In vitro α-amylase and α-glucosidase Inhibition, Antioxidant and GC-MS Profiling of *Ziziphus oenoplia*

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Abstract
Ziziphus oenoplia (L.) Mill., a traditional medicinal plant of the Rhamnaceae family, was examined by inhibiting α-amylase and α-glucosidase, the phytochemical content, antioxidant capacity and in vitro antidiabetic potential. Sequential solvent extraction of ethanolic leaf extracts produced a rich profile of secondary metabolites including flavonoids, phenolics, alkaloids and terpenoids, as determined by qualitative and quantitative phytochemical screening. Total phenolic content was measured at 151.21 ± 7.78 mg GAE/g with flavonoids at 34.90 ± 3.67 mg. QE/g/High quantities of phenolics and flavonoids were responsible for the dose-dependent radical neutralization shown by antioxidant activity, which was evaluated using DPPH scavenging and reducing power tests.

The extract showed considerable inhibition of α-glucosidase (IC50 = 1.44 ± 0.01 mg/mL) and α-amylase (IC50 = 1.61 ± 0.05 mg/mL), indicating that it may be able to control postprandial hyperglycemia. Gas chromatography-mass spectrometry (GC-MS) revealed 38 chemicals that supported the plant's bioactivity including fatty acids (linolenic acid), nitrogen-containing heterocycles and phytosterols (campesterol, γ-sitosterol). These findings validate the ethnomedicinal use of Z. oenoplia for diabetes management and highlight its therapeutic promise, warranting further in vivo and mechanistic studies.

Keywords: *Ziziphus oenoplia*, antidiabetic activity, α-amylase and α-glucosidase inhibition, phytochemical screening, antioxidant activity, GC-MS analysis.

Introduction

A chronic metabolic disease known as diabetes mellitus (DM) is typified by high blood glucose levels brought on by compromised insulin activity. Diabetes has nearly doubled in incidence worldwide since 1980 and the disease burden is rising alarmingly, especially in low- and middle-income nations[2]. Over 82 million people in developing countries are expected to have diabetes by the year 2030, compared to 48 million in industrialized countries[26,49]. Over 82 million people in developing countries are expected to have diabetes by the year 2030, compared to 48 million in industrialized countries[22,31]. Chronic hyperglycemia is the outcome of insulin resistance and insufficiency in type 2 diabetes, sometimes referred to as non-insulin-dependent diabetes. It is responsible for 90% of all instances of diabetes worldwide[13,14].

Insulin therapy and oral hypoglycemic medications are used to treat diabetes; however, synthetic medications have drawbacks such as decreased effectiveness and drug resistance. For their potential to provide complete metabolic regulation, natural substitutes such as thiazolidinediones, biguanides, α-glucosidase inhibitors, sulfonylureas and non-sulfonylurea secretagogues are being investigated[6,12,37].

Polyphenols, carotenoids, lignans, coumarins and glucosinolates are examples of plant-derived bioactive chemicals that have proven essential in drug development because of their low cost, low toxicity and broad availability[40]. The antidiabetic potential of medicinal plants is considerable, especially those that include flavonoids, terpenoids, saponins, carotenoids, alkaloids and glycosides. Because they are safe and have little toxicity, medicinal plants continue to be a good substitute for synthetic ones[34,45].

The medicinal plant *Ziziphus oenoplia* (L.) Mill. which is a member of the Rhamnaceae family, has significant ethnomedicinal relevance, known for its therapeutic applications in traditional medicine[24]. The plant's leaves, flowers and fruits have antimicrobial and wound-healing properties, while its stem bark is used for sore throats[35]. Its active compounds, including ziziphines, have significant biomedical applications. The plant has numerous therapeutic benefits, including antiulcer, antioxidant, anthelmintic and antiplasmodial properties. It was traditionally used for treating gastrointestinal disorders, hypotension and urinary ailments in rural areas[10,23,25,41].

Its bioactive compounds including flavonoids, alkaloids, saponins and tannins, have potential in modern medicine, particularly in diabetes management. Several significant anti-diabetic targets such as intestinal maltase-glucoamylase, liver receptor homolog-1 (NR5A2), retinol-binding protein-4 (RBP-4), intestinal maltase-glucoamylase, pancreatic α-amylase (AM2A), peroxisome proliferator-activated receptor alpha (PPAR-α), dipeptidyl peptidase-4 (DPP-4), intestinal maltase-glucoamylase and protein tyrosine phosphatase non-receptor, have all been experimentally linked to the *Ziziphus oenoplia* plant. *Ziziphus oenoplia's* therapeutic qualities such as its flavonoids and tannins which have strong antioxidant activity and lower oxidative stress, are shown by

phytochemical investigations. These attributes help to prevent chronic illnesses including diabetes and liver ailments[28,44,47].

The study sought to determine phytochemicals and evaluate *Z. oenoplia's* potential anti-diabetic properties. To treat type 2 diabetes, it concentrated on blocking the α-glucosidase and α-amylase enzymes. Inhibiting these enzymes lowers blood sugar levels and controls hyperglycemia, which is a reaction to type 2 diabetes medication.

Material and Methods

Plant Selection and Authentication: A robust sample of *Ziziphus oenoplia* (L.) Mill. was collected during November and December from Kolli Hills, Namakkal district, Tamil Nadu, India. Identification was confirmed through morphological characteristics and validated by the Scientist "F" and Head of Office at the Botanical Survey of India, TNAU Campus, Coimbatore. The assigned specimen identifier is BSI/SRC/5/23/2024-25/Tech.-200.

Preparation of plant extract: *Ziziphus oenoplia* (L.) Mill leaves were dried to eliminate moisture before being milled into a fine powder. This powder was then sieved to enhance the surface area of the plant material exposed to solvents. Four separate solvents petroleum ether, ethyl acetate, ethanol and water, were used in a sequential extraction procedure on the leaf powder. Continuous active reflux was used for the extraction process, keeping the temperature below each solvent's boiling point for five hours per solvent. Anhydrous conditions were used to collect and dry the solvent extracts to avoid contamination and deterioration.

