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Research Article

NUTRITIONAL ANALYSIS, *IN VITRO* AND *IN SILICO* STUDIES ON ANTIOXIDANT AND ANTI-DIABETIC POTENTIAL OF *COCCINIA GRANDIS* L. FRUIT

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ABSTRACT

Coccinia, commonly known as ivy gourd (Coccinia grandis L.), is a medicinal food plant with numerous health benefits. It is widely used in traditional medicine, particularly in Ayurveda and folk medicine, for managing conditions such as inflammation and oxidative stress. The precise biochemical mechanisms through which Coccinia exerts its therapeutic effects are not yet fully understood, and there is limited research on its long-term safety and potential side effects. Investigating these lesser-reported aspects of Coccinia is crucial for filling the knowledge gaps, developing natural therapeutics, and validating its role in modern healthcare. The aim of this study was to evaluate the nutritional profile, antioxidant activity, and the anti-diabetic potential of the fruit of Coccinia grandis L. through in vitro and in silico approaches. From the study, the fruit extract of C. grandis possesses a good nutritional profile like protein (1.2 g/100 g) and fiber (1.2 g/100 g). There are 38 ± 0.45 µg/g of total alkaloids, 84 ± 0.25 µg/g of total phenols and 98 ± 0.31 µg/g of total flavonoids in the fruit extract. When compared to standard (butylated hydroxytoluene), the extract exhibits the strong antioxidant activity of 94.4% at 250 µg/mL. The results of the in vitro assay highlighted that the extract has low inhibition activity on α-amylase (4.6% at 250 µg/mL) and moderate inhibition against α-glucosidase (53% at 250 µg/mL) when compared to the standard acarbose. The phytoconstituent quercetin was quantified in the fruit extract (0.06 % w/w) using HPTLC method. Further, the computational in silico analysis of quercetin binding affinity with alpha amylase (-15.866 kcal/mol) and alpha glucosidase glucosidase (-14.147 kcal/mol) enzymes and the in silico results are consistent with in vitro experimental data. The study concluded that the fruit of C. grandis presents a good source of nutrients with antioxidant and anti-diabetic potentials.

1. Introduction

Coccinia grandis L., commonly known as ivy gourd or scarlet gourd, has been studied for its medicinal and nutritional properties. Earlier studies have reported that it is rich in vitamins such as vitamin A, C, and B-complex, along with essential minerals like iron, calcium, phosphorus, and potassium. It also contains dietary fiber and essential amino acids, making it a valuable functional food (Tardy *et al*., 2020). The fruit is known to contain various phytochemicals, including flavonoids, alkaloids, terpenoids, saponins, tannins, and phenolics. Additionally, it has a high content of β-carotene, lutein, and lycopene, contributing to its antioxidant properties (Lee and Joo, 2022). Pharmacologically, *C. grandis* exhibits significant antioxidant activity due to the presence of polyphenols and flavonoids, which contribute to its free-radical scavenging ability. Studies have also shown its anti-diabetic potential, as it helps lower blood glucose levels by enhancing insulin sensitivity. Furthermore, the fruit possesses anti-inflammatory and hepatoprotective properties, helping to reduce oxidative stress-related inflammation. It also demonstrates antimicrobial activity against various pathogens, making it a promising candidate for medicinal applications (Pekamwar *et al*., 2013).

Despite the existing research on *C. grandis*, further studies are necessary to provide a more comprehensive understanding of its health benefits. There is a need for detailed nutritional profiling to validate its role as a functional food. *In vitro* and *in silico* studies will help assess its antioxidant and anti-diabetic potential more precisely. *In vitro* studies will evaluate antioxidant properties, along with anti-diabetic potential through α-amylase and α-glucosidase inhibition assays. *In silico* studies will include molecular docking and computational analysis to predict the interaction of bioactive compounds with diabetic and oxidative stress-related targets.

Moreover, many traditional uses of *C. grandis* lack scientific validation, creating a gap between traditional knowledge and scientific evidence. This study aims to bridge that gap by providing robust scientific data supporting its medicinal applications. Additionally, identifying bioactive compounds in the fruit could lead to the development of natural antioxidant and anti-diabetic supplements, contributing to pharmaceutical and nutraceutical advancements. By conducting nutritional analysis, *in vitro*, and *in silico* studies, this work aims to establish *C. grandis* as a scientifically validated functional food with significant antioxidant and anti-diabetic properties.

The International Diabetes Federation published statistics in 2021 that showed 1 in 11 persons in Southeast Asia had diabetes mellitus (DM), with over half (46 million) going untreated. By 2045, it is expected that there will be 152 million diabetics, underscoring the urgent need for all-encompassing regional action. With an 8.3% prevalence rate, one in seven (90 million) persons worldwide have diabetes in India alone (IDF, 2024). According to the WHO report in 2021, noncommunicable diseases accounted for seven out of the ten top causes of death worldwide. DM is one of the seven noncommunicable diseases (WHO, 2024). DM is a group of metabolic disorders of carbohydrates, proteins, and lipids. It is characterized by hyperglycemia resulting from a defect in beta cells of the pancreas. This leads to affect insulin secretion, failure of insulin action, or both. Untreated conditions, DM can cause microvascular complications such as damage to the eye, which can cause retinopathy; damage to the kidney, which leads to nephropathy; and damage to the nerve, which results in neuropathy. It can also cause macrovascular complications such as myocardial infarction, cerebrovascular accident, thrombosis, and atherosclerosis (Deshpande *et al*., 2008). DM is categorized into two types: insulin-dependent (IDDM) and non-insulin-dependent (NIDDM) DM. IDDM is called type I DM, and NIDDM is known as type II DM (CDC, 2024). Due to immunological reactions occurring in type I DM, beta cells are selectively destroyed. There

