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# Evaluation of *In Silico*, Antidiabetic and Antioxidant Properties of *Syzygium cumini* fruit Extract

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## A B S T R A C T

Diabetes mellitus represents a significant global health challenge requiring innovative therapeutic approaches. This study investigates the phytochemical composition, antidiabetic potential, and antioxidant activities of *Syzygium cumini* fruit extracts through comprehensive in vitro and in silico methodologies. Both aqueous and ethanolic extracts were prepared and analyzed for bioactive compounds. Antidiabetic activity was evaluated using α-amylase and α-glucosidase inhibition assays. Molecular docking studies were conducted to predict the binding affinity of identified compounds with key diabetic target proteins. Results revealed significant presence of bioactive compounds in both extracts. The extracts demonstrated substantial α-amylase ($IC_{50}$: 45.2 µg/ml) and α-glucosidase ($IC_{50}$: 38.7 µg/ml) inhibitory activities. Strong antioxidant potential was observed with DPPH scavenging activity reaching 87.3% at 500 µg/ml concentration. Molecular docking analysis revealed promising binding interactions between major compounds and diabetic target proteins, supporting the traditional use of *S. cumini* in diabetes management.

**Keywords:** Diabetes mellitus, Conventional medications, *C. anacardioides*, Blood glucose levels.

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## INTRODUCTION

Diabetes mellitus (DM) is a prevalent endocrine disorder characterized by chronic metabolic imbalances in carbohydrate, fat, and protein metabolism, leading to elevated blood sugar levels (hyperglycemia). This condition arises from insufficient production of insulin, impaired insulin action, or a combination of both factors (Tran et al., 2020). Due to its high frequency, morbidity and mortality it is getting the third 'killer' of humanity after cancer and cardiovascular conditions (Verma et al., 2012) . Current therapies for DM includes the use of oral hypoglycemic agents (meglitinides, DPP-4 inhibitors, biguanides, α-glucosidase inhibitors, sulfonylureas, thiazolidinediones and incretin mimetics) along with exercise or diet that basically suppress microvascular as well as macrovascular complications and also upgrade the glycemic control (Lorenzati et al., 2010).Oral drugs pose health risks with various side effects such as gastrointestinal, cutaneous, hematological issues, hypoglycemic coma, kidney and liver disturbances. Moreover, they are unsuitable for pregnant individuals. The medical system faces a challenge in managing diabetes without adverse effects. Despite advances in health sciences, patients are increasingly seeking natural products with antidiabetic properties (Ullah et al., 2022).

Plants have long been recognized as valuable sources of medicinal compounds, many synthetic drugs are derived from them (Alam et al., 2022). Medicinal herbs, better replacement therapeutic agents, used as precautionary measures as well as cure of multiple diseases due to their

pharmacological as well as nutritional properties (Karimi et al., 2015). Phytochemicals are bioactive compounds naturally occurring in plants. While they do not provide direct nutritional value, they play vital roles in various physiological functions within the human body, contributing to overall health and well-being (Abbas et al., 2015).

Australia is home to a wide range of plant species, thanks to the country's varied climate, which spans from tropical to temperate. Indigenous Australians have long used local flora to treat a variety of health problems, including coughs, fever, headaches, cold, diabetes, cancer, heart attacks, influenza and inflammation. One such plant is Tuckeroo (*Cupaniopsis anacardioides*) (Pham et al., 2022). This plant has dark green leaves, greenish-white flowers, a short trunk and orange, round fruit. Native Australian fruits, in particular, hold potential for the discovery of medicinal compounds. This fruit is often eaten by birds and also edible for humans, may contain various bioactive compounds that may be beneficial to human health (Pham et al., 2017). This tropical plant belongs to the Sapindaceae family, which also includes other medicinal plants like *Litchi chinensis* and *Dimocarpus longan* widely used in East Asia (Pham et al., 2020).

The Sapindaceae family is basically a group of flowering plants that includes around 2000 species found in a variety of climates around the world. Many members of this family have been researched for their potential medicinal properties. The most common pharmacological properties described for this family are antioxidant, anti-diabetic and anti-inflammatory effects (Díaz and Rossini, 2012).

*C. anacardioides* fruit contain various phytochemical natural compounds like total phenolic, flavonoids, proanthocyanidins (Pham *et al.* 2022) and linear triterpens (Díaz and Rossini 2012) etc. Phytochemical analysis, antioxidant and anticancer activites of *C. anacardioides* fruit have been reported whereas hypoglycemic and hypolipidemic effect of *C. anacardioides* fruit has not been reported in literature, so the present study aimed to be carried out to evaluate the potency of fruit extracts as hypolipidemic and hypoglycemic agent. Additionally, pancreatic tissue sections stained with hematoxylin-eosin have also been examined histologically.