Qualitative estimation of phytoconstituents: The crucial stage in determining the chemical composition of unrefined plant extracts is phytochemical screening, which identifies the main groups of bioactive chemicals that give them their therapeutic qualities. Steroids, reducing sugars, alkaloids, phenolic compounds, hydrocarbons, tannins, flavonoids, amino acids, terpenoids and cardioglycosides are examples of these bioactive secondary metabolites.

Harborne[16] outlined standard biochemical test procedures as a quick and economical way to identify particular types of substances based on their chemical reactivity, such as coloring and precipitation.

Determination of total flavonoids: Quercetin was used as a reference component in an AlCl3 colorimetric test based on the methodology of Amjad et al[4] to quantify the total quantity of flavonoids. A milliliter of the material was combined with a solution of methanol extract that contained 2% $AlCl_3$. For 15 minutes, the mixture was kept at 30 °C to enable the aluminum chloride and flavonoids to form a combination. A 430 nm measurement of the complex was made using an ultraviolet-visible spectrophotometer. Quercetin concentrations were used to generate a calibration curve. The plant extract's total flavonoid concentration (TFC) was measured in milligrams per gram of dry weight, or quercetin equivalent (QE).

Quantitative Evaluation of Total Phenolic Compounds in *Ziziphus oenoplia*: Phenolic content concentration was measured in the study using 2% sodium carbonate solution added to the test samples and they were then incubated for two minutes at 25 to 30°C. The reaction mixture was then allowed to run for half an hour at room temperature after 100 µL of 50% Folin-Ciocalteu reagent was added. A calibration curve built using gallic acid was used to calculate the total phenolic content after the absorbance was measured at 720 nm. Gallic acid equivalents (GAE) were used to express the results.

Antioxidant activity: DPPH radical scavenging activity: To assess the free radical scavenging ability of plant extracts, the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) test is a technique that is somewhat modified from the Brand-Williams et al[7] approach. The stable free radical DPPH was dissolved in methanol to solve with a concentration of 100 µM. The reaction mixtures were placed at 23°C for 30 minutes after different quantities of the DPPH solution (3 mL), ranging from 20 to 100 µg/mL of the compounds under investigation, were added. When an antioxidant is present, the stable DPPH radical is reduced which lowers the absorbance at 517 nm.

A quantitative indicator of antioxidant potential is the color shift from deep violet to yellow, which denotes the scavenging activity. Ascorbic acid is utilized as a positive control. The absorbance of the test and control samples is used to determine the proportion of DPPH radical scavenging activity.

Reducing power: The potential of bioactive substances in plant extracts to donate electrons is commonly assessed using the reducing power test. One milliliter of plant extract, two and a half milliliters of 0.2 M phosphate buffer at pH 6.6 and two and a half milliliters of potassium ferricyanide were used in the experiment, maintained at 50°C for 20 minutes. The mixture was centrifuged for 10 minutes at 3000 rpm for 2.5 milliliters of trichloroacetic acid (100g/l). The final step was mixing 2.5 ml of the supernatant solution with 0.5 ml of $FeCl_3$ (1g/l) and 2.5 ml of distilled water. A UV-visible spectrophotometer was used to test the resultant solution's absorbance at 700 nm[49].

***In vitro* anti-diabetic activity**

α-amylase inhibition assays: 500 µL of *Z. oenoplia* extract and the standard medication acarbose at different doses (100, 200, 300, 400 and 500 µg/mL) were incubated in DMSO with 500 µL of α-amylase solution in a sodium phosphate buffer, pH 6.9 and 0.006 M at 37°C for 10 minutes as part of the α-amylase inhibition experiment. After adding 1% dinitro salicylic solution and heating it for five minutes at 100°C, the process was stopped. A spectrophotometer measuring absorbance at 540 nm was used to determine the

% inhibition[18]. Three duplicates of the experiment were run. The formula can be employed for calculating the outcomes.

$$\% \text{ of inhibition of } \alpha\text{-amylase} = \frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs. of control}} \times 100$$

α-glucosidase inhibition assays: Inhibition of the α-glucosidase assay involved a reaction mixture containing acarbose and 1 mL of the extracts combined with 0.1 units of α-glucosidase at various concentrations. 500 microliters of 3 mM p-nitrophenyl glucopyranoside were added and the mixture was incubated at 37°C for 25 minutes. One milliliter of 0.02 M Na_2CO_3 was introduced to halt the response and the mixture was then incubated for ten minutes at 25°C. After that, the reaction mixture was diluted to 10 milliliters using 0.02 M sodium phosphate buffer with a pH of 6.9 and 0.006 M NaCl. Monitoring the release of p-nitrophenol from p-nitrophenyl glucopyranoside at 405 nm allowed for the evaluation of α-glucosidase's inhibitory action[35].

$$\% \text{ of inhibition of } \alpha\text{-glucosidase} = \frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs. of control}} \times 100$$

GC-MS Analysis of *Ziziphus oenoplia*: `The Perkin Elmer Clarus 500 system, which has an AOC-20i autosampler and a gas chromatograph connected to a mass spectrometer, was used to perform the GC-MS analysis of the ethanolic extract of *Ziziphus oenoplia.* At 70 eV, the separation was carried out using an Elite-1 fused silica capillary column running in electron impact mode. Helium gas was used as the carrier gas; it was 99.999% pure. After starting at 110 °C and holding it there for two minutes, the oven temperature was raised by 10 °C every minute until 280 °C, where it remained for nine minutes. After setting the injector temperature to 250 °C, the injection volume was 2 µL with a split ratio of 10:1.

A mass range of 40 to 450 Da was covered by the mass spectra, which were obtained at 70 eV with a scan interval of 0.5 seconds. Turbo Mass Ver 5.2.0 was used to process the data, which included chromatogram analysis and mass spectra. The National Institute of Standards and Technology (NIST) library's mass spectra were compared to identify the compound.