is no insulinogenic stimuli that causes hypoinsulinemia. Juvenile suffering from type I DM. The imbalance between blood sugar absorption and insulin secretion causes type II DM. Type II DM is controlled by delaying postprandial hyperglycemia via secretion of insulin from beta cells, optimizing insulin action, lowering glucogenesis and gluconeogenesis, interfering with carbohydrate metabolism, blocking ATP-gated potassium channels, stimulating peroxisome proliferator-activated receptor activity (PPAR), and modifying glucagon-like peptide-1 (GLP-1) (Casa-Villegas *et al*., 2018). In the present study, therapeutic approaches for controlling postprandial hyperglycemia are designed to inhibit gastrointestinal carbohydrate-hydrolyzing enzymes alpha amylase and alpha glucosidase. Alpha amylase has the property of cleaving the glycosidic linkage of alpha (1,4) in homo- and heteropolysaccharides to produce oligosaccharides (Ogunyemi *et al*., 2022).

Alpha-glucosidase breaks the alpha 1,4-glycosidic linkage in oligosaccharides to produce monosaccharides (Patil *et al*., 2017). Only monosaccharides are absorbed via the intestinal mucosa cells. When carbohydrate-hydrolyzing enzymes are blocked, blood glucose concentrations fall, diminishing the insulin requirement (Ekor, 2014), which can assist to prevent the production of Advanced Glycation End-Products (AGEs). AGEs are a risk factor for complications of DM. Most of the pharmacotherapeutics reveal that DM is not a cure because the rising prevalence causes difficulties. The use of phytotherapeutics as a supplement to existing pharmaceuticals to control DM and its complications (Bharti *et al*., 2018). Many herbs have anti-diabetic properties. *Coccinia grandis* (Cucurbitaceae), also have revealed that this plant is capable of acting as an antipyretic, analgesic, antibacterial, anti-inflammatory, antidiabetic, antioxidant, antidyslipidemic, antituberculosis, antieczema, antiulcer, hepatoprotective, anticancer, and antitussive agent (DAF, 2016). There are few studies on the phytoconstituents that are responsible for suppressing the carbohydrate-digesting enzymes that control DM. A balanced diet throughout life supports growth, development, and aging while also lowering the risk of chronic disease, resulting in general health and well-being (Patil *et al*., 2017). Experimental screening processes are time-consuming and expensive; computational screening has received a lot of attention in recent years. Hence, in this present study, the anti-diabetic effect of a flavonoid compound identified by HPTLC from the fruits of *C. grandis* L. was determined by an experimental *in vitro* and *in silico* approach to explore the antidiabetic targets by inhibiting alpha amylase and alpha glucosidase enzymes.

2. Materials and methods

2.1. Collection of plant material

Fruits of *C. grandis* L. were collected from Thanjavur, Tamil Nadu, India. To get rid of contaminants, the collected fruits cleaned several times with distilled water. After being, sliced fruits were spread out on plain paper and allowed to dry in the shade for ten days at room temperature, then ground into a fine powder using a grinder mixture. Powered materials used in further work.

2.2. Preparation of the extract

The 100 g of powdered material of *C. grandis* fruit was extracted with 1 L sterile distilled water at room temperature for 24 h, filtered using Whatman 42 filter paper and freeze dried. The powder was dissolved in 80 percent hydro-alcohol with a know concentration, again filterd and used for further experiments.

2.3. Determination of moisture content

The moisture content of a sample was determined by weighing the sample before and after drying in a preheated oven at 105°C for 6 hours. The sample was first weighed along with the crucible, and then after drying, it was cooled in a desiccator to prevent moisture absorption. The moisture content was calculated by comparing the weight loss, which

corresponds to the amount of water evaporated from the sample.

2.4. Determination of total ash

The crucible was weighed and placed over the electric burner and the crucible was partially opened. The sample was charred with initial expulsion of smoke and the crucible was placed in a muffle furnace and heated to 600^{o}C and kept for 2 hours. Organic matter in the sample burnt to left the minerals. The crucible was removed from the furnace and cooled it in a dessicator to room temperature and weighed.

2.5. Nutritional profile

Determination of total carbohydrates (Morris, 1948), total proteins (Lowry *et al.,* 1952), total lipids (Bligh and Dyer, 1959), total energy value (Sands, 1974) and total fibers (Maynard, 1970) were carried out in the *C. grandis* fruit sample. The total carbohydrate content was determined using the anthrone method. In the presence of sulfuric acid, carbohydrates undergo hydrolysis into simple sugars, which further dehydrate to form furfural derivatives. These derivatives react with anthrone to produce a blue-green complex that was measured spectrophotometrically at 620 nm.

The Lowry method is based on the reaction of protein molecules with the Folin–Ciocalteu reagent. The method involves two steps: first, proteins react with copper ions under alkaline conditions (Biuret reaction), forming a complex; second, the complex undergoes reduction by the Folin-Ciocalteu reagent, producing a blue color that was measured spectrophotometrically at 660 nm. The intensity of the color was proportional to the protein concentration in the sample. The Bligh and Dyer method was used as solvent extraction technique for determining total lipids in *C. grandis* fruit. Chloroform-methanol-water system was employed to efficiently extract lipids while minimizing non-lipid contaminants. Lipids were extracted into the chloroform phase, separated from the aqueous phase, and quantified gravimetrically. The total lipid content was calculated by the formula: Total Lipid Content (%) = (Weight of lipids / Weight of sample) x 100. Total energy value was calculated using the formula: Total Energy (kcal/100g) = (Carbohydrates x 4) + (Proteins x 4) + (Lipids x 9).