## MATERIALS AND METHODS

### Experimental station

The experimental study was conducted at Pakistan Council of Scientific and Industrial Research, Lahore.

### Collection and processing of plant material

Identified Fruits of *Syzygium cumini* were collected from Botanical Garden, GC University, Lahore. Fruits were first washed with tap water and then with distilled water to make them free of debris and dust particles. They were shade dried and grounded to powder with the help of an electrical grinder and kept in air-tight container until use.

50g of dried, powdered fruits were dissolved in a volume of 500ml of distilled water for aqueous extract and 95% ethanol for ethanolic extract, was placed in an incubator and maintained at 37°C with continuous shaking for a duration of 24 hours. They were then centrifuged at 4000 rpm for 15 min. Residue were removed by filtration using whatman no.1 filter paper and then filtrate was evaporated in the rotary evaporator under low temperature (45°C) and reduced pressure until it is completely dry and then saved at 4º C for further use (Jeyaseelan et al., 2012).

### Phytochemical Analysis

The qualitative phytochemical analysis of both extracts were performed for the detection of flavonoids, phenolics, tannins, alkaloids, terpenoids, saponins, carbohydrates and glycosides according to the procedures described by (De et al., 2010).

### Phytochemical Analysis

Qualitative phytochemical screening was performed to detect major classes of secondary metabolites including alkaloids, flavonoids, phenolic compounds, terpenoids, saponins, tannins, carbohydrates, and glycosides using standard protocols (Ewenighi, 2015).

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**Alkaloids Detection:** Wagner's test and Mayer's test were employed. Formation of brown/reddish precipitate indicated alkaloid presence.

**Flavonoids Detection:** Alkaline reagent test was performed Yellow coloration indicated flavonoid presence, which disappeared upon acidification.

**Phenolic Compounds Detection:** Ferric chloride test involved adding 2-3 drops of 5% ferric chloride solution to 2ml plant extract. Blue, green, or black coloration indicated phenolic compounds.

**Terpenoids Detection:** Salkowski test was conducted byReddish-brown coloration indicated terpenoid presence.

**Saponins Detection:** Froth test involved vigorous shaking of 2ml plant extract with 6ml distilled water. Persistent froth formation indicated saponin presence.

**Tannins Detection:** Ferric chloride test was performed by adding 2-3 drops of 5% ferric chloride to 2ml plant extract.

Blue-black or green precipitate indicated tannin presence. (Akhtar *et al.* 2018).

**Antidiabetic Activity Assessment**

**α-Amylase Inhibition Assay**

The α-amylase inhibitory activity was determined using the dinitrosalicylic acid (DNS) method (Miller, 1959). Porcine pancreatic α-amylase (1 unit/ml) in phosphate buffer (pH 6.9) was pre-incubated with different concentrations of plant extracts (50-500 µg/ml) at 25°C for 10 minutes. Starch solution (0.5%) was added as substrate, and the reaction mixture was incubated at 25°C for 10 minutes. The reaction was terminated by adding DNS reagent and boiling for 5 minutes. Absorbance was measured at 540 nm using a UV-visible spectrophotometer. Acarbose served as positive control. Inhibition percentage was calculated using the formula:

% Inhibition = [(Acontrol - Asample) / Acontrol] × 100

(Ewenighi, 2015).

**α-Glucosidase Inhibition Assay**

α-Glucosidase inhibitory activity was assessed using p-nitrophenyl-α-D-glucopyranoside (pNPG) as substrate (Kim et al., 2005). Different concentrations of plant extracts (50-500 µg/ml) were pre-incubated with α-glucosidase enzyme (1 unit/ml) in phosphate buffer (pH 6.8) at 37°C for 15 minutes. pNPG solution (5 mM) was added, and the mixture was incubated at 37°C for 20 minutes. The reaction was stopped by adding sodium carbonate (0.1 M), and absorbance was measured at 405 nm. Acarbose was used as reference standard (Patel et al., 2012).

**Antioxidant Activity Evaluation**

**DPPH Radical Scavenging Assay**

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined. Different concentrations of plant extracts (50-500 µg/ml) were mixed with DPPH solution Absorbance was measured at 517 nm. Ascorbic acid served as positive control

**ABTS Radical Scavenging Assay**

The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical cation decolorization assay The ABTS•+ solution was diluted with phosphate buffer to achieve an absorbance of 0.70 ± 0.02 at 734 nm. Plant extracts at various concentrations were mixed with ABTS•+ solution and incubated for 6 minutes before absorbance measurement (Mahmoud et al., 2017).