Results and Discussion

Preliminary Phytochemical screening of *Z. oenoplia*: Bioactive substances found in plants, known as phytochemicals, have anti-inflammatory, antidiabetic and antioxidant properties[8]. With extractability reliant on solvent polarity, preliminary phytochemical screening qualitatively detects secondary metabolites such as alkaloids, flavonoids, glycosides, saponins, tannins, phenols, steroids, triterpenoids, amino acids and reducing sugars[18]. The phytochemical profile and possible pharmacological activities of *Ziziphus oenoplia* (Rhamnaceae), a medicinal plant with traditional antidiabetic and antioxidant qualities, were evaluated by screening it with petroleum ether, chloroform, ethyl acetate, ethanol and aqueous solvents[19]. *Ziziphus oenoplia* extracts were subjected to a preliminary phytochemical screening, which found a wide range of secondary metabolites in five different solvent systems: petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extracts (Table 1).

Petroleum ether, ethyl acetate, ethanolic and aqueous extracts all had high levels of alkaloids whereas the chloroform extract had a modest amount. Both ethanolic and aqueous extracts included high levels of glycosides, flavonoids, saponins, tannins, phenols, amino acids and reducing sugars. The ethyl acetate extract had moderate levels of phenols and triterpenoids and high levels of flavonoids, saponins and tannins. In petroleum ether and ethyl acetate extracts, steroids were found in high concentrations, whereas in chloroform extract and ethanolic and aqueous extracts, they were found in moderate amounts. The presence of triterpenoids was modest in aqueous, chloroform and ethyl acetate extracts, but substantial in petroleum ether and ethanolic extracts. Petroleum ether and chloroform, which are non-polar and moderately polar extracts, were devoid of reducing sugars, glycosides, flavonoids, saponins, tannins, phenols and amino acids.

Table 1
Preliminary Phytochemical screening of *Z. oenoplia*

S.N.	Phytochemical Test	Petroleum Ether Extract	Chloroform Extract	Ethyl Acetate Extract	Ethanolic Extract	Aqueous Extract
1	Alkaloids	+++	++	+++	+++	+++
2	Glycosides	– – –	– – –	– – –	+++	+++
3	Flavonoids	– – –	– – –	+++	+++	+++
4	Saponins	– – –	– – –	+++	+++	+++
5	Steroids	+++	++	+++	– – –	– – –
6	Tannins	– – –	– – –	+++	+++	+++
7	Phenols	– – –	– – –	++	+++	+++
8	Triterpenoids	+++	++	++	+++	++
9	Amino Acids	– – –	– – –	– – –	+++	+++
10	Reducing Sugars	– – –	– – –	– – –	+++	+++

Legend: (+++) Strongly Present**,** (++) Moderately Present, (+) Weakly Present**,** (– – –) Absent

Table 2
Quantitative Phytochemical Analysis of *Ziziphus oenoplia* Extract

S.N.	Phytochemicals	*Z. oenoplia* Extract
1	Total phenol (mg GAE/g)	151.21 ± 7.78
2	Flavonoids (mg QE/g)	34.90 ± 3.67

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

Ziziphus oenoplia's phytochemical profile differed considerably between solvent systems, indicating how solvent polarity affected the secondary metabolite extraction process. The extensive dispersion of alkaloids in *Z. oenoplia* is suggested by their substantial presence in all extracts, but especially in petroleum ether, ethyl acetate, ethanol and aqueous solvents. As alkaloids block enzymes that hydrolyze carbohydrates, such as α-amylase and α-glucosidase, they are known to have antidiabetic properties[43]. Their presence across solvent polarities indicates a diverse alkaloid composition, potentially contributing to the plant's pharmacological activities. Metabolites were found to be distributed in a variety of ways. Alkaloids were found to be moderately prevalent in chloroform and highly prevalent in petroleum ether, ethyl acetate, ethanolic and aqueous extracts. This suggests that alkaloids play a role in enzyme inhibition and may have antidiabetic effects[43].

Amino acids, reducing sugars, glycosides, flavonoids, saponins, tannins and phenols were all highly present in polar extracts (both ethanolic and aqueous). Ethyl acetate exhibited moderate levels of phenols and triterpenoids along with significant levels of flavonoids, saponins and tannins. Polar compounds, especially flavonoids and phenols, contribute to antioxidant activity, mitigating diabetes-related oxidative stress[8].

Saponins may support hypoglycemic effects[11]. Steroids, found in non-polar (petroleum ether) and moderately polar (chloroform, ethyl acetate) extracts, align with their lipophilic nature and potential anti-inflammatory benefits[38]. Triterpenoids, present across all extracts, may enhance antidiabetic and antioxidant activities[19]. The absence of polar compounds in non-polar and moderately polar solvents highlights solvent-specific extraction, emphasizing the need for diverse solvents to capture the full phytochemical spectrum[17]. These findings suggest that *Z. oenoplia*'s polar extracts are rich in antioxidants while alkaloids and triterpenoids may drive enzyme inhibitory effects supporting its traditional use.

Phytochemical quantitative analysis of *Z. oenoplia* extract: *Ziziphus oenoplia* extract's quantitative phytochemical examination showed notable amounts of total phenolic and flavonoid content (Table 2). 151.21 ± 7.78 mg GAE/g of extract was the total phenolic content which indicates a high concentration of phenolic chemicals. The flavonoid content was 34.90 ± 3.67 mg QE/g, suggesting a moderate but notable presence of flavonoids in the extract. Quantitative analysis of *Ziziphus oenoplia* extract revealed a high total phenolic content of 151.21 ± 7.78 mg GAE/g, indicating a significant presence of phenolic compounds.

Strong antioxidants phenols may neutralize reactive oxygen species, which is essential for controlling oxidative stress in diabetics[8]. This substantial phenolic content supports the traditional use of *Z. oenoplia* for antioxidant effects and suggests its potential in alleviating oxidative stress-related conditions.

The flavonoid content was measured at 34.90 ± 3.67 mg QE/g, reflecting a notable contribution to the phenolic profile. α-amylase and α-glucosidase are important enzymes in the digestion of carbohydrates and flavonoids are known to block them helping to regulate blood sugar levels[4,8]. This concentration aligns with the plant's antidiabetic potential, particularly in polar extracts like ethanol where flavonoids are efficiently extracted[17]. The interplay between phenolics and flavonoids enhances the extract's antioxidant and antidiabetic properties.

