



RESEARCH ARTICLE

Identification of bioactive compounds from the ethnomedicinal plant *Senna alata* (L.) Roxb. (fabaceae) through *in vitro* and molecular docking analysis against α -glucosidase enzyme: A diabetic drug target

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Abstract

Senna alata (L.) Roxb. belongs to the family Fabaceae, is reported to have traditional use to treat diabetics and is selected for the study. Preliminary phytochemical analysis was carried out in the selected plant, indicating comparatively higher amounts of phenol, flavonoid, tannin and saponin in quantification. The antidiabetic activity of the plant was analyzed and the result indicated that the acetone and methanolic extract showed the lowest IC₅₀ values in α -amylase and α -glucosidase assays respectively. The methanolic extract, which showed an IC₅₀ (39.977 μ g/ml) value similar to the standard (35.151 μ g/ml), was selected for HR-LCMS analysis. HR-LCMS analysis indicated compounds that exhibit antidiabetic properties, including rutin, kaempferol, rhein and luteolin in the extract. Molecular docking analysis revealed 5 compounds showing better binding affinity namely 5-methoxyhydnocarpin-D, quercetin 3-rhamnoside-7-glucoside, marimetin, kaempferol and luteolin, than the standard drugs voglibose and acarbose. The present *in vitro* antidiabetic study against 5NN8 target protein was supported by molecular docking analysis. Therefore, further study of bioactive compounds identified through HR-LC MS can help develop future drug leads. Using such medicinal plants can support the improvement of the healthcare system as they do not have many side effects. *S. alata* is an important medicinal plant, but at the same time, it has become a weed in different parts of Kerala. Validation of medicinal properties and identification of bioactive molecules can help the sustainable utilization of the plant.

Keywords

Senna alata, α amylase, α glucosidase, HR-LCMS technique

Introduction

Diabetes is a major threat faced by humans that leads to related disorders with high rates of mortality (1). Diabetes mellitus is one of the major public health problems which are commonly seen worldwide, caused as a result of defects in insulin secretion and/or insulin action and leads to metabolic disorder of multiple etiologies distinguished by the failure of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism (2). It is regarded as one of the world's top 5 main causes of death (3). The disease has been now reported to be increasing in emerging and many developing countries at pandemic proportions. The global problem of diabetes mellitus stances massive social expenses and has chief implications for all healthcare structures. As per the report in 2006, India is among the highest

affected emerging economy country, with 41 million diabetes cases and there will be a projected rise to 70 million by the year 2025 (4). It has been estimated that 425 million adults aged between 20-79 years were living with diabetes mellitus (5). This estimate shows a worrisome indication that diabetes mellitus has attained the status of a global pandemic. According to International Diabetes Federation (IDF) report, the third, uppermost risk factor for premature mortality is elevated blood glucose, following high blood pressure. Therefore, appropriate efforts should be made for its management. Insulin injections and oral antidiabetic agents (sulfonylureas, biguanides, α -glucosidase inhibitors and troglitazones) have been used for the management of diabetes. Still, these antidiabetic drugs do not give a long-term glycaemic control without causing any adverse side effects. This has ultimately led to the exponential increase in the application of alternative and complementary options in herbal remedies that have high efficacy, cheaper with little or no adverse side effects (6). Herbal medicines have provided the primary and safest therapeutic approach since prehistoric times, which has shown a remarkable role in primary healthcare development (7). There is a number of plants that have shown antidiabetic potential through a remarkable reduction in blood glucose levels (8). Phytoconstituents with remarkable antidiabetic potential have been reported in a number of *in vitro*, *in vivo* and clinical studies. The phytoconstituents such as berberine from the root extract of *Berberis aristata*, baccic acid from the root ethanolic extract of *Bumelia sartorum*, scirpusin B from the stem bark of *Callistemon rigidus* and curcumin from the *Curcuma longa* are few examples reported (9).

During the last few years, researchers worldwide focused on identifying naturally originated potent α -glucosidase enzyme inhibitors with potentially fewer adverse side effects than clinically used drugs (10). Diabetic dyslipidemia results in excess free fatty acids (FFA), which are converted to triglycerides (TGs) in the liver. Accumulation of fat results in an increased small dense low-density lipoprotein cholesterol (LDLc) and TGs levels and decreased high-density lipoprotein cholesterol (HDLc), both of which contribute to diabetes-related cardiovascular risk. Hyperlipidemia is now well recognized as a significant risk factor for the early onset of diabetes and its consequences (11).