**Ferric Reducing Antioxidant Power (FRAP) Assay**

FRAP assay was conducted Plant extracts were mixed with FRAP reagent and incubated at 37°C for 4 minutes. Absorbance was measured at 593 nm, and results were expressed as ascorbic acid equivalents.

**Molecular Docking Studies**

Molecular docking was performed using AutoDock Vina software. Docking parameters were set to default values, and the best conformations were selected based on binding energy scores**.**

**Statistical Analysis**

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation. $IC_{50}$ values were calculated using GraphPad Prism software. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was employed for statistical comparisons. Statistical significance was set at $p < 0.05$.

supplementary I.

**RESULTS**

The extraction yields varied significantly between solvents, with ethanolic extraction showing higher efficiency compared to aqueous extraction. Qualitative phytochemical screening revealed the presence of multiple classes of secondary metabolites in both extracts. (Table 1).

Table 1: Qualitative analysis of phytochemicals of Aqueous and Ethanolic extracts of *Cupaniopsis anacardioides* fruits.

| Serial No. | Compounds | Test Name | Aqueous | Ethanolic |
|---|---|---|---|---|
| 1. | Flavonoids | Alkaline Reagent Test | ++ | +++ |
| 2. | Phenolic compounds | Ferric Chloride Test | ++ | +++ |
| 3. | Alkaloids | Wagner’s Test | _ | _ |
| 4. | Terpenoids | Salkowski’s Test | + | ++ |
| 5. | Saponins | Froth Test | +++ | +++ |
| 6. | Carbohydrates | Molish’s Test | + | ++ |
| 7. | Tannins | Ferric Chloride Test | ++ | +++ |
| 8. | Glycosides | Keller Killiani Test | + | ++ |

+++ = appreciable amount; ++ = moderate amount; + = trace amount; - = completely absent

Table 2: α-Amylase and α-Glucosidase inhibitory activity of *Syzygium cumini* fruit extracts at different concentrations.

| Extract Type | Concentration (µg/ml) | α-Amylase (% Inhibition) | α-Glucosidase (% Inhibition) |
|---|---|---|---|
| Aqueous Extract (ASE) | 50 | 23.4 ± 1.8 | 28.7 ± 2.1 |
| Aqueous Extract (ASE) | 100 | 38.9 ± 2.3 | 45.2 ± 2.8 |
| Aqueous Extract (ASE) | 250 | 58.7 ± 2.9 | 68.4 ± 3.1 |
| Aqueous Extract (ASE) | 500 | 76.3 ± 2.5* | 82.6 ± 1.9* |
| Ethanolic Extract (ESE) | 50 | 31.2 ± 2.1 | 36.8 ± 2.4 |
| Ethanolic Extract (ESE) | 100 | 48.6 ± 2.7 | 54.3 ± 2.9 |
| Ethanolic Extract (ESE) | 250 | 71.8 ± 3.2 | 78.9 ± 2.6 |
| Ethanolic Extract (ESE) | 500 | 89.7 ± 1.8** | 91.4 ± 2.1** |
| Acarbose (Standard) | 50 | 92.3 ± 1.4 | 94.7 ± 1.2 |

Data are expressed as mean ± SD (n=3). Aqueous extract (ASE) and ethanolic extract (ESE) showed a dose-dependent inhibition of both enzymes. Acarbose was used as the standard inhibitor. Significance: $p < 0.05$ (*), $p < 0.01$ (**).