The high phenolic yield is likely due to the use of a polar solvent such as ethanol, which effectively extracts polar compounds[38]. Compared to other medicinal plants, *Z. oenoplia*'s phenolic content exceeds thresholds associated with strong antioxidant activity (>100 mg GAE/g), while its flavonoid levels (>30 mg QE/g) suggest potential to limit enzyme activity[19,43]. Low standard deviations confirm data reliability, though the lower flavonoid content indicates that other phenolics like tannins may dominate. GC-MS analysis and other forms of further characterization are required to pinpoint certain bioactive substances. These results underscore *Z. oenoplia*'s therapeutic promise, with high phenolic content driving antioxidant capacity and flavonoids supporting antidiabetic effects, warranting further bioactivity and mechanistic studies.

Antioxidant activity of *Z. oenoplia*: Using the DPPH radical scavenging experiment, the antioxidant activity of *Ziziphus encolpia* extract was evaluated and the outcomes were contrasted (Table 3). At 50 µl, the percentage inhibition of *Z. oenoplia* extract was 8.63%; at 250 µl, it increased to 22.40%, demonstrating dose-dependent DPPH radical scavenging action. However, the optical density (OD) values for the sample did not consistently decrease with concentration, suggesting variability in scavenging efficiency. The standard showed a stronger antioxidant effect, with scavenging activity rising from 23.6% at 2 µl to 75.2% at 10 µl accompanied by a consistent decrease in OD from 0.945 to 0.357. Its phenolic (151.21 mg GAE/g) and flavonoid (34.90 mg QE/g) content drive the dose-dependent

increase in scavenging from 8.63% at 50 µl to 22.40% at 250 µl[8]. Compared to the standard's 75.2% inhibition at 10 µl, the extract's lower efficacy reflects the complexity of crude extracts where non-antioxidant components may reduce potency[38]. Inconsistent optical density trends suggest experimental variability or constituent interactions affecting scavenging kinetics[32].

For plant extracts, antioxidant activity is essential because it combats oxidative stress and reactive oxygen species (ROS) which are connected to long-term diseases like diabetes[8]. The DPPH assay evaluates this potential by measuring radical scavenging through reduced absorbance at 517 nm, reflecting the electron-donating capacity of compounds like phenolics and flavonoids[32]. Tropical medicinal plant *Ziziphus oenoplia* (Rhamnaceae) has long been utilized for its antidiabetic and antioxidant properties, which are ascribed to secondary metabolites like flavonoids and phenols[19]. Its antioxidant capacity may be evaluated by evaluating its DPPH scavenging activity across concentrations, which may enhance its α-amylase and α-glucosidase inhibitory actions. It is compared to a reference (like ascorbic acid) to assess its potential for treatment.

Recent studies contextualize these results. A study on *Ziziphus mauritiana* reported 30–50% DPPH inhibition at 200 µg/ml with similar phenolic levels, indicating that *Z. oenoplia*'s lower activity may stem from flavonoid diversity[39]. Likewise, *Syzygium cumini* extracts achieved 60% inhibition at 250 µg/ml, driven by comparable flavonoids, suggesting *Z. oenoplia*'s flavonoids contribute less significantly[29]. Despite this, the extract's dose-dependent scavenging supports its role in managing oxidative stress, complementing its antidiabetic potential via α-amylase and α-glucosidase employ additional assays (e.g. FRAP) and using GC-MS to identify key antioxidants[39].

Reducing Power of *Z. oenoplia*: The reducing power assay assesses antioxidant capacity by measuring a plant extract's ability to reduce Fe^{3+} to Fe^{2+}, indicated by increased absorbance at 700 nm, reflecting electron donation by compounds like phenolics and flavonoids[32]. This capacity helps to neutralize reactive oxygen species, mitigating oxidative stress in conditions like diabetes. *Ziziphus oenoplia* (Rhamnaceae), valued traditionally for its antioxidant and antidiabetic effects, contains phenols and flavonoids that likely contribute to its reducing power[19]. This assay quantifies its antioxidant potential, complementing other methods like DPPH scavenging and comparison with a standard (e.g. ascorbic acid) evaluates its therapeutic efficacy. Ascorbic acid was used as a reference and the reducing power of the ethanolic extract of *Ziziphus oenoplia* was evaluated using absorbance at 700 nm (Table 4).

The *Z. oenoplia* extract exhibited a dose-dependent increase in reducing power, with absorbance rising from 0.139 ± 0.02 at 10 mg/L to 0.346 ± 0.08 at 500 mg/L. The control showed minimal absorbance (0.054 ± 0.01). Ascorbic acid demonstrated significantly higher reducing power, with absorbance increasing from 0.298 ± 0.05 at 5 mg/L to 1.100 ± 0.06 at 15 mg/L.

The reducing power assay demonstrated moderate antioxidant capacity in *Ziziphus oenoplia* ethanolic extract with absorbance rising dose-dependently from 0.139 at 10 mg/L to 0.346 at 500 mg/l likely driven by phenolics (151.21 mg GAE/g) and flavonoids (34.90 mg QE/g) that reduced Fe^{3+} to Fe^{2+} [32]. This aligns with its traditional use for oxidative stress relief[19]. Compared to ascorbic acid (absorbance 1.100 at 15 mg/L), the extract's lower potency reflects the complexity of crude extracts where non-antioxidants may dilute efficacy[38].

