$C_6H_{12}O_4$	1,2,3,5-Cyclohexanetetrol, (1.alpha.,2.beta.,3.alpha.,5.beta.)-	1,472
9	29.026	192	$C_7H_{12}O_6$	Quinic acid	1,852
10	32.209	180	$C_{10}H_{12}O_3$	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	1,653
11	39.124	280	$C_{18}H_{32}O_2$	13-Hexyloxacyclotridec-10-en-2-one	2,325
12	39.881	296	$C_{19}H_{36}O_2$	9-Octadecenoic acid, methyl ester, (E)-	2,085
13	43.928	268	$C_{17}H_{32}O_2$	1,4-Dioxaspiro [4.14] nonadecane	2,171
14	47.959	340	$C_{20}H_{36}O_4$	Ethyl stearate, 9,12-diepoxy	2,281
15	49.185	308	$C_{20}H_{36}O_2$	Linoleic acid ethyl ester	2,193
16	49.265	884	$C_{57}H_{10}4O_6$	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E, E, E)-	6,149
17	49.935	372	$C_{20}H_{36}O_6$	Dicyclohexano-18-crown-6	2,856
18	51.911	406	$C_{25}H_{42}O_4$	Fumaric acid, 2-octyl tridec-2-yn-1-yl ester	2,802

Table 4: Identification of bioactive activities of the active compounds from the hydroalcoholic extract of *Z. oenopolia* fruit: data obtained by GC-MS techniques

S. no	Compound name	Biological activity	Chemical structure (from PubChem)
1	Heneicosane	Antimicrobial activity [34]	
2	Quinic acid	Antimicrobial activity [35]	
3	9-Octadecenoic acid, methyl ester,	Anti-inflammatory, antiandrogenic cancer preventive, dermatitigenic hypocholesterolemic, 5-alphareductase inhibitor, anemiagenic insectifuge, flavor [36]	
4	Linoleic acid ethyl ester	Anti-inflammatory, hypocholesterolemic, cancer preventive hepatoprotective, nematicide, insectifuge, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, insectifuge [35]	

3.2 *In vitro* and *in silico* anti-diabetic activity of *Z. oenopolia* fruit hydroalcoholic extract through inhibition of α-amylase and α-glucosidase enzymes

Potential anti-diabetic agents include α-amylase and α-glucosidase enzyme inhibitors, which can regulate hyperglycemia and lower the risk of diabetes. The study used a hydroalcoholic extract of *Z. oenopolia* fruit to test how well it blocked α-glucosidase and α-amylase enzymes in a laboratory setting. Recently, several researchers have studied a range of plant materials using the inhibitory activities of the α-amylase and α-glucosidase enzymes. The review's results are given in Table 5. The α-amylase IC_{50} ranged from 170 to 610 µg/mL

Table 5: Review of IC_{50} of various plant extracts through *in vitro* inhibitory activity on anti-diabetic targets α-amylase and α-glucosidase

S. No	Plant	α-amylase (IC50) (mg/mL)	α-glucosidase (IC50) (mg/mL)	References
1	*Quercus coccifera* (methanol extract)	0.17	0.38	Jaber [40]
2	*Cleistocalyx nervosum* (aqueous extract)	0.61 ± 0.09	0.44 ± 0.05	Chukiatsiri et al. [41]
3	*Cleistocalyx nervosum* (ethanolic extract)	0.42 ± 0.07	0.23 ± 0.04	Chukiatsiri et al. [41])
4	*Withania frutescens* (hydro-ethanolic extract)	0.40 ± 0.124	0.180 ± 0.018	Mechchate et al. [42]
5	*Chloroxylon swietenia* (aqueous extract)	446.7 ± 3.63	373.3 ± 4.41	Ramana Murty Kadali et al. [43]
6	*Chloroxylon swietenia* (ethanolic extract)	233.3 ± 4.17	236.7 ± 1.67	Ramana Murty Kadali et al. [43]