The determination of total fibre was done by the Maynard method (1970), which involves sequential digestion of the sample with acid and alkali to remove digestible components, leaving behind the indigestible fiber fraction. First, a weighed sample (2-5 g) was boiled in 1.25% sulfuric acid for 30 minutes, maintaining a constant volume by adding hot distilled water as needed. The mixture was then filtered through Whatman No. 41 filter paper or a sintered glass crucible, and the residue was washed thoroughly with hot distilled water. The residue was then transferred to a beaker containing 1.25% sodium hydroxide and boiled for another 30 minutes before being filtered and washed again with hot distilled water, 95% ethanol, and acetone to remove non-fiber components. The purified residue was dried at 105°C for 6 hours, cooled in a desiccator, and weighed (W_1). To determine the fiber content, the dried residue was ashed in a muffle furnace at 550°C for 4–5 hours, cooled in a desiccator, and reweighed (W_2). The total fiber content was calculated using the formula: Total Fiber Content (%) = ((W1−W2) / Sample weight) x 100.

2.6. Phytochemical analysis

Alkaloids content of aqeous fruit extract was estimated by Evans (2002) method. Phenol content was determined using modified Folin-Ciocalteu's method described by Singleton and Rossi (1965). Flavonoid content measured through aluminium chloride method observed by Chang et al. (2002).

2.7. *In vitro* assays

The antioxidant capacity of the fruit sample is tested based on the procedure described by Evans (1991), which utilizes the DPPH method. In this method, a 0.1 mM DPPH solution was prepared and mixed with the fruit

extract. The DPPH radical, initially violet in color, was reduced by antioxidants present in the sample, leading to a color change towards colorlessness or light yellow. After incubating the mixture for 30 minutes at room temperature, the absorbance was measured at 517 nm using a spectrophotometer. The degree of scavenging activity was calculated by comparing the decrease in absorbance to a control.

The alpha-amylase inhibitory activity of the fruit sample was determined by using 4-nitrophenyl α-D-glucopyranoside (CNPG) as a substrate (Gella et al., 1997). The method involves incubating the fruit extract with alpha-amylase, followed by the addition of CNPG. If the enzyme is inhibited, the hydrolysis of CNPG was reduced, leading to a lower production of 4-nitrophenol. The amount of 4-nitrophenol released was measured spectrophotometrically, and the inhibition of alpha-amylase was quantified by comparing the absorbance with a control sample. The percentage inhibition was calculated using the formula:

$$\text{Inhibition Percentage} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The inhibition of alpha-glucosidase was evaluated by measuring the ability of the fruit extract to inhibit the hydrolysis of p-nitrophenyl-α-D-glucopyranoside (p-NPG) (Dewi et al., 2007). The fruit extract was incubated with alpha-glucosidase and p-NPG, and the resulting release of p-nitrophenol was measured at a specific wavelength of 405 nm. A decrease in the amount of p-nitrophenol indicates the inhibition of the enzyme. The percentage inhibition of alpha-glucosidase was calculated by comparing the absorbance values.

2.8. HPTLC analysis

Hydrochloroform extract was prepared for HPTLC analysis to identify both hydrophilic and lipophilic compounds present in the sample. The aqueous extract was used to obtain hydrophilic bioactive compounds, such as polyphenols and flavonoids, which are often responsible for the observed antioxidant and anti-diabetic activity. Analyzing the aqueous extract was used in the *in vitro* assays and hence HPTLC test is crucial because it allows for the identification and profiling of the compounds responsible for the biological effects observed, such as alpha-amylase, alpha-glucosidase inhibition and free radical scavenging. This analysis not only helps correlate the chemical composition with the bioactivity but also ensures the quality control and standardization of the extract by providing a chemical fingerprint.

The test sample (0.5 g) was taken in a fractional funnel with 50 mL of water and added chloroform to extract. After shaking, the chloroform fraction was collected and dried and the dried sample was dissolved in chloroform again. The standard quercetin sample was prepared using 20 mg of quercetin in 10 mL of methanol and 1 mL of standard was diluted to 10 mL. Linomat5 (CAMAG) applicator was used for applying 1 to 4 µL of standard and 5 to 25 µL of sample to a precoated silica gel 60 F254 HPTLC plates (Merck) with a uniform thickness of 0.2 mm. The plate was developed in the Toluene: Ethyl acetate: Formic acid (5:4:1) solvent system to a distance of 8 cm. CAMAG TLC Scanner3 was used to scan the plate at 254 nm and CAMAG REPROSTAR3 was used to observe the plate under UV light at 254 nm and 366 nm.

2.9. *In silico* studies (molecular docking)

GalaxyDockWeb (2024), an online web tool was used for flexible docking study. The structure of the identified compound (Quercetin; $C_{15}H_{10}O_7$; M.W: 302.23) by HPTLC in the fruit extract taken as a ligand and its 3D coordinate was downloaded (Figure1) from the PubChem database to check the binding affinity with the carbohydrate hydrolyzing enzymes of alpha-amylase and alpha-glucosidase. The 3D crystal structures of alpha-amylase (PDB ID: 2QV4) and alpha-glucosidase (PDB ID: 5NN5) were downloaded from the PDB database. Using Biovia Discovery Studio Client 2021, the target

proteins were prepared by eliminating ligands and other non-receptor entities that might occupy surrounding space. Water was removed from the crystal structures to refine them. In the Discovery Studio Visualiser (DSV), 3D structures of protein targets created and saved in PDB format. The number and bond length of hydrogen bonds and binding amino acid residues were visualized in the form of a 2D interaction using the Discovery studio visualizer after docking.

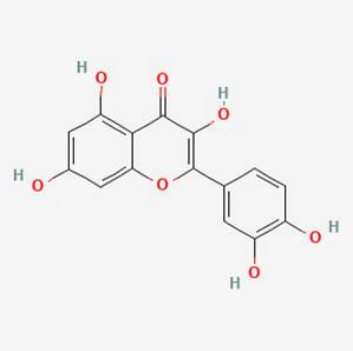


Figure 1. Chemical structure of quercetin (PubChem ID: 5280343).