Table 3
Antioxidant activity of *Ziziphus oenoplia* extract and standard in DPPH assay

Concentration (µl)	OD 517 nm			% Activity	
	(Sample)	(Standard)		(Sample)	(Standard)
50	1.011	2 µl	0. 945	8.63	23.6
100	1.019	4 µl	0.753	6.7	37.0
150	0.905	6 µl	0.572	17.5	58.3
200	0.916	8 µl	0.450	18.5	69.1
250	0.871	10 µl	0.357	22.4	75.2

Control OD 571nm

Table 4
Reducing power of *Ziziphus oenoplia* ethanolic extract and ascorbic acid

Sample	Concentration (mg/L)	Absorbance (700 nm)
Control	0	0.054 ± 0.01
Ethyl Alcohol Extract of *Ziziphus oenoplia*	10	0.139 ± 0.02
	200	0.249 ± 0.04
	500	0.346 ± 0.08
Ascorbic Acid (Standard)	5	0.298 ± 0.05
	10	0.670 ± 0.06
	15	1.100 ± 0.06

Recent studies contextualize these results. A study on *Ziziphus mauritiana* reported absorbance of 0.5–0.8 at 0.5 mg/L, linked to higher phenolics (212.15 mg GAE/g), suggesting *Z. oenoplia*'s slightly lower efficiency[3]. Similarly, *Ziziphus jujuba* extracts showed 0.6 absorbance at 0.1 mg/L, driven by flavonoids[29]. *Z. oenoplia*'s modest reducing power at higher concentrations indicates moderate activity among Ziziphus species. Low standard deviations (±0.02–0.08) confirm reliability, though variability at 500 mg/L suggests matrix effects.

This reducing power complements the extract's DPPH scavenging (22.40% at 250 µl) and antidiabetic enzyme inhibition, supporting its multifaceted role in diabetes management[8]. Future studies should calculate IC50, combine assays (e.g. FRAP) and use GC-MS to identify key compounds[3].

Evaluation of *in vitro* inhibition against key anti-diabetic enzymes α-amylase and α-glucosidas: An important tactic for type 2 diabetes management is to inhibit the enzymes α-amylase and α-glucosidase, which hydrolyze carbohydrates. As enzyme inhibitors, natural compounds high in flavonoids and phenolics, such as *Ziziphus oenoplia*, have less adverse effects than manufactured medications like acarbose. The extract's efficacy is supported by its IC50 values compared to acarbose benchmarks. IC50 values for the *in vitro* inhibitory actions of acarbose and *Ziziphus oenoplia* ethanolic extract against α-amylase and α-glucosidase are shown in table 5.

IC50 values for α-amylase and α-glucosidase were 0.65 mg/mL and 0.153 mg/mL respectively indicating significant inhibition by acarbose. Based on α-amylase and α-glucosidase IC50 values of 1.61 ± 0.05 mg/mL and 1.44 ± 0.01 mg/mL respectively, the *Z. oenoplia* extract demonstrated moderate inhibitory efficacy as in figures 1 and 2.

In vitro assays revealed that *Ziziphus oenoplia* ethanolic extract moderately inhibits α-amylase (IC50 = 1.61 ± 0.05 mg/mL) and α-glucosidase (IC50 = 1.44 ± 0.01 mg/mL), indicating potential to lower postprandial glucose. This activity likely stems from its phenolic (151.21 mg GAE/g) and flavonoid (34.90 mg QE/g) content, which disrupts enzyme function[32].

Stronger α-glucosidase inhibition minimizes side effects from excessive starch breakdown[19]. Compared to acarbose (IC50 = 0.153 mg/mL for α-amylase, 0.65 mg/mL for α-glucosidase), the extract is less potent, likely due to inactive compounds in crude extracts[32], but it may offer a safer alternative.

Recent studies provide context. *Ziziphus mauritiana* extracts showed IC50 values of 0.8–1.2 mg/mL (α-amylase) and 0.9–1.5 mg/mL (α-glucosidase) with higher phenolics[3] while *Syzygium cumini* reported 1.0 mg/mL and 0.7 mg/mL respectively, driven by flavonoids[29]. *Z. oenoplia*'s comparable IC50 values suggest efficient bioactives despite lower phenolic content. Low standard deviations ensure reliable results, complementing its DPPH scavenging (22.40%) and reducing power (0.346 absorbance), supporting multifaceted antidiabetic potential[8]. Further studies should refine IC50 estimates, explore synergies via fractionation, validate *in vivo* and identify inhibitors using LC-MS[4].

GC-MS identified compounds in *Ziziphus oenoplia* ethanolic extract: A wide variety of phytochemicals were identified by the GC-MS analysis of *Ziziphus oenoplia* ethanolic extract, which was carried out using a Perkin Elmer Clarus 500 system with an Elite-1 fused silica capillary column. Retention times (RT) for the identified compounds, CAS numbers, molecular formulas, peak areas, match scores and relative abundances (Area%-T and Area%-M) are presented in table 6.