in both alcoholic and water-based extracts, while the α-glucosidase IC_{50} ranged from 180 to 440 µg/mL in both [40–43]. The current inhibitors of *Z. oenopolia* fruit hydroalcoholic extract have identified α-amylase (IC_{50} = 328.76 µg/mL) and α-glucosidase (IC_{50} = 337.28 µg/mL), while the standard drug (acarbose) was evaluated *in vitro* for α-amylase (IC_{50} = 281.95 µg/mL) and α-glucosidase (IC_{50} = 304.72 µg/mL). These findings suggest that *Z. oenopolia* fruit hydroalcoholic extract has the potential to be used as an anti-diabetic medication. In both the α-amylase and α-glucosidase tests (Figure 3), the hydroalcoholic extract of *Z. oenopolia* fruit had a dose-dependent inhibitory effect, with correlation coefficient statistics agreeing at R^2 = 0.979 for the α-amylase assay and R^2 = 0.981 for the α-glucosidase assay. According to Sakulkeo et al., scopoletin, *N*-trans-feruloyltyramine, and *N-trans*-coumaroyltyramine were three separate compounds that had IC_{50} values of 110.97, 29.87, and 0.92 µg/mL, respectively [15]. Additionally, the crude extract of *Neuropeltis racemosa* stems demonstrated potent α-glucosidase inhibition at 2 mg/mL (96.09%). Recently, Jaber [40] and Mechchate et al. [42] reported *in vitro* α-amylase inhibitory activity using the standard drug acarbose (IC_{50} = 590 and 717 µg/mL), while Daou et al. [44] and Jaber [40] reported *in vitro* α-glucosidase inhibitory activity using the standard drug acarbose (IC_{50} = 151.14 and 1.01 mg/mL).

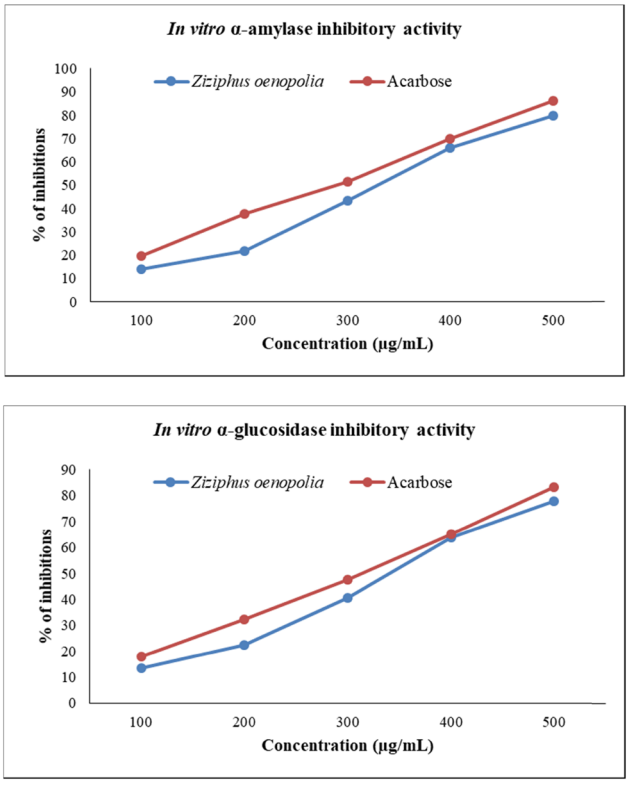


Figure 3: *In vitro* anti-diabetic activity of the hydroalcoholic extract of *Z. oenopolia* fruit through inhibition of α-amylase and α-glucosidase enzymes.