3. Results and discussion

3.1. Nutritional profile

The rate of absorption and the quality of food are determined by the amount of moisture available in the food. The reported moisture value (3.2%) indicates that the fruits could be stored at room temperature for a long time (Table 1). The amount of minerals likely to be found in food substances is largely determined by the amount of ash present in the food. The reported value of ash (2.8 g/100 g) indicated that *C. grandis* fruit is a good source of minerals. Protein-rich diets are shown to be an effective weight-loss tool, hence sustained satiety is essential for promoting weight loss and inducing a negative energy balance (Moon and Koh, 2020). According to the analysis report, the current test sample of *C. grandis* fruits contains proteins (1.2 g/100 g), lipids (0.2 g/100 g) and carbohydrates (3.1 g/100 g). In addition, *C. grandis* is also identified to contain high-fiber sources (1.2 g/100 g). Increased fiber consumption has also been linked to a lower risk of metabolic syndrome and factors that raise the risk of heart disease and diabetes (Lattimer and Haub, 2010). Hence, *C. grandis* fruit may be added to our daily diets to reduce the risk of diseases like heart and diabetes.

Table 1. Proximate and Nutritional profiles in the extract.

S. No.	Constituents	Quantity
1.	Moisture	3.2 %
2.	Ash	2.8 g/100 g
3.	Proteins	1.2 g/100 g
4.	Lipids	0.2 g/100 g
5.	Carbohydrates	3.1 g/100 g
6.	Fibre	1.2 g/100 g
7.	Energy	19 kcal

Table 2. Organic constituents present in the extract. Values are mean ± S.D (n = 3).

S. No.	Organic constituents	Quantity (µg/g)
1.	Total alkaloids	38 ± 0.45
2.	Total flavonoids	98 ± 0.31
3.	Total phenols	84 ± 0.25

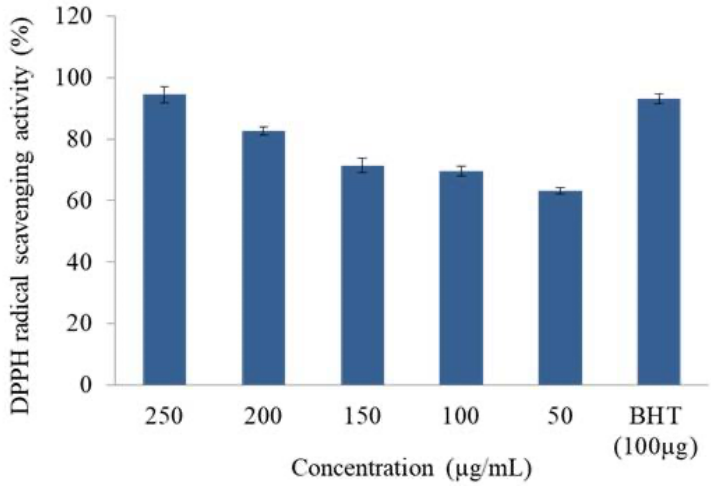


Figure 2. Free radical-scavenging capacities of the *C. grandis* fruit extract.

3.2. Phytochemical analysis

Total alkaloids, phenolics, and flavonoids content showed in Table 2. Heavy metal atoms in Dragendorff reagents bind with the nitrogen atoms of alkaloids to produce ionic complex. Thiourea and bismuth of nitric acid medium form a yellow bismuth complex with the ionic compound (Raal *et al.,* 2020). Based on the fact, alkaloid content reported as (38 ± 0.45 µg/g) and it can contribute to various health benefits, such as anti-inflammatory, analgesic and anti-cancer properties. Some alkaloids also have the potential to regulate blood sugar levels, improve digestion and support the immune system. However, while alkaloids can provide therapeutic effects, they must be consumed in controlled amounts, as excessive intake can lead to toxicity. Phenolics reacts with Folin-Ciocalteu reagent to form blue chromophore that was quantitatively measured and the maximum absorption of chromophore based on pholic concentration found in the extract (84 ± 0.25 µg/g). C-4 keto groups of flavonoid reacts with aluminium chloride to forms acid stable yellow complex (Pérez *et al.*, 2023). Consider these mechanism, *C. grandis* fruit extract showed profound amount of flavonoids (98 ± 0.31 µg/g). Phenolic acid and flavonoids are antioxidants found in plants. These antioxidants can nutralize the activity of free radicals to produce chelated metal complex.

3.3. *In vitro* antioxidant assay

The phenolic compounds act as reducing agents, hydrogen donors and singlet oxygen quenchers and have the redox property which are crucial role for their antioxidant activity and they also can chelate metals (Deshpande *et al*., 2008). The DPPH method used to unraveling the free radical scavenging activity of *C. grandis* L. fruit extract. Depends on electron-transfer reaction, DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) produces violet colour in alcohol. This free radicals reduced in the presence of phonolics and flavonoids in the fruit extract to form yellow solution, which comprehensively indicates the free radicals are quenched (Baliyan *et al.,* 2022). The absorpance of the extract observed and reported

in Figure 2 as the percent in dry weight extract µg/mL. When compared to BHT (93.1% at 100 µg/mL), the extract exhibited the significant free radical scavenging activity at 250 µg/mL of 94.4%. At 250 µg/mL, the extract had the significant total phenolics and flavonoids content, as well as the highest free radical-scavenging activity of 94.4%. Hence, this delved study strongly proved that *C. grandis* L fruit extract possesses efficient antioxidant properties.