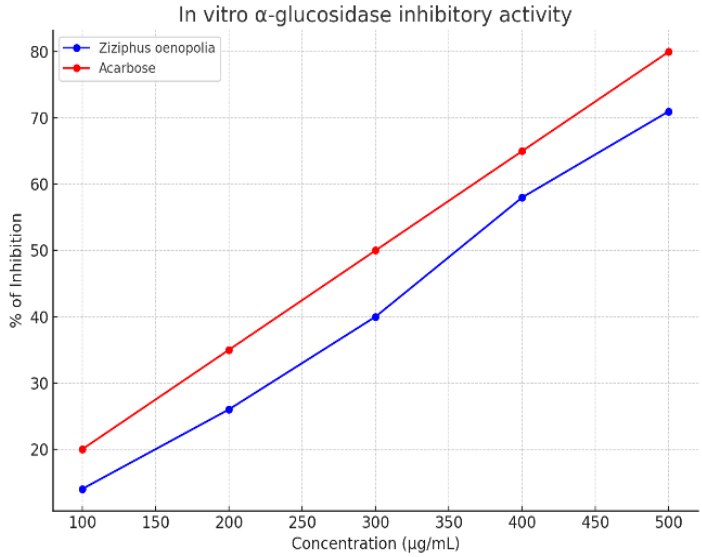

Figure 1: *In vitro* inhibitory activity on anti-diabetic targets α-glucosidase

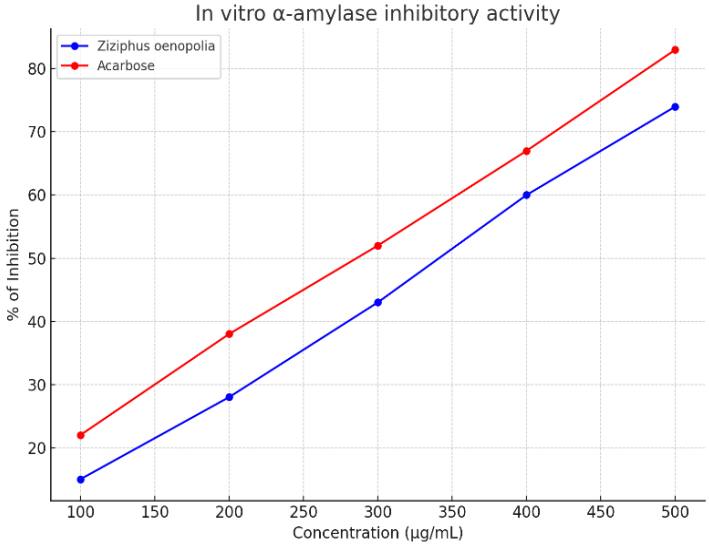

Figure 2: *In vitro* inhibitory activity on anti-diabetic targets α-amylase

Table 5
***In vitro* inhibitory activity of *Ziziphus oenoplia* extract and acarbose against α-amylase and α-glucosidase**

S.N.	Sample	α-amylase (IC50) (mg/mL)	α-glucosidase (IC50) (mg/mL)
1	Acarbose	0.153	0.65
2	*Ziziphus oenoplia extract*	1.61 ± 0.05	1.44 ± 0.01

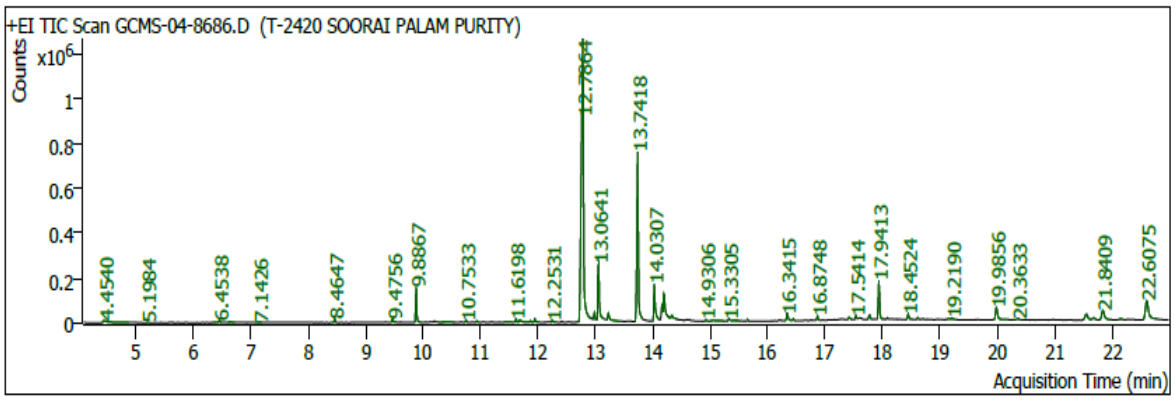

Figure 3: GC-MS analysis of *Ziziphus oenoplia* ethanolic extract

A total of 38 compounds were detected, accounting for 99.86% of the total peak area. Major constituents included 1H-[1]Pyrindine-3-carbonitrile, 4-ethyl-2-oxo-2,5,6,7-tetrahydro- (RT 12.7864, 42.85% Area%-T), 1H-Pyrrolo[2,1-b]quinazolin-9-one, 3-hydroxy-2,3-dihydro- (RT 13.7418, 16.42% Area%-T), γ-Sitosterol (RT 22.6075, 4.31% Area%-T), 9,12,15-Octadecatrienoic acid, (Z, Z, Z)- (RT 14.1973, 4.15% Area%-T) and phytol (RT 14.0307, 3.61% Area%-T). Other notable compounds included n-Hexadecanoic acid (RT 13.0641, 6.57% Area%-T), squalene (RT 17.9413, 3.31% Area%-T), campesterol (RT 21.5520, 1.14% Area%-T) and stigmasterol (RT 21.8409, 1.72% Area%-T). The presence of glycerin (RT 4.4540, 0.88% Area%-T), benzoic acid derivatives and various esters further highlighted the extract's chemical diversity.

38 compounds were found in the ethanolic extract of *Ziziphus oenoplia* by GC-MS analysis; the main ingredients were 1H-[1]pyridine-3-carbonitrile, 4-ethyl-2-oxo-2,5,6,7-tetrahydro- and 1H-pyrrolo[2,1-b]quinazolin-9-one, 3-hydroxy-2,3-dihydro-. The plant's anti-inflammatory, antioxidant and antidiabetic effects are supported by its terpenoids, fatty acids and phytosterols.

Ziziphus oenoplia ethanolic extract GC-MS analysis revealed a diverse phytochemical profile, featuring phytosterols, fatty acids, terpenoids and nitrogen-containing heterocycles. Phytosterols such as γ-sitosterol (4.31%), campesterol (1.14%) and stigmasterol (1.72%) align with findings from *Ziziphus mauritiana*, where they supported their function in treating diabetes and cardiovascular difficulties by contributing to antioxidant and anti-inflammatory effects[30,42]. Similarly, 9,12,15-octadecatrienoic acid (linolenic acid, 4.15%) mirrors its anti-inflammatory properties reported in *Ziziphus jujuba*[33].