The binding interactions between α-amylase (PDB: 2QV4) and specific phytochemicals from *Z. oenopolia* fruit hydroalcoholic extract, including heneicosane (−4.80 kcal/mol), quinic acid (−6.40 kcal/mol), 9-octadecenoic acid, methyl ester (−5.40 kcal/mol), linoleic acid ethyl ester (−5.50 kcal/mol), and acarbose (−7.30 kcal/mol), as well as α-glucosidase (PDB: 3A4A) binding interactions with heneicosane (−4.10 kcal/mol), quinic acid (−6.50 kcal/mol), 9-octadecenoic acid, methyl ester (−4.90 kcal/mol), linoleic acid ethyl ester (−4.50 kcal/mol), and acarbose (−7.40 kcal/mol) are displayed

Table 6: Molecular docking of phytochemicals and anti-diabetic targets using PyRx (Autodock Vina) tools, LD_{50} (ProTox-II), and analysis of the correlation matrix (*N* = 4)

		Binding affinity (kcal/mol)	
Ligand	**LD_{50} (mg/kg)**	**2QV4**	**3A4A**
Heneicosane	750	−4.80	−4.10
Quinic acid	9,800	−6.40	−6.50
9-Octadecenoic acid, methyl ester,	3,000	−5.40	−4.90
Linoleic acid ethyl ester	20,000	−5.50	−4.50
Correlation matrix (*N* = 4)	R = 0.955 or R^2 = 0.9123		
Acarbose (standard drug)	2,000	−7.30	−7.40

Table 7: ADME properties of phytochemicals and acarbose of *Z. oenopolia* fruit using swiss ADME

ADME	Heneicosane	Quinic acid	9-Octadecenoic acid, methyl ester	Linoleic acid ethyl ester	Acarbose (standard drug)
Physicochemical properties					
Formula	$C_{21}H_{44}$	$C_7H_{12}O_6$	$C_{19}H_{36}O_2$	$C_{20}H_{36}O_2$	$C_{25}H_{43}NO_{18}$
Molecular weight	296.57 g/mol	192.17 g/mol	296.49 g/mol	308.50 g/mol	645.60 g/mol
No. heavy atoms	21	13	21	22	44
No. arom. heavy atoms	0	0	0	0	0
Fraction Csp3	1.00	0.86	0.84	0.75	0.88
Num. rotatable bonds	18	1	16	16	13
No. H-bond acceptors	0	6	2	2	19
No. H-bond donors	0	5	0	0	14
Molar refractivity	103.06	40.11	94.26	98.59	137.92
TPSA	0.00 Å²	118.22 Å²	26.30 Å²	26.30 Å²	329.01 Å²
Lipophilicity					
Log $P_{o/w}$ (iLOGP)	5.85	−0.12	4.75	5.03	−1.99
Log $P_{o/w}$ (XLOGP3)	10.99	−2.37	7.45	7.34	−8.82
Log $P_{o/w}$ (WLOGP)	8.44	−2.32	6.20	6.36	−8.72
Log $P_{o/w}$ (MLOGP)	7.60	−2.14	4.80	4.93	−7.45
Log $P_{o/w}$ (SILICOS-IT)	8.43	−1.82	6.54	6.80	−6.36
Consensus Log $P_{o/w}$	8.26	−1.75	5.95	6.09	−6.67
Water solubility					
Log *S* (ESOL)	−7.41	0.53	−5.32	−5.32	2.57
Solubility	1.14×10^{-5} mg/mL	6.48×10^{2} mg/mL	1.43×10^{-3} mg/mL	1.47×10^{-3} mg/mL	2.41×10^{5} mg/mL
Class	Poorly soluble	Highly soluble	Moderately soluble	Moderately soluble	Highly soluble
Log *S* (Ali)	−10.96	0.43	−7.83	−7.72	2.69
Solubility	3.29×10^{-9} mg/mL	5.12×10^{2} mg/mL	4.34×10^{-6} mg/mL	5.88×10^{-6} mg/mL	3.18×10^{5} mg/mL
Class	Insoluble	Highly soluble	Poorly soluble	Poorly soluble	Highly soluble
Log *S* (SILICOS-IT)	−8.34	2.08	−6.09	−5.77	6.23
Solubility	1.37×10^{-6} mg/mL	2.30×10^{4} mg/mL	2.40×10^{-4} mg/mL	5.23×10^{-4} mg/mL	1.10×10^{9} mg/mL
Class	Poorly soluble	Soluble	Poorly soluble	Moderately soluble	Soluble
Pharmacokinetics					
GI absorption	Low	Low	High	High	Low
BBB permeant	No	No	No	No	No
P-gp substrate	No	Yes	No	No	Yes
CYP1A2 inhibitor	Yes	No	Yes	Yes	No
CYP2C19 inhibitor	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	Yes	No
CYP2D6 inhibitor	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	No	No
	−0.31 cm/s	−9.15 cm/s	−2.82 cm/s	−2.97 cm/s	−16.50 cm/s