3.4. *In vitro* anti-diabetic assay

At 250 µg/mL, the extract had 4.6% inhibition against α-amylase and its inhibitory activity on alpha-amylase was comparable to that of standard acarbose (3.6% at 100 µg/mL) (Table 3). The phenolic and flavonoid compounds in the *C. grandis* fruit extract competatively inhibit the alpha-amylase activity by competitively binding to its active site, preventing the enzyme from breaking down starch, and potentially altering its structure, thereby reducing starch digestion. Alpha-amylase involved in several biological activities, including the digestion of carbohydrates. Many crude drugs reduce alpha-amylase activity (Kalita *et al*., 2018). Natural α-amylase inhibitors slow down the digestion of carbohydrates, which lowers blood glucose absorption and consequently leads to postprandial hyperglycemia. Polyphenolic compounds are abundant in plants and have attracted a lot of attention because of their inhibitory activity against digestive enzymes, which is achieved by the presence of hydroxyl groups in polyphenolic structures (Grundy *et al*., 2016). Two mechanistic pathways have been proposed for alpha-amylase inhibition: (i) the formation of an enzyme-inhibitor complex and (ii) reducing the rate of glucose diffusion from the active site by slowing the digestion and absorption of carbohydrates and viscous water-soluble dietary fibres (Alqahtani *et al*., 2019). The inhibitory activity of *C. grandis* fruit extract against alpha-amylase enzyme is represented in Table 3. Inhibition of alpha-amylase activity found to be increasing in a dose-dependent manner.

Table 3. Alpha-amylase inhibition assay of the extract. Values are mean ± S.D (n = 3)

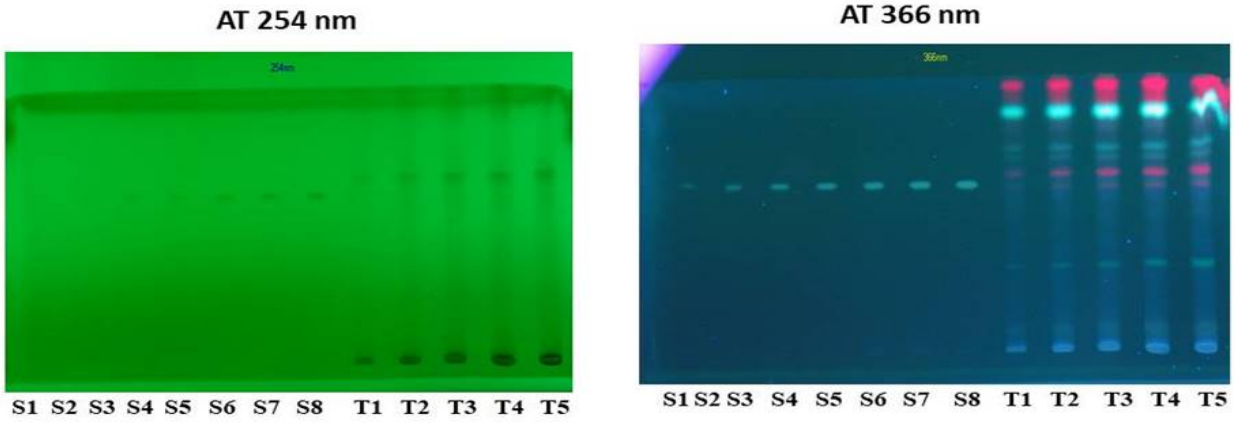
S. No.	Concentration (µg /mL)	% Inhibition
1	250	4.6 ± 0.7
2	200	4.4 ± 1.7
3	150	3.4 ± 1.0
4	100	2.1 ± 0.7
5	50	1.6 ± 0.6
6	Acarbose (100 µg)	3.6 ± 1.5

Table 4. Alpha-glucosidase inhibition assay of the extract. Values are mean ± S.D (n = 3)

S. No.	Concentration (µg /mL)	% Inhibition
1	250	53 ± 0.67
2	200	45 ± 0.43
3	150	37 ± 0.46
4	100	26 ± 0.62
5	50	13 ± 0.15
6	Acarbose (100 µg)	44 ± 0.47

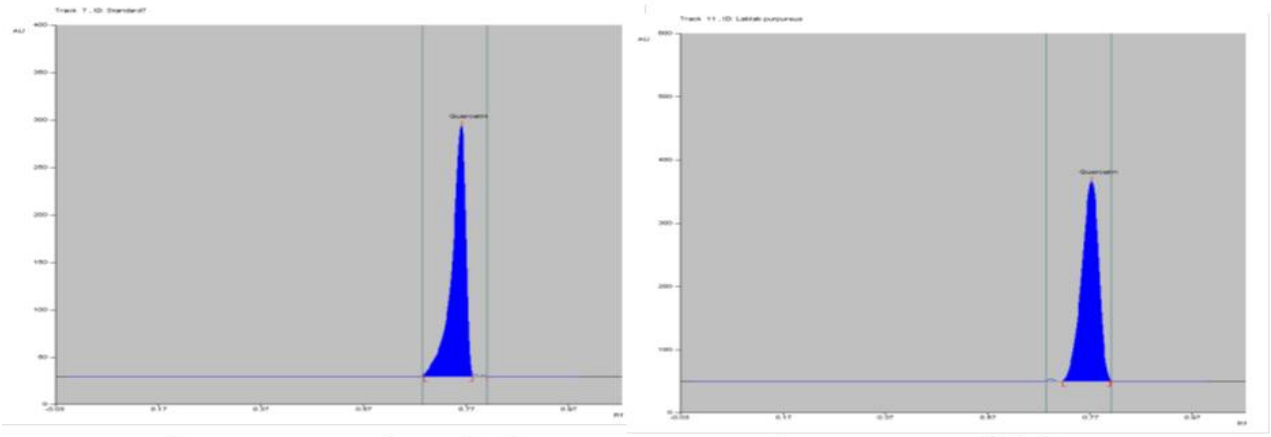
Alpha-glucosidase is a membrane-bound enzyme that catalyses the conversion of oligosaccharides to glucose and is found in the epithelium of the small intestine. Inhibition of the alpha-glucosidase enzyme essential for the control the conversion of glucose from oligosaccharides. The conversion committed for the control of diabetes (Gong *et al*., 2020). Inhibition of alpha-glucosidase enzyme depends on the hydrogen scavenging capacity because the hydrogen is required for hydrolysis of the alpha-(1→4)-glycosidic linkage. As a result, the inhibitors prevent the binding of hydrogen ion to the catalytic site of the enzyme (Alqahtani *et al*., 2019; Ansari and Khodagholi, 2013). The α-glucosidase inhibitory activity of the aqueous extract of *C. grandis* L. fruits extract shown in Table 4. As a result, the fruit extract found to have moderate inhibitory activity against the alpha-glucosidase enzyme. The percentage inhibition of *C. grandis* fruit extract at 50-250 µg/mL concentrations showed a dose-dependent increase in percentage inhibition (13-53%). Hence, the inhibition activity of fruit extract of *C. grandis* against alpha-glucosidase would delay carbohydrate degradation that results in a decrease glucose absorption and a reduction in the postprandial blood glucose level. Standard acarbose (100 µg/mL) showed 44% α-glucosidase inhibitory activity. In comparison to acarbose, this study indicates that the extract of *C.grandis* possesses a very formidable inhibitor activity against the enzymes alpha-amylase and alpha-glucosidase.