Table 6
GC-MS identified compounds in *Ziziphus oenoplia* ethanolic extract

RT (min)	Compound Name	CAS#	Formula	Area	Match Score	Area%-T (%)	Area%-M (%)
4.4540	Glycerin	56-81-5	$C_3H_8O_3$	72190	65.5	0.88	2.06
5.1984	Phosphinic acid, diethyl-, methyl ester	1000306-03-5	$C_5H_{13}O_2P$	2299	77.9	0.03	0.07
6.3760	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	28564-83-2	$C_6H_8O_4$	2534	73.9	0.03	0.07
6.4538	Phenol, 2-propyl-	644-35-9	$C_9H_{12}O$	13004	86.9	0.16	0.37
6.6426	Benzoic acid, ethyl ester	93-89-0	$C_9H_{10}O_2$	8627	88.8	0.11	0.25
7.1426	Ethanol, 2-phenoxy-	122-99-6	$C_8H_{10}O_2$	6496	64.4	0.08	0.19
8.4647	Benzene, 1,3,5-trimethyl-2-propyl-	4810-04-2	$C_{12}H_{18}$	19845	82.7	0.24	0.57
9.4756	2',4'-Dihydroxy-3'-methylpropiophenone	63876-46-0	$C_{10}H_{12}O_3$	25764	73.8	0.31	0.73
9.8867	Benzoic acid, 4-ethoxy-, ethyl ester	23676-09-7	$C_{11}H_{14}O_3$	202245	97.6	2.47	5.77
10.4533	Carbonic acid, allyl butyl ester	1000314-64-8	$C_8H_{14}O_3$	17420	65.3	0.21	0.50
10.7533	Megastigmatrienone	38818-55-2	$C_{13}H_{18}O$	10092	77.0	0.12	0.29
10.9421	4,4,5,8-Tetramethylchroman-2-ol	82391-05-7	$C_{13}H_{18}O_2$	6134	64.9	0.07	0.17
11.4310	4(1H)-Quinazolinone	491-36-1	$C_8H_6N_2O$	8354	71.9	0.10	0.24
11.6198	Phenol, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, (R)-	30199-26-9	$C_{15}H_{22}O$	23109	75.8	0.28	0.66
11.6865	Tetradecanoic acid	544-63-8	$C_{14}H_{28}O_2$	29478	79.1	0.36	0.84
11.8754	2H-Pyran-2-carboxaldehyde, 3,4-dihydro-2,5-dimethyl-	1920-21-4	$C_8H_{12}O_2$	9938	55.7	0.12	0.28
11.9531	2,3-Bis(1-methylallyl)pyrrolidine	1000306-54-0	$C_{12}H_{21}N$	21601	69.9	0.26	0.62
12.2531	1-(1-Butoxypropan-2-yloxy)propan-2-yl heptanoate	1000367-12-8	$C_{17}H_{34}O_4$	12728	60.6	0.16	0.36
12.7864	1H-[1]Pyrindine-3-carbonitrile, 4-ethyl-2-oxo-2,5,6,7-tetrahydro-	1000303-30-7	$C_{11}H_{12}N_2O$	3507228	87.5	42.85	100.00
12.9864	Diphenylsulfone	127-63-9	$C_{12}H_{10}O_2S$	69279	93.9	0.85	1.98
13.0641	n-Hexadecanoic acid	57-10-3	$C_{16}H_{32}O_2$	537836	90.7	6.57	15.34
13.2308	2-Pyrrolidinone, 1-phenyl-	4641-57-0	$C_{10}H_{11}NO$	132580	56.6	1.62	3.78
13.7418	1H-Pyrrolo[2,1-b]quinazolin-9-one, 3-hydroxy-2,3-dihydro-	1000302-68-2	$C_{11}H_{10}N_2O_2$	1343910	92.8	16.42	38.32
14.0307	Phytol	150-86-7	$C_{20}H_{40}O$	295443	96.0	3.61	8.42
14.1973	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	463-40-1	$C_{18}H_{30}O_2$	339330	88.1	4.15	9.68
14.3418	Octadecanoic acid	57-11-4	$C_{18}H_{36}O_2$	53961	81.5	0.66	1.54
14.9306	Cyclohexanecarboxaldehyde, 3,3-dimethyl-5-oxo-	65080-66-2	$C_9H_{14}O_2$	6962	65.4	0.09	0.20
15.0639	Carbonic acid, 2-dimethylaminoethyl methyl ester	1000331-47-3	$C_6H_{13}NO_3$	29973	50.3	0.37	0.85
15.3305	5-(4-Butoxy-phenyl)-2H-[1,2,4]triazol-3-ylamine	1000317-50-0	$C_{12}H_{16}N_4O$	35885	70.2	0.44	1.02
15.6527	Hexanedioic acid, bis(2-ethylhexyl) ester	103-23-1	$C_{22}H_{42}O_4$	7254	74.2	0.09	0.21
16.3415	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	23470-00-0	$C_{19}H_{38}O_4$	72553	73.5	0.89	2.07
16.4526	Phthalic acid, di(2-propylpentyl) ester	1000377-93-5	$C_{24}H_{38}O_4$	11302	76.7	0.14	0.32
16.8748	Benzenamine, N,N-dimethyl-2-(1,2,3,9-tetrahydropyrrolo[2,1-b]quinazolin-3-yl)-	33903-13-8	$C_{19}H_{21}N_3$	24328	69.5	0.30	0.69

17.4192	Octadecanoic acid, 2,3-dihydroxypropyl ester	123-94-4	$C_{21}H_{42}O_4$	17564	61.6	0.21	0.50
17.5414	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	137-89-3	$C_{24}H_{38}O_4$	21743	86.6	0.27	0.62
17.7858	Pyrrolo[2,1-b]quinazolin-9(1H)-one, 3-[2-(dimethylamino)phenyl]-2,3-dihydro-	33903-15-0	$C_{19}H_{19}N_3O$	35898	63.9	0.44	1.02
17.9413	Squalene	111-02-4	$C_{30}H_{50}$	270771	96.0	3.31	7.72
18.4524	Heneicosane	629-94-7	$C_{21}H_{44}$	40868	85.8	0.50	1.17
18.6191	Squalene	111-02-4	$C_{30}H_{50}$	12054	67.5	0.15	0.34
19.2190	2-Amino-3-benzyl-5-(4-methoxyphenyl)pyrazine-1-oxide	123437-73-0	$C_{18}H_{17}N_3O_2$	27258	51.8	0.33	0.78
19.9856	Hexacosane	630-01-3	$C_{26}H_{54}$	150410	89.9	1.84	4.29
20.3633	dl-α-Tocopherol	10191-41-0	$C_{29}H_{50}O_2$	15759	80.1	0.19	0.45
21.5520	Campesterol	474-62-4	$C_{28}H_{48}O$	93648	80.6	1.14	2.67
21.6854	2,7-Naphtrydine-4-carbonitrile, 5,6,7,8-tetrahydro-3-benzylamino-7-ethyl-1-diethylamino-	1000277-03-8	$C_{22}H_{29}N_5$	27088	53.3	0.33	0.77
21.8409	Stigmasterol	83-48-7	$C_{29}H_{48}O$	140660	81.3	1.72	4.01
22.1520	Undecane, 3,8-dimethyl-	17301-30-3	$C_{13}H_{28}$	10491	66.4	0.13	0.30
22.6075	γ-Sitosterol	83-47-6	$C_{29}H_{50}O$	352882	88.7	4.31	10.06