(Continued)

Table 7: *Continued*

ADME	Heneicosane	Quinic acid	9-Octadecenoic acid, methyl ester	Linoleic acid ethyl ester	Acarbose (standard drug)
Log K_p (skin permeation)					
Druglikeness					
Lipinski	Yes; 1 violation: MLOGP > 4.15	Yes; 0 violation	Yes; 1 violation: MLOGP > 4.15	Yes; 1 violation: MLOGP > 4.15	No; 3 violations: MW > 500, NorO > 10, NHorOH > 5
Ghose	No; 1 violation: WLOGP > 5.6	No; 1 violation: WLOGP < −0.4	No; 1 violation: WLOGP > 5.6	No; 1 violation: WLOGP > 5.6	No; 4 violations: MW > 480, WLOGP < −0.4, MR > 130, #atoms > 70
Veber	No; 1 violation: rotors > 10	Yes	No; 1 violation: rotors > 10	No; 1 violation: rotors > 10	No; 2 violations: rotors > 10, TPSA > 140
Egan	No; 1 violation: WLOGP > 5.88	Yes	No; 1 violation: WLOGP > 5.88	No; 1 violation: WLOGP > 5.88	No; 1 violation: TPSA > 131.6
Muegge	No; 3 violations: XLOGP3 > 5, heteroatoms < 2, rotors > 15	No; 2 violations: MW < 200, XLOGP3 < −2	No; 2 violations: XLOGP3 > 5, rotors > 15	No; 2 violations: XLOGP3 > 5, rotors > 15	No; 5 violations: MW > 600, XLOGP3 < −2, TPSA > 150, H-acc > 10, H-don > 5
Bioavailability Score	0.55	0.56	0.55	0.55	0.17
Medicinal chemistry					
PAINS	0 alert	0 alert	0 alert	0 alert	0 alert
Brenk	0 alert	0 alert	1 alert: isolated_alkene	1 alert: isolated_alkene	2 alerts: aldehyde, isolated_alkene
Leadlikeness	No; 2 violations: rotors > 7, XLOGP3 > 3.5	No; 1 violation: MW < 250	No; 2 violations: rotors > 7, XLOGP3 > 3.5	No; 2 violations: rotors > 7, XLOGP3 > 3.5	No; 2 violations: MW > 350, rotors > 7
Synthetic accessibility	2.84	3.34	3.16	3.34	7.25

Table 8: Phytochemicals and acarbose (standard) interactions of *Z. oenopolia* fruit with anti-diabetic target 2QV4 (α-amylase) and 3A4A (isomaltase)