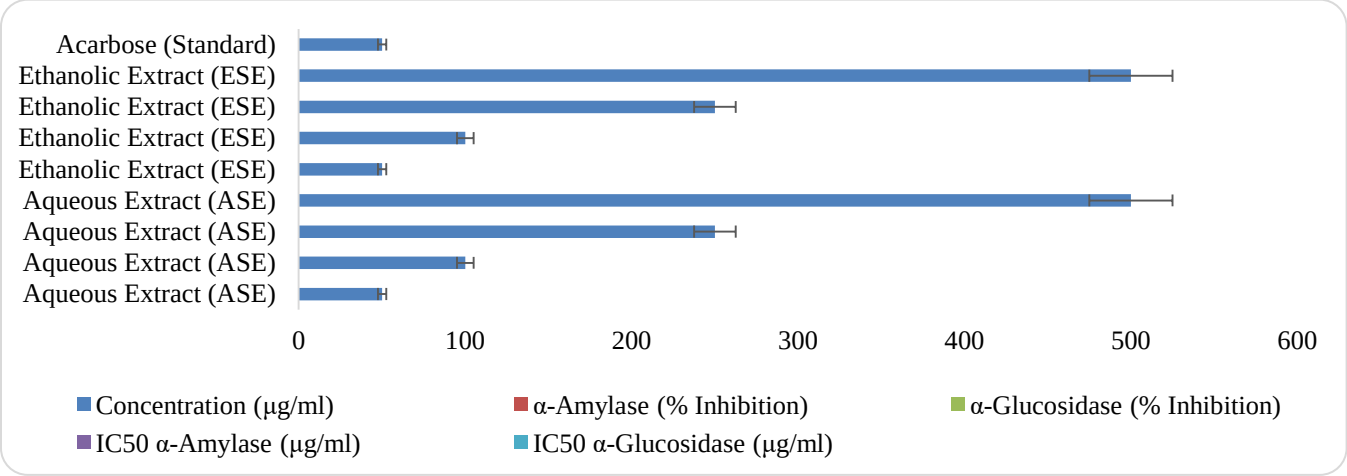


Figure 1: α-Amylase and α-Glucosidase inhibitory activity of *Syzygium cumini* fruit extracts at different concentrations

**Antidiabetic Activity**

**α-Amylase Inhibition** Both aqueous and ethanolic extracts demonstrated significant α-amylase inhibitory activity in a dose-dependent manner (Figure 1). The ethanolic extract exhibited superior inhibitory potential with an $IC_{50}$ value of 45.2 ± 2.1 µg/ml compared to the aqueous extract $IC_{50}$: 67.8 ± 3.4 µg/ml).

At the highest tested concentration (500 µg/ml), ethanolic extract achieved 89.7 ± 1.8% inhibition, while aqueous extract showed 76.3 ± 2.5% inhibition. The standard drug acarbose demonstrated an IC50 value of 38.9 ± 1.6 µg/ml (Table 2).

Table 3: α-Amylase and α-Glucosidase $IC_{50}$ of *Syzygium cumini* fruit extracts at different concentrations.

| Extract Type | Concentration (µg/ml) | $IC_{50}$ α-Amylase (µg/ml) | $IC_{50}$ α-Glucosidase (µg/ml) |
|---|---|---|---|
| Aqueous Extract (ASE) | 50 | 67.8 ± 3.4 | 52.4 ± 2.8 |
| Aqueous Extract (ASE) | 100 | | |
| Aqueous Extract (ASE) | 250 | | |
| Aqueous Extract (ASE) | 500 | | |
| Ethanolic Extract (ESE) | 50 | 45.2 ± 2.1 | 38.7 ± 1.9 |
| Ethanolic Extract (ESE) | 100 | | |
| Ethanolic Extract (ESE) | 250 | | |
| Ethanolic Extract (ESE) | 500 | | |
| Acarbose (Standard) | 50 | 38.9 ± 1.6 | 31.2 ± 1.4 |

Data are expressed as mean ± SD (n=3). Aqueous extract (ASE) and ethanolic extract (ESE) showed a dose-dependent inhibition. Significance: $p < 0.05$ (*), $p < 0.01$ (**).

**α-Glucosidase Inhibition activity of plant extract**

α-Glucosidase inhibitory activity followed similar patterns, with ethanolic extract showing greater potency ($IC_{50}$: 38.7 ± 1.9 µg/ml) compared to aqueous extract (IC50: 52.4 ± 2.8 µg/ml). Maximum inhibition achieved by ethanolic extract was 91.4 ± 2.1% at 500 µg/ml concentration, while aqueous extract reached 82.6 ± 1.9% inhibition (Figure 1). Acarbose reference standard exhibited an IC50 value of 31.2 ± 1.4 µg/ml (Table 3).

**Antioxidant Activity**

**DPPH Radical Scavenging Activity**Both extracts demonstrated substantial DPPH radical scavenging potential Ethanolic extract showed superior activity with IC50 value of 78.4 ± 2.7 µg/ml compared to aqueous extract (IC50: 96.2 ± 3.1 µg/ml). At 500 µg/ml concentration, ethanolic extract achieved 87.3 ± 2.2% scavenging activity, while aqueous extract showed 79.1 ± 2.8%. Ascorbic acid standard demonstrated $IC_{50}$ value of 52.6 ± 1.8 µg/ml. (Figure 2).