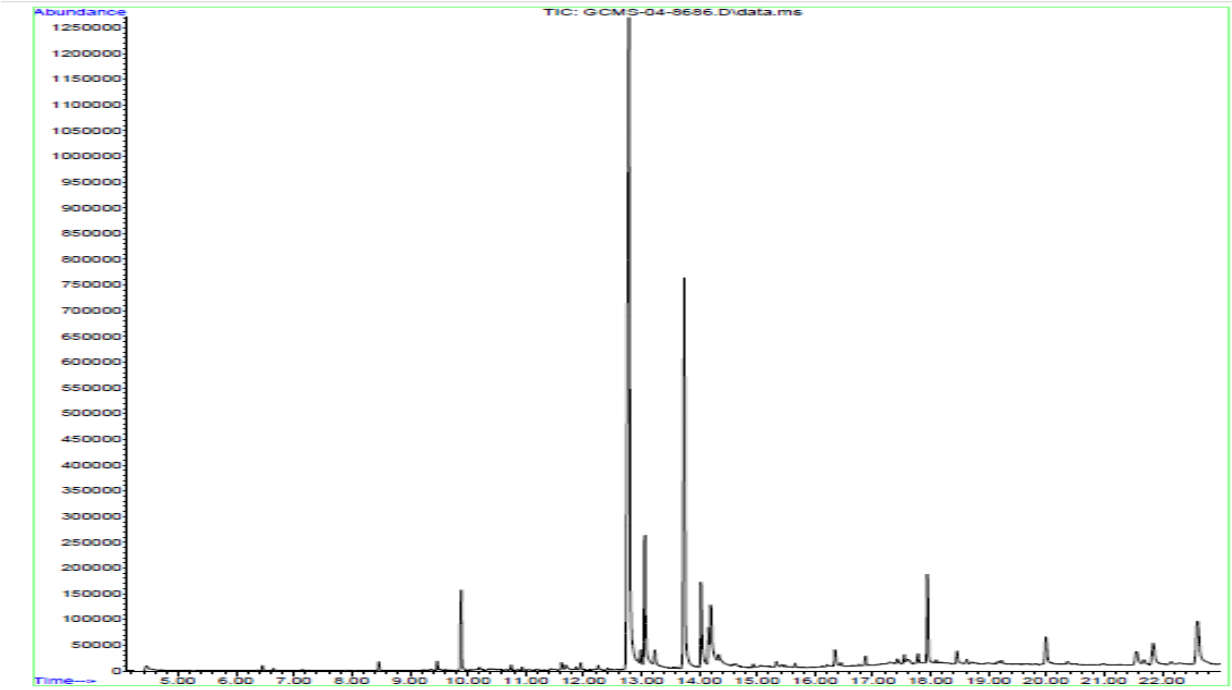

Figure 4: GC-MS analysis of *Ziziphus oenoplia* ethanolic extract

Nitrogen-containing heterocycles including 1H-[1]Pyrindine-3-carbonitrile, 4-ethyl-2-oxo-2,5,6,7-tetrahydro- (42.85%) and 1H-Pyrrolo[2,1-b]quinazolin-9-one, 3-hydroxy-2,3-dihydro- (16.42%), are notable for their rarity in *Ziziphus* species, yet resemble alkaloids in *Ziziphus nummularia* with anticancer potential[1,20].

Phytol (3.61%) and squalene (3.31%) corroborate their antioxidant and hepatoprotective roles in *Hibiscus sabdariffa* and *Ziziphus lotus*[9,27,46]. n-Hexadecanoic acid (6.57%) aligns with its anti-inflammatory and antimicrobial properties in *Alangium salviifolium* and *Salsola kali*[5,46]. Compared to *Ziziphus glabrata*, which emphasized alkaloids, *Ziziphus oenoplia* exhibits greater diversity, likely due to plant part or environmental factors[30]. The high identification rate (99.86%) underscores methodological robustness. However, the bioactivity of heterocycles remains understudied and bioassays as used in *Luffa cylindrica* are needed[46]. Seasonal and geographical variations, noted in *Ziziphus mauritiana*, also require exploration[30].

Conclusion

This investigation thoroughly assessed the antioxidant capacity, phytochemical composition and *in vitro* antidiabetic activities of *Ziziphus oenoplia* (L.) Mill. ethanolic leaf extract, underscoring its therapeutic promise in diabetes management. The results of phytochemical screening showed a wide variety of secondary metabolites such as flavonoids, alkaloids, phenols and triterpenoids with quantitative analysis confirming high phenolic (151.21 ± 7.78 mg GAE/g) and moderate flavonoid (34.90 ± 3.67 mg QE/g) content. The extract exhibited dose-dependent antioxidant activity in DPPH (22.40% at 250 µl) and reducing power assays (absorbance 0.346 at 500 mg/L), attributed to its phenolic and flavonoid constituents, which mitigate oxidative stress associated with diabetes.

In vitro assays demonstrated moderate inhibition of α-amylase (IC50 = 1.61 ± 0.05 mg/mL) and α-glucosidase (IC50 = 1.44 ± 0.01 mg/mL), suggesting its potential to regulate postprandial hyperglycemia, albeit less potently than acarbose. GC-MS analysis identified 38 compounds, with dominant constituents such as 1H-[1]Pyrindine-3-carbonitrile, 4-ethyl-2-oxo-2,5,6,7-tetrahydro- (42.85%), phytosterols and fatty acids, likely contributing to its bioactivity. These findings validate the traditional use of *Z. oenoplia* in ethnomedicine and highlight its multifaceted pharmacological potential. Still, additional research is necessary to separate bioactive substances, clarify their processes and confirm their effectiveness through *in vivo* tests to forward their use in diabetic treatments.

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