Ligand	Amino acid-binding residues (α-amylase PDB ID: 2QV4)	Amino acid-binding residues (isomaltase PDB ID: 3A4A)
Heneicosane	Asp 197, His 299, Arg 195, **Glu 233**, **Ala 198**, **Tyr 62**, **His 201**, Ile 235, His 101, **Leu 162**, **Trp 58**, His 305, **Thr 163**, Trp 59, **Leu 165**, Gln 63	Phe 563, Phe 494, Tyr 566, Glu 497, Lys 568, Pro 488, Asn 489, Pro 567, Lys 569, Lys 373, Asn 565, Gly 564.
Quinic acid	**Trp 58**, **Tyr 62**, **Leu 165**, His 101, **Thr 163**, **Leu 162**, **Ala 198**, **His 201**, Asp 197, **Glu 233**, Arg 195, Asp 300, His 299	Asp 233, Lys 156, Ser 236, Trp 238, Gly 161, Thr 237, Asn 235, Glu 422, His 423, Ile 419, Glu 429. Phe 314, Asn 317
9-Octadecenoic acid, methyl ester	Trp 59, His 305, **Trp 58**, **Leu 165**, **Tyr 62**, Asp 300, Asp 197, **Ala 198**, **Leu 162**, **Glu 233**, Ile 235, **His 201**, His 101, His 299, Gln 63, **Thr 163**	Ser 157, Ser 241, Ser 240, Asp 242, Lys 156, Asn 415, Tyr 158, Tyr 316, Phe 314, Glu 411, Arg 315, Gln 279, Phe 303, Leu 313, His 280, Pro 312
Linoleic acid ethyl ester	**Trp 58**, **Tyr 62**, **Thr 163**, Asp 300, Trp 59, **Leu 165**, Gln 63, Val 234, Ile 235, Lys 200, Tyr 151, **His 201**, **Glu 233**, **Ala 198**, **Leu 162**	Pro 567, Asn 489, Pro 488, Lys 568, Gly 564, Phe 563, Tyr 566, Phe 494, Glu 497, Lys 373, Asn 565
Acarbose (standard)	Lys 200, Tyr 151, **His 201**, **Leu 162**, **Glu 233**, **Ala 198**, Asp 197, Asp 300, His 101, **Tyr 62**, Gln 63, **Leu 165**, Gly 104, Gly 164, **Thr 163**, Trp 59, **Trp 58**, His 305, Gly 306	Gly 269, Val 266, Arg 270, Glu 271, Ile 272, Trp 15, Ile 262, Asn 259, Met 273, Glu 296, Leu 297, Gln 260, Ser 298, His 295, Thr 274, Arg 263, Asn 264

in Table 6 using the PyRx (Autodock Vina) tool; the ADME properties of *Z. oenopolia* fruit phytochemicals are shown in Table 7 using Swiss ADME. The study presented in Table 8 investigates the interaction between *Z. oenopolia* fruit phytochemicals and acarbose (a standard anti-diabetic drug) with the key anti-diabetic target enzyme, 3A4A (isomaltase). Table 7 illustrates a comparative study of the ADME properties of *Z. oenopolia* fruit phytochemicals with acarbose, a standard anti-diabetic drug, using the SwissADME tool. The table briefly compares the properties such as physicochemical properties, lipophilicity, solubility, pharmacokinetics, druglikeness, and medicinal chemistry of *Z. oenopolia* fruit phytochemicals and Acarbose (Standard drug).

Figure 4 shows that the phytochemicals in *Z. oenopolia* fruit prevent both α-amylase and α-glucosidase enzymes from working normally. This indicates that the fruit of *Z. oenopolia* was able to block α-glucosidase enzyme activity both *in vitro* and *in vivo*. Thus, it can be used as a drug for diabetes. According to our understanding, there exists a robust positive correlation (R^2 = 0.912) between the anti-diabetic targets. This suggests that the hydroalcoholic extract of *Z. oenopolia* fruit possesses an inhibitory mechanism comparable to that of α-amylase and α-glucosidase, which regulate hyperglycemia. The binding affinities (kcal/mol) of a few of the chosen phytochemicals, as expressed in the target protein, are displayed