People with diabetes mellitus (DM), hypertension and extreme obesity are more prone to become infected with COVID-19 and are at a higher risk of complications and mortality (12). It was found that individuals with DM had a higher risk of *Severe acute respiratory syndrome* (SARS) and *Middle East respiratory syndrome* (MERS). DM affects 34.2 million people in the United States, or 10.5 % of the population (Centers for Disease Control and Prevention, 2020). 26.8% of individuals aged 65 and over had DM, putting them at a greater risk of dying from COVID-19 (Centers for Disease Control and Prevention, 2020). Hypertension and severe obesity affect 68.4% and 15.5% of people with diabetes, respectively (13). So, necessary care and

treatments should be provided to diabetic patients during the current scenario.

Senna alata (Synonym: *Cassia alata* Linn.), is a widely distributed herb coming under the family Fabaceae and sub family-Caesalpinioideae. In India, they are commonly known as Carrion Crow Bush, Winged Senna, Em-press Candle plant, etc. It is found in habitats such as river banks, lakeshores and forests. *S. alata* has been identified as a medicinal plant used to cure many ailments and diseases in many parts of the world. Apart from being cultivated for ornamental purposes, the plant is also grown for medicinal purposes; reports suggest that *S. alata* is extensively used for the treatment of gastrointestinal conditions, skin diseases, allergic reactions, internal and external infections and inflammatory conditions. They are grown by farmers mainly for medicinal purposes. For treating various skin diseases (pruritus, ringworm, rashes, itching and eczema), bronchitis and asthma *S. alata* has been widely used as folk medicine in several countries (14). The plant is traditionally used in the treatment of diabetes, malaria, typhoid, tinea infections, asthma, scabies, blotch, herpes, ringworms and eczema. Leaves and flowers of *S. alata* are used in the process of regulation of lipid absorption, obesity and fat levels in blood serum. The aqueous leaf extracts significantly reduce serum cholesterol, serum leptin, blood glucose levels, triglyceride, hepatic triglyceride and insulin levels in Wistar mice (15). Due to the reported traditional use of *S. alata* to treat diabetics, this plant was selected for the present study.

Materials and Methods

Collection of plant material and extraction

The plant for the present study *Senna alata* (Fig. 1) was collected from Madavoor, Kerala, India, in 2020. Plant material was washed in the laboratory with distilled water to remove adhering dust particles, then shade dried at room temperature (26 ± 2 °C). The leaves were crushed and powdered using a grinder and passed through a 0.3 mm mesh sieve.



Fig. 1. *Senna alata* leaves with inflorescence (Latitude : 8.6650054 ; Longitude : 76.7855686)

Approximately 13.0 g of powdered plant material was subjected to successive extraction using Soxhlet apparatus with petroleum ether, chloroform, acetone, methanol and water (150 ml each). After each extraction, plant material was air dried overnight at room temperature for

complete evaporation of the extraction solvent before subsequent extraction with another solvent. The individual extracts were collected, filtered through Whatman No.1 filter paper and concentrated using a rotary evaporator (Rotavapor R210; BUCHI, Flawil, Switzerland) under reduced pressure at 40 °C to obtain a crude extract that was collected in a sterile container and stored at -4 °C for further analyses.

Preliminary phytochemical analysis

The preliminary phytochemical analysis of secondary metabolites was done using the following method described by Gul *et al.* (16).

Quantitative estimation of secondary metabolites

Estimation of total saponin content

Vanillin sulfuric acid assay is used (17) to determine the total saponin content of the extract. To 0.5 ml of an aqueous solution of the sample, 0.5 ml of freshly prepared vanillin of 8% (8 g in 100 ml) and then 5 ml of sulfuric acid of 72% (28.8 ml made up to 40 ml) were added, and thoroughly mixed in an ice water bath. The mixture was then boiled in a warmed water bath at 60 °C for 10 min and then cooled again in ice-cold water. Absorbance at 535 nm was recorded against the blank reagent along with standard saponin using a UV spectrophotometer (UV- 1700- (E) 230EC- Shimadzu).

Estimation of total phenolic content

The total amount of phenolic content was determined using the Folin- Ciocalteu reagent (18). Aliquots of the extracts were taken in a 10 ml glass tube and made up to a volume of 3 ml with distilled water. Then 0.5 ml folin ciocalteu reagent (1:1 with water) and 2 ml Na₂CO₃ (20%) were added sequentially in each tube. A blue color was developed in each tube because the phenols undergo a complex redox reaction with phosphomolibdic acid in Folin ciocalteu reagent in an alkaline medium which resulted in a blue-colored complex, molybdenum blue. The test solutions were warmed for 1 min, cooled and absorbance was measured at 650 nm against the reagent used as a blank.