**ABTS Radical Scavenging Activity**

ABTS radical scavenging assay revealed similar trends with ethanolic extract (IC50: 65.3 ± 2.4 µg/ml) outperforming aqueous extract (IC50: 84.7 ± 3.2 µg/ml) **(**Figure 2).

**FRAP Activity**

Ferric reducing power increased proportionally with extract concentration. Ethanolic extract exhibited higher reducing power (1247.3 ± 45.2 µg ascorbic acid equivalents/mg extract) compared to aqueous extract (896.7 ± 38.9 µg ascorbic acid equivalents/mg extract) at 500 µg/ml concentration. (Table 4).

Table 4: Antioxidant activity of *Syzygium cumini* fruit extracts determined by DPPH, ABTS, and FRAP assays. Data are expressed as mean ± SD (n=3). Both aqueous extract (ASE) and ethanolic extract (ESE)

| Extract Type | Concentration (µg/ml) | DPPH Scavenging (%) | ABTS Scavenging (%) | FRAP (µg AAE/mg) | $IC_{50}$ DPPH (µg/ml) | $IC_{50}$ ABTS (µg/ml) |
|---|---|---|---|---|---|---|
| Aqueous Extract (ASE) | 50 | 28.6 ± 2.1 | 32.4 ± 2.3 | 234.7 ± 18.9 | 96.2 ± 3.1 | 84.7 ± 3.2 |
| Aqueous Extract (ASE) | 100 | 42.8 ± 2.8 | 47.9 ± 2.9 | 425.3 ± 22.4 | | |
| Aqueous Extract (ASE) | 250 | 65.4 ± 3.1 | 69.7 ± 3.2 | 648.9 ± 28.7 | | |
| Aqueous Extract (ASE) | 500 | 79.1 ± 2.8* | 85.4 ± 2.3* | 896.7 ± 38.9* | | |
| Ethanolic Extract (ESE) | 50 | 35.7 ± 2.4 | 41.2 ± 2.6 | 298.4 ± 21.3 | 78.4 ± 2.7 | 65.3 ± 2.4 |
| Ethanolic Extract (ESE) | 100 | 52.9 ± 2.9 | 58.6 ± 3.1 | 534.8 ± 26.7 | | |
| Ethanolic Extract (ESE) | 250 | 74.6 ± 3.3 | 79.8 ± 2.8 | 823.6 ± 33.2 | | |
| Ethanolic Extract (ESE) | 500 | 87.3 ± 2.2** | 92.8 ± 1.7** | 1247.3 ± 45.2** | | |
| Ascorbic Acid (Standard) | 100 | 94.8 ± 1.5 | 96.2 ± 1.3 | 1456.9 ± 52.8 | 52.6 ± 1.8 | 48.3 ± 2.1 |