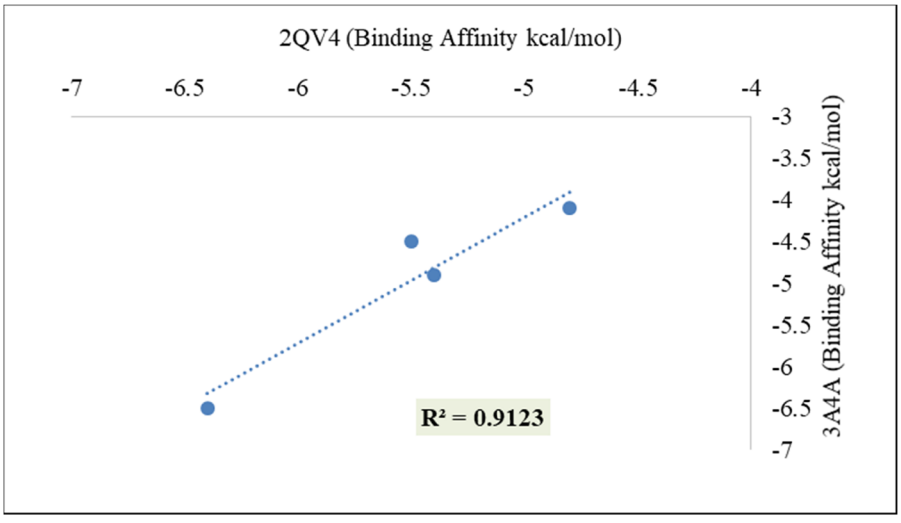


Figure 4: Correlation matrix (N = 4) between the anti-diabetic targets.