3.5. HPTLC analysis



S1-S8: 1 to 4µl of Standard Quercetin; T1-T5: 5 to 25µl of sample Solution

A band R_f value of 0.61 corresponding to Quercetin is visible in test solution tracks



Chromatogram of standard

Chromatogram of fruit extract

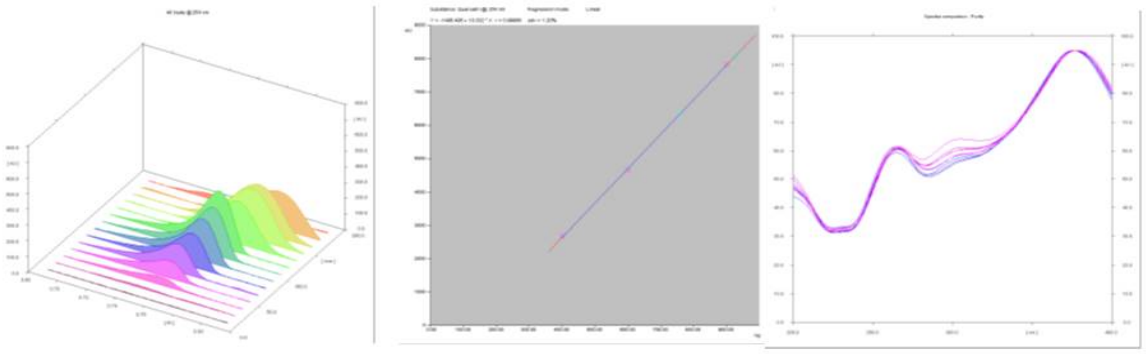


Figure 3. Identification and quantification of quercetin in *C. grandis* fruit extract.

The mobile phase used for the HPTLC investigation was Toluene: Ethyl acetate: Formic acid with the 5:4:1 ratio found to be suitable to furnish sharp, compact and intense peak at R_f = 0.60 (Figure 3). This analysis helped to separate the most abundant compound, quercetin in the extract. The peaks of the standard and the extract compared by aligning each other at wavelength 254 nm. The regression/correlation coefficient (r) were obtained as Regression via Height, Y = -27.8 + 0.2; r = 0.99 & sdv = 1.4; Regression via Area, Y = -501.2 + 3.8; r = 0.99 & sdv = 0.46. The (%) recovery for quercetin in the extract was found as 0.06% w/w.

3.6. Molecular docking studies

Cartoon diagrams of quercetin bound alpha-amylase and alpha-glucosidase structures and 2D representation of various interactions between the quercetin and active site residues of enzymes are shown in Figures 4a & b and Figures 6a & b, respectively. The drug quercetin exhibited good inhibitory binding affinities against two human carbohydrate metabolic enzymes alpha-amylase (-15.866 kcal/mol) and alpha-glucosidase (-14.147 kcal/mol). The binding pose of the quercetin molecule and its interacting residues within the catalytic site of alpha-amylase is shown in Figure 5c. Based on the previous research report of alpha-amylase crystal structure, it possesses three important catalytic residues, Asp197, Glu233, and Asp300 in the active site of alpha-amylase. According to a computational docking study, the alpha-amylase showed six hydrogen bond interactions between the amino acid residues including the catalytic residues reported earlier and the quercetin drug. The donor hydroxy groups of quercetin molecule showed hydrogen bond interactions with the residues of Thr163, Lys200, Asp300, and Glu233 with the distances of 2.98, 2.90, 3.07, and 2.72 & 3.1 Å, respectively. The quercetin molecule also involved as an acceptor to have hydrogen bond interaction with a distance of 3.48Å to amino acid Lys200. Proença *et al.*(2019), reported similar hydrogen bond interactions for other flavonoid compounds with alpha-amylase in his study. Apart from these interactions, quercetin molecule identified as hydrophobic core owing to its aromatic rings present in the basic skeleton of the structure and also it contributed significantly to have the interactions at the active site by contacting the residues of Trp58,Trp59, Tyr62,Tyr151, His299, Arg195, Ser199, and Val234. Interesting to note that few special type interactions like π…alkyl interactions (Drug…Leu162, Leu165, and Ala198), π…π T-shaped interactions (His201), π…sigma interactions (Ile235) and π…Anion interactions (Glu233) also contributed significantly to the drug binding affinity and stabilize the drug molecule at the active site of alpha-amylase.