Significance: $p < 0.05$ (*), $p < 0.01$ (**). AAE = Ascorbic Acid Equivalents

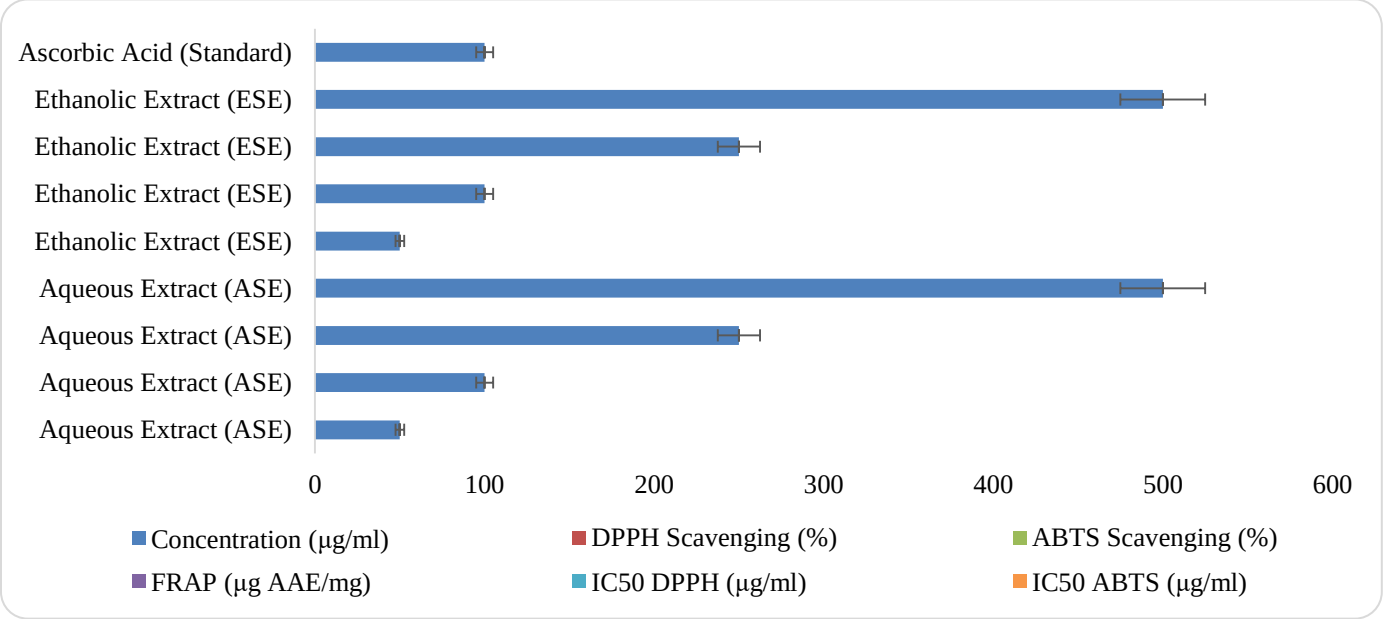


Figure 2: Antioxidant activity of *Syzygium cumini* fruit extracts determined by DPPH, ABTS, and FRAP assays.

Table 5: Molecular docking analysis of major phytochemicals from *Syzygium cumini* fruits against key target proteins involved in diabetes (α-Amylase, α-Glucosidase, and DPP-4).

| Compound | α-Amylase (1HNY) Binding Energy (kcal/mol) | α-Glucosidase (3A4A) Binding Energy (kcal/mol) | DPP-4 (2RGU) Binding Energy (kcal/mol) | Average Binding Energy |
|---|---|---|---|---|
| Quercetin | -8.4 ± 0.2** | -8.7 ± 0.3** | -7.9 ± 0.2** | -8.33 |
| Ellagic Acid | -7.8 ± 0.3* | -8.1 ± 0.2** | -7.2 ± 0.3* | -7.70 |
| Gallic Acid | -6.9 ± 0.2* | -7.3 ± 0.3* | -6.5 ± 0.2* | -6.90 |
| Myricetin | -7.5 ± 0.2* | -7.8 ± 0.2* | -7.1 ± 0.3* | -7.47 |
| Acarbose (Standard) | -7.2 ± 0.3 | -7.6 ± 0.2 | N/A | -7.40 |

Significance: $p < 0.05$ (*), $p < 0.01$ (**). AAE = Ascorbic Acid Equivalents

**Molecular Docking**

**Molecular Docking Results**

Molecular docking studies revealed favorable binding interactions between major phytochemicals and target proteins (Table 5). Quercetin showed the strongest binding affinity with α-amylase (-8.4 kcal/mol), α-glucosidase (-8.7 kcal/mol), and DPP-4 (-7.9 kcal/mol). Ellagic acid demonstrated good binding scores with α-amylase (-7.8 kcal/mol) and α-glucosidase (-8.1 kcal/mol). The binding conformations revealed multiple hydrogen bonds and hydrophobic interactions contributing to complex stability **(**Figure 3).