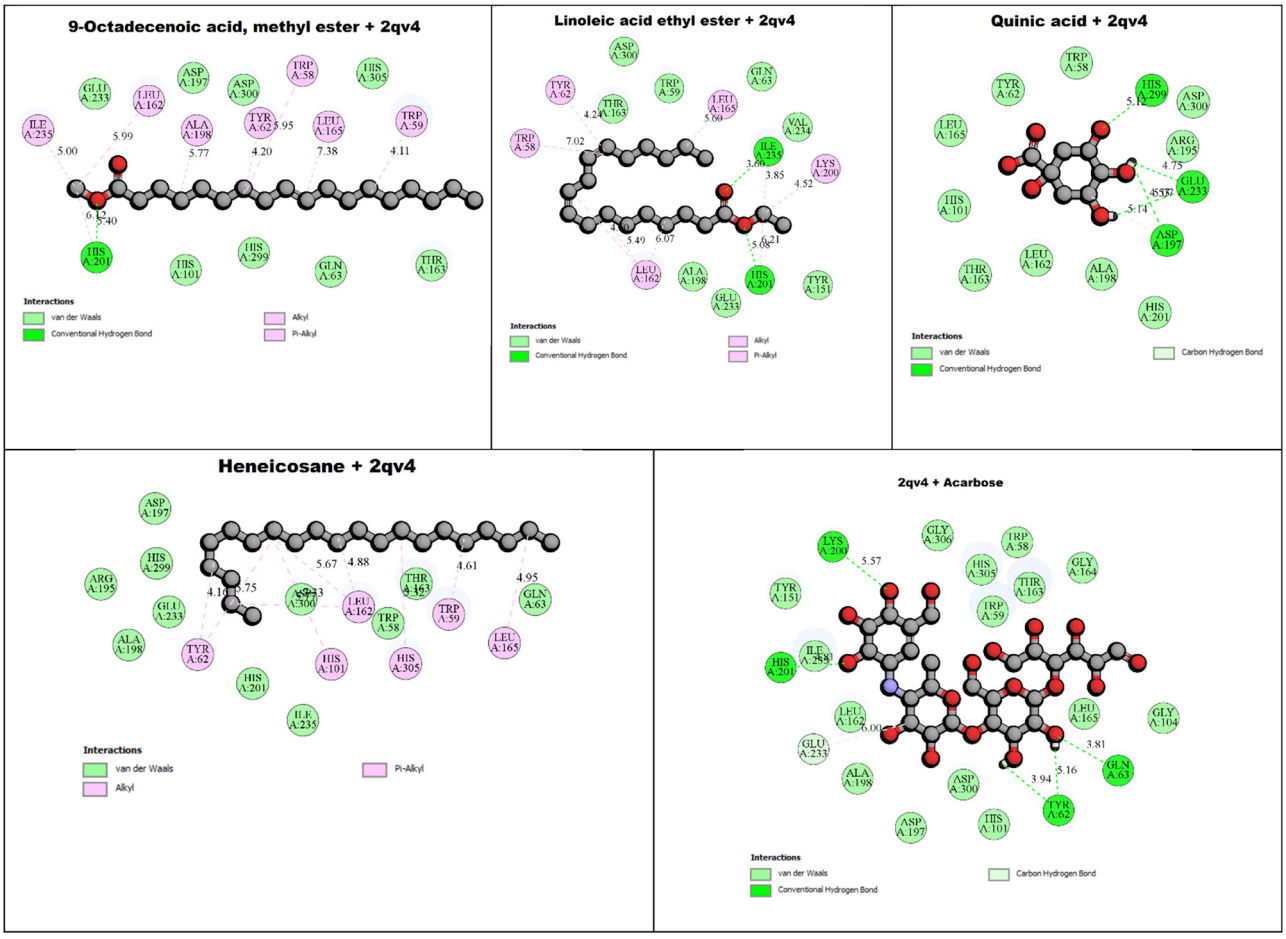


Figure 5: 2D view of phytochemicals and acarbose of *Z. oenopolia* fruit interaction with the anti-diabetic target 2QV4 (α-amylase).

in Table 5. Figures 5 and 6 show a 2D picture of how the chosen ligand interacts with the amino acid residues of the target protein. This may show that the acarbose and phytochemicals in *Z. oenopolia* fruit stop the enzymes α-amylase and α-glucosidase from functioning.

Inhibition of α-amylase (PDB ID: 2QV4) [15] and α-glucosidase (PDB ID: 3A4A) [45] has been recently identified as the target proteins. Lolok et al. [46] investigated the molecular docking of stigmasterol and β-sitosterol, which were separated from *Morinda citrifolia*, with α-amylase (PDB ID: 2QV4), and Akshatha et al. [16] explored similar results of phytochemical interaction with α-amylase (PDB ID: 2QV4). The former study examined the molecular docking of plant-derived α-glucosidase inhibitors (PDB ID: 3A4A), while the latter examined the molecular docking of new 3-amino-2,4-diarylbenzo (4,5) imidazo (1,2-a) pyrimidines against α-glucosidase (PDB ID: 3A4A) [47]. Similarly, Murugesu et al. [48] reported that LC-MS was the best method for identifying α-glucosidase (PDB ID: 3A4A) inhibitors in *Clinacanthus nutans* leaves. In conclusion, this study showed that the hydroalcoholic extract of *Z. oenopolia* fruit can inhibit the activity of α-amylase and α-glucosidase enzymes both *in vitro* and *in silico*, suggesting that it could be used as a potential diabetes drug.

4 Conclusions

The phytochemicals present in the fruit of *Z. oenopolia* are equally involved in the inhibitory activities of α-amylase and α-glucosidase, as evidenced by computational and statistical approaches. As a result of the significant positive link between the anti-diabetic targets, it can be inferred that the hydroalcoholic extract obtained from the fruit of *Z. oenopolia* possesses an inhibitory mechanism comparable to that of α-amylase and α-glucosidase, which is responsible for the regulation of increased blood sugar levels. This results in the presence of phytochemicals, including flavonoids, terpenoids, phenols, steroids, tannins, and saponins. Phytochemicals are effective and environment-friendly treatments.