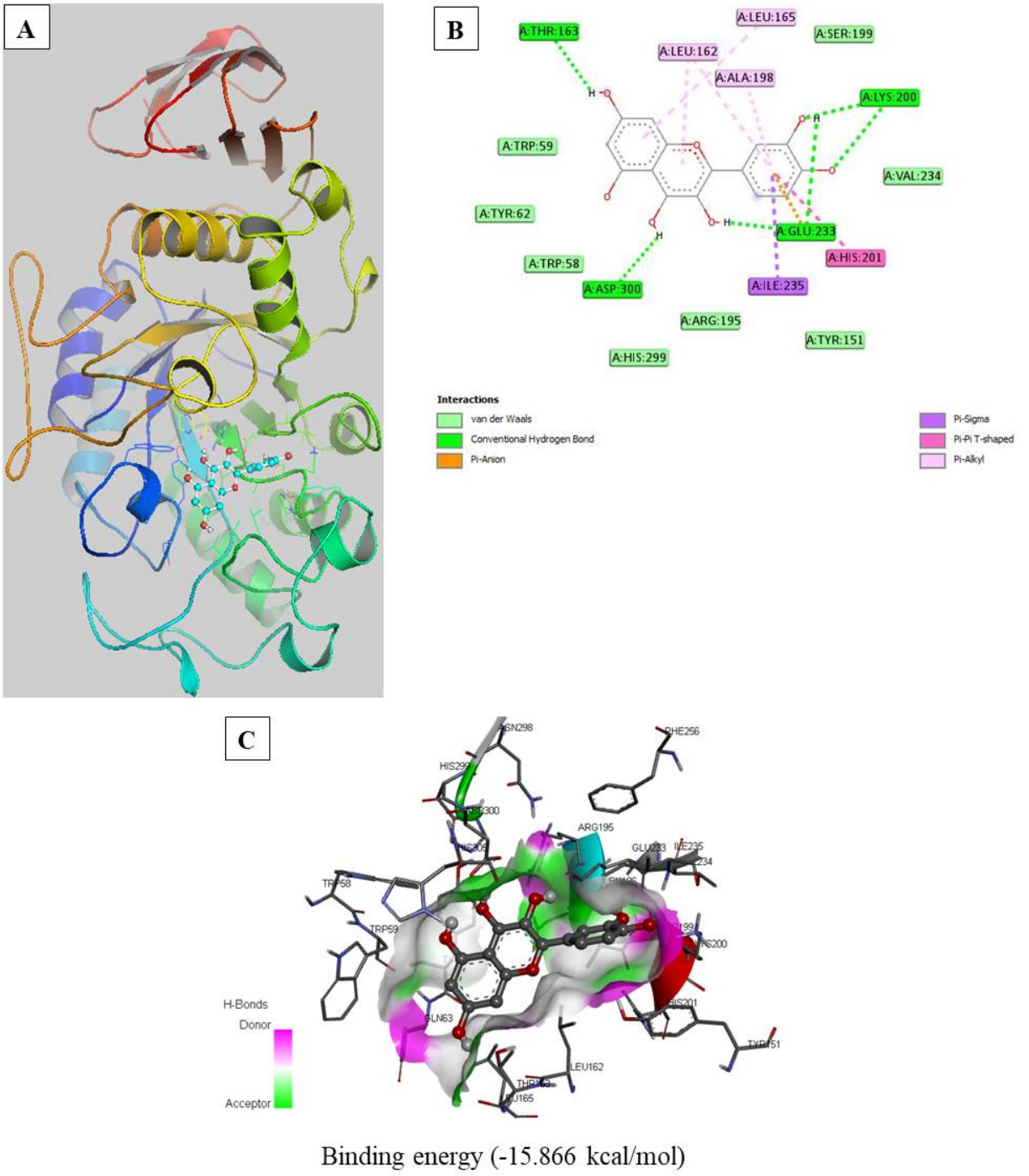


Figure 4. (a) Binding pose of quercetin in the catalytic site of alpha-amylase. (b) Two dimensional representation of quercetin with interacting residues of alpha-amylase. (c) Binding mode of quercetin along with active residues of alpha-amylase at the active site. Here pink, green and white colours represent the donor, acceptor, and hydrophobic regions, respectively.

A similar computational docking study for quercetin against the alpha-glucosidase enzyme, which is another carbohydrate metabolic enzyme secreted in the intestine was also done. The docked pose of quercetin in the active site of alpha-glucosidase is shown in Figure 5a and a two-dimensional representation of various intermolecular interactions between the quercetin and alpha-glucosidase is shown in Figure 5b. Observation of docked pose in the

alpha-glucosidase active site showed five hydrogen bonds with various receptor residues (Asp282, Asp404, Asp616, and Arg600).