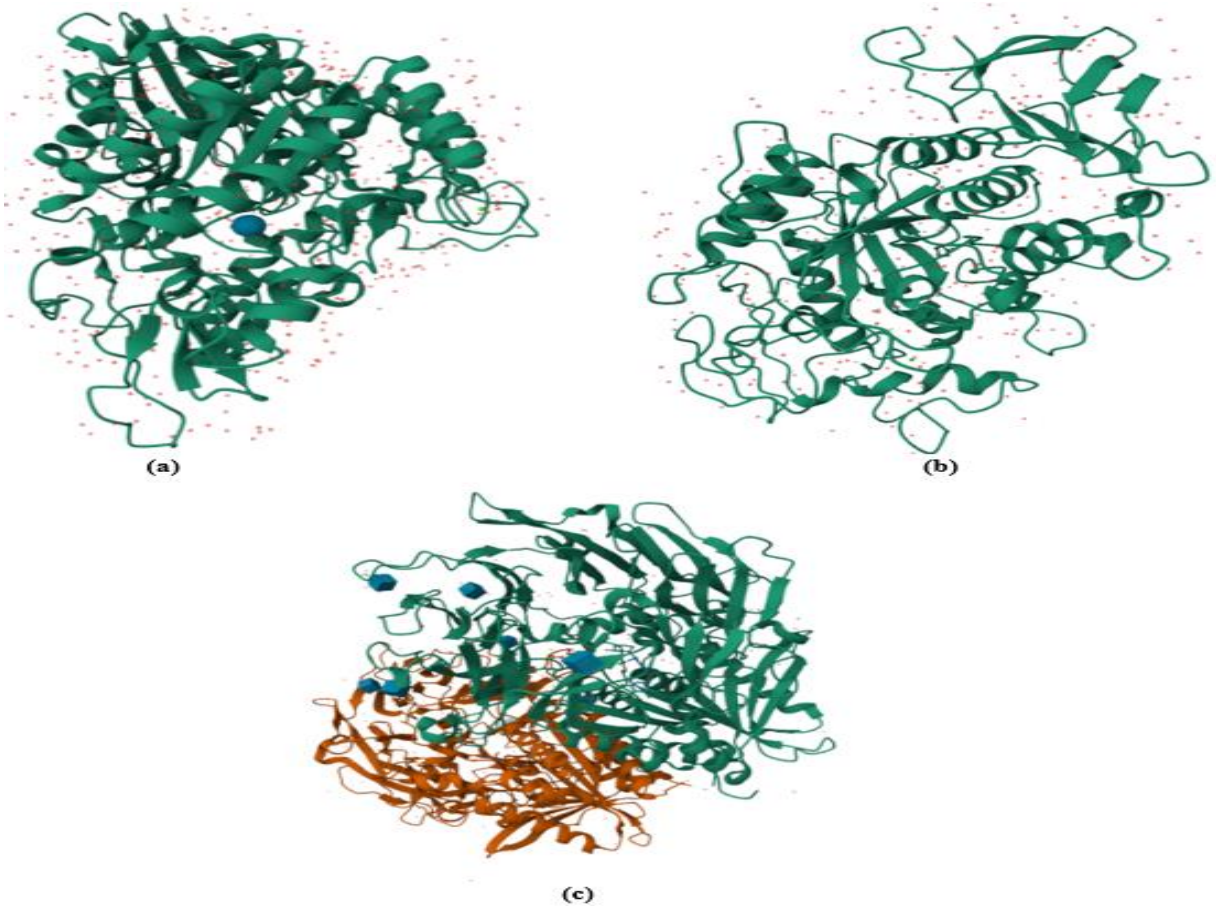


Figure 3. Key target PDB proteins involved in diabetes (α-Amylase (1HNY), α-Glucosidase (3A4A), and DPP-4 (2RGU).

Analysis of binding poses revealed that quercetin forms hydrogen bonds with key amino acid residues in the active sites of all target proteins. In α-amylase, quercetin interacted with Asp197, Glu233, and Asp300, while in α-glucosidase, interactions occurred with Asp214, Asp349, and Arg442. These interactions mirror the binding patterns of known inhibitors, supporting the predicted antidiabetic activity (Figure 4).