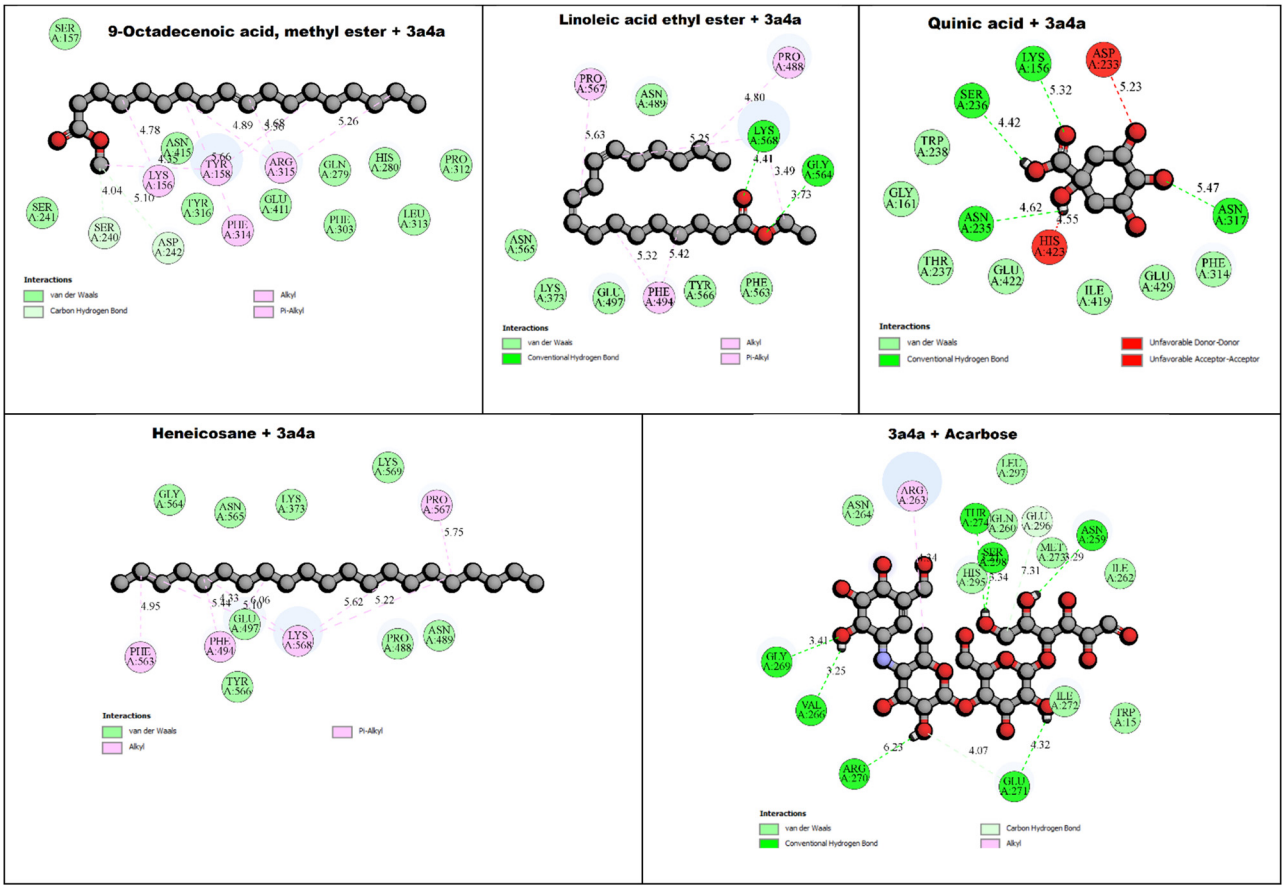


Figure 6: 2D view of phytochemicals and acarbose of *Z. oenopolia* fruit interaction with the anti-diabetic target 3A4A (isomaltase).

Acknowledgments: This study was partly supported by the Kaohsiung Armed Forces General Hospital (KAFGH_A_-113003). The authors extend their appreciation to the Researchers Supporting Project number (RSPD2024R677), King Saud University, Riyadh, Saudi Arabia, for financial support.

Funding information: This study was partly supported by Kaohsiung Armed Forces General Hospital (KAFGH_A_-113003).

Author contributions: Conceptualization, R.S.V., S.B., and R.V; methodology and software, R.S.V., S.B., and S.B.; validation, A.H.H., Z.H.W., formal analysis data curation, R.S.V, S.B. and C.H.Y.; investigation, R.S.V., and R.V.; writing – original draft, R.S.V., S.B., and R.V; writing – review and editing R.S.V., S.B., R.V; C.H.Y and Z.H.W.; finding acquisition, C.H.Y. All authors have read and agreed to the published version of the manuscript.

Conflict of interest: The authors declare no conflict of interest.

Ethical approval: The conducted research is not related to either human or animal use.

Data availability statement: All data used to support the finding of this study are available from the corresponding author upon request.

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