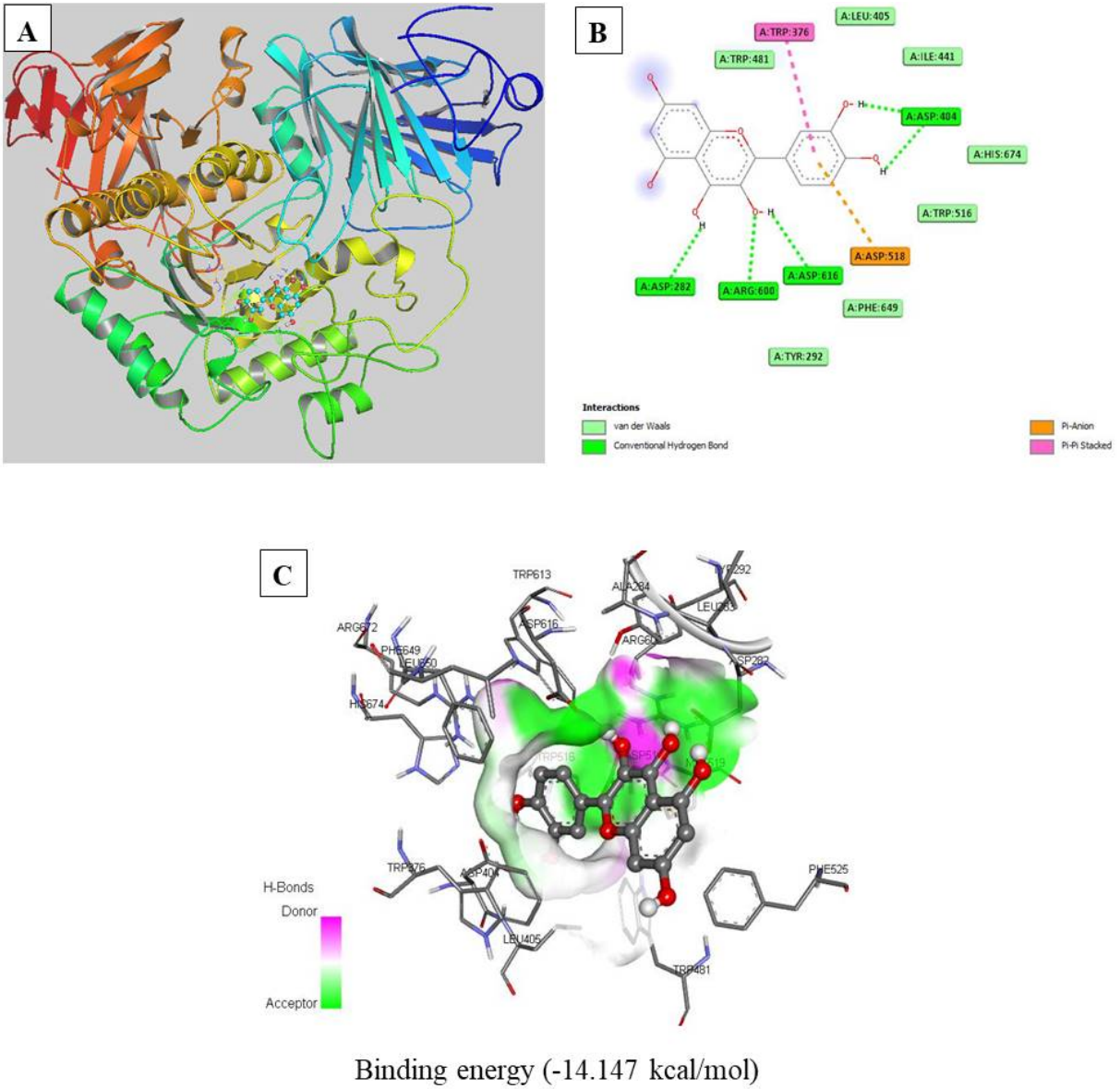


Figure 5. (a) Binding pose of quercetin in the catalytic site of alpha-glucosidase. (b) Two-dimensional representation of quercetin with interacting residues of alpha-glucosidase. (c) Binding mode of quercetin along with active residues of alpha- glucosidase at the active site. Here, pink, green, and white colours represent the donor, acceptor, and hydrophobic regions, respectively

Many hydroxy groups of quercetin donate the hydrogens to form hydrogen bonds by interacting the alpha-glucosidase residues of Asp282, Asp616, and Asp404 with the distances of 2.52, 2.71, and 2.50 & 2.72Å, respectively. Similarly, quercetin also accepts two hydrogen bonds from the residues of Arg600 and His674 with distances of 2.7 and 3.3Å, respectively. Apart from these non-bonded interactions, a few residues of alpha-glucosidase namely Tyr292, Phe649, Trp516, His674, Ile441, Leu405, and Trp481 are also contributing vander Waals short contacts with the quercetin molecule and helps to fit the drug well at the active site. Interestingly, few special types of interactions π…anion (between drug &

Asp518) and π…π stacking interaction (between drug & Trp376) also significantly contribute the energy to drug stability and anti-diabetic activity. Hence, the drug quercetin extracted from *C. grandis* fruit demonstrated significant inhibitory activity against human carbohydrate metabolic enzymes alpha-amylase and alpha-glucosidase and it was actively pronounced as an anti-diabetic agent through *in vitro* and *in-silico* molecular docking studies.

4. Conclusions

The comprehensive study proved that *C. grandis*, is one of the highest nutrient such as carbohydrates, proteins and lipids containing fruit which possess the most valuable phytochemical Quercetin with the high medicinal potency. From the study, the fruit extract of *C. grandis* possesses a good nutritional profile such as nutrients, ash and fibre content. The amounts of individual phytoconstituents, alkaloids, phenols, and flavonoids were identified as rich in the *C. grandis* fruit extract. The extract exhibits the strong free radical scavenging ability due to the presence of active phytoconstituents such as phenols and flavonoids. The *in vitro* assay highlighted that the the extract has inhibition activity on alpha-amylase and alpha-glucosidase. The antioxidants reduce oxidative stress and inflammation, improve insulin sensitivity, and helps to regulate blood sugar level. As the results of present meticulous studies, phytoconstituents quercetin molecule in the *C. grandis* fruit extract was proved to possess the strong inhibitory activity against alpha-amylase and alpha-glucosidase enzymes by *in-silico* approach. Thus, quercetin present in the current plant extract proved to possess the *in vitro* anti-diabetic activity and in these aspects are also taken into account while developing novel alpha-amylase and alpha-glucosidase inhibitors.

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