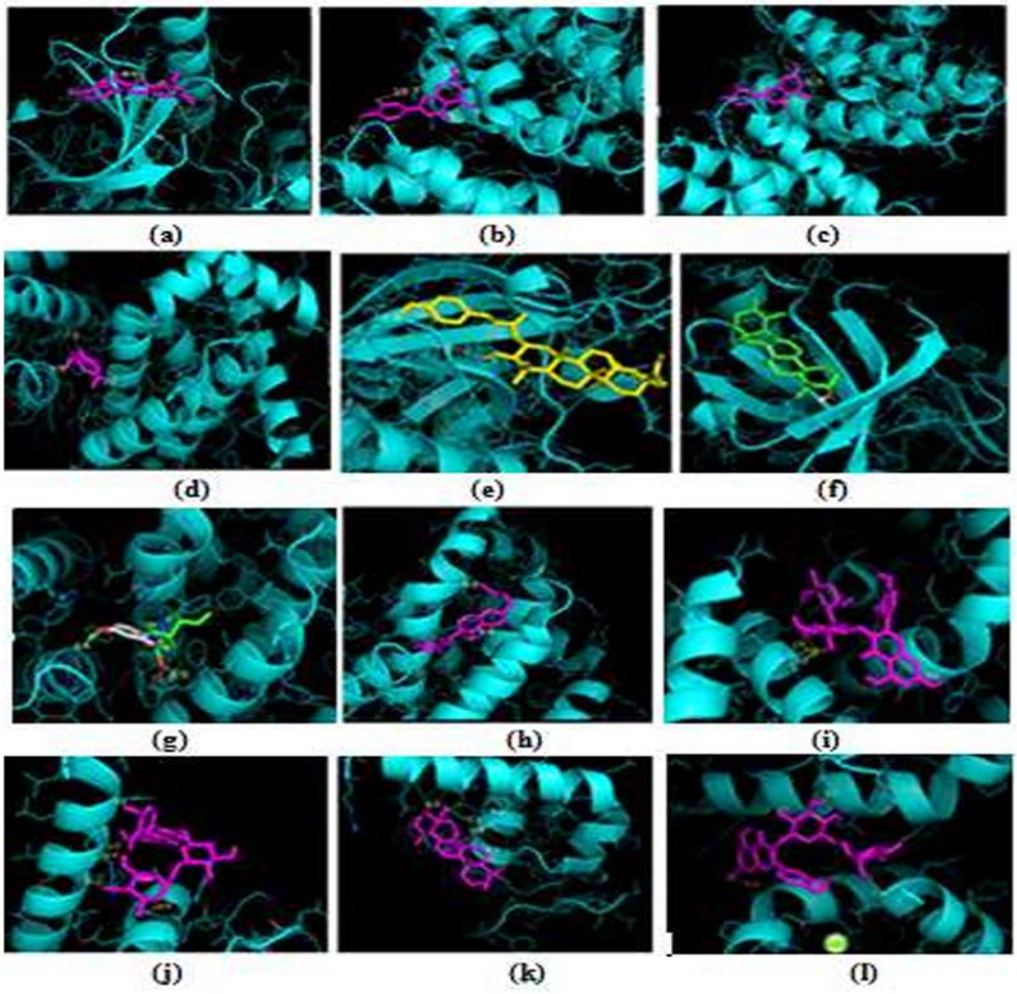


Figure 4. Representative docking interaction images of the top phytochemicals (Quercetin, Ellagic Acid, Gallic Acid, Myricetin) with α-Amylase (1HNY), α-Glucosidase (3A4A), and DPP-4 (2RGU).

**DISCUSSION**

with 90% having type 2 diabetes, and its prevalence, particularly high in Pakistan, is projected to reach 700 million by 2045, necessitating urgent preventive measures and therapies (Tran *et al*., 2020; Azeem *et al*., 2022; Ardalani *et al*., 2021; Verma *et al*., 2012). This enhanced effect is attributed to the high extraction efficiency of phytochemicals such as flavonoids, saponins, terpenoids, and phenolic compounds. The significant α-amylase and α-glucosidase inhibitory activities observed in this study align with the traditional use of *S. cumini* fruits in diabetes management. These enzymes play crucial roles in carbohydrate digestion and glucose absorption, making them important therapeutic targets for postprandial glucose control. The inhibition of these enzymes can effectively reduce glucose spikes after meals, a critical aspect of diabetes management. Flavonoids, particularly quercetin and myricetin identified in *S. cumini* fruits, have been extensively studied for their antidiabetic properties. These compounds exert their effects through multiple mechanisms including enzyme inhibition, insulin sensitization, and glucose transporter modulation. The molecular docking results support these mechanisms, showing favorable binding interactions between flavonoids and target proteins. The potent antioxidant activity demonstrated by both extracts is particularly relevant in diabetes management, as oxidative stress plays a central role in diabetic complications. The ability to scavenge free radicals and reduce oxidative damage can potentially prevent or delay diabetic complications including nephropathy, retinopathy,

and cardiovascular diseases. The molecular docking studies provide valuable insights into the potential mechanisms of action. The strong binding affinities observed between major phytochemicals and target proteins suggest that multiple compounds may work synergistically to produce the observed effects. This multi-target approach is advantageous in diabetes management, as it addresses various aspects of the disease simultaneously.

These compounds may contribute to the overall antidiabetic activity observed in the study. The integration of experimental and computational approaches in this study provides a robust foundation for understanding the therapeutic potential of *S. cumini* fruit extracts. While in vitro studies demonstrate biological activities, molecular docking predicts potential mechanisms, creating a comprehensive picture of the compounds' therapeutic profiles.

The findings of this study support the traditional use of *S. cumini* fruits in diabetes management and provide scientific evidence for their therapeutic potential. The multi-target approach offered by these natural extracts, combined with their antioxidant properties, makes them attractive candidates for comprehensive diabetes management strategies.

## CONCLUSION

This comprehensive study demonstrates the significant antidiabetic and antioxidant potential of *Syzygium cumini* fruit extracts. The ethanolic extract showed superior activity across all evaluated parameters, attributed to its higher phytochemical content. The multi-target approach offered by these natural extracts, combined with their safety profile, makes them promising candidates for developing novel antidiabetic therapeutics. Further in vivo studies and clinical trials are warranted to fully establish their therapeutic efficacy and safety in human populations.

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