Estimation of total flavonoid content

Total flavonoid contentment was estimated by using the Aluminium Chloride method (19). To 1 ml of sample, added 75µl of 5% sodium nitrite (500 mg in 10 ml). After 6 min of incubation, 150 µl of 10% aluminum chloride solution (6 g in 60 ml) was added and the solution was kept undisturbed for 5 min, followed by the addition of 0.5 ml of 1 M sodium hydroxide and 2.5 ml distilled water. The solution was mixed well and absorbance was read at 510 nm with standard quercetin. The result expressed as mg of flavonoid as quercetin equivalents (QEE).

Determination of total tannin content

To 1 ml of the extract in a 100 ml volumetric flask containing 75 ml water, added 5 ml of Folin-Denis reagent, 10 ml of sodium carbonate solution and made up to 100 ml with water, shaken well and allowed to stand for 30 min. The

color developed was read at 700 nm. A standard graph was plotted by using 0- 100 µg of tannic acid (20).

Estimation of antidiabetic activity

α-Glucosidase inhibitory assay

The inhibitory activity of the sample on α-glucosidase enzyme was determined according to the method described by Kim *et al.* (21). The 200 µl of diluted α-glucosidase (0.067 U/ml) was pre-incubated with the varying concentrations of sample for 10 min. The substrate solution p-nitrophenyl α-D-glucopyranoside (pNPG) was prepared in 0.1 M Sodium phosphate buffer (pH 6.9). Then 200 µl of 3.0 mM pNPG prepared in 0.1M sodium phosphate buffer (pH 6.9) was added as a substrate to start the reaction. The reaction mixture was incubated at 37 °C for 20 min and stopped by adding 2 ml of 0.1 M Na₂CO₃. The α-glucosidase activity was determined by measuring the yellow-colored para- nitrophenol released from pNPG at 400 nm. The results were expressed as a percentage of inhibition. The same procedure was done with acarbose (1mg/ml), as standard.

$$\text{Inhibitory activity (\%)} = (B-T/B-C) \times 100$$

Where, B is the absorbance of the blank, T is the absorbance in the presence of the test substance, C is the absorbance of control.

α- Amylase inhibitory assay by DNSA method

The antidiabetic activity of the test samples was determined according to the method described in the Worthington Enzyme Manual with slight modifications (22). In brief, 500µL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing 0.5 mg/ml of α-amylase enzyme and different concentrations (in µg) of the test sample as enzyme inhibitor were pre-incubated at 37 °C for 10 min. After the pre-incubation, 500 µL of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube and incubated at room temperature for 5 min. The reaction was stopped using 1.0 ml of dinitrosalicylic acid (DNSA) reagent. The test tubes were incubated in a boiling water bath for 5 min and then cooled to room temperature. The volume of the reaction mixture was made up to 10 ml by adding distilled water, and the absorbance was measured at 540 nm using UV-Visible spectrophotometer.

$$\text{Percentage inhibition} = \frac{B-A \times 100}{(B-C)}$$

C- Absorbance of the Control with starch and without alpha amylase, B- Absorbance of the Control with starch and alpha amylase, A- Absorbance of the Test.

HR-LCMS Analysis

The phytochemicals present in the extract were evaluated using high-resolution liquid chromatography and mass spectrometry (HR-LCMS) analysis (23). The extract was prepared in methanol and then subjected to HR-LCMS analysis. The HR-LCMS of the sample was carried out in Sophisticated Analytical Instrument Facility (SAIF), IIT Bombay, Pawai- Mumbai. Chemical fingerprints of plant

extract were prepared by Agilent high-resolution liquid chromatography and mass spectrometry model- G6550A with 0.01% mass resolution. The acquisition method was set to be MS- minimum range 50 (M/Z) and maximum 1000 dalton (M/Z) with scanning rate for each spectrum per second.

Molecular docking techniques

Preparation of target protein

The 3D structure of α -glucosidase (PDB ID: 5NN8) (24) was retrieved from Protein Data Bank (<https://www.rcsb.org/>), in PDB format and visualized using Bio via discovery studio. Protein optimization was done using Auto dock version 4.2., saved in PDB format for molecular docking analysis.

Ligand preparation

The 3D structure of the selected ligand molecule was generated from CORINA online tool (https://www.mn-am.com/online_demos/corina_demo) by uploading the canonical SMILE (simplified molecular-input line-entry system) of each ligand molecule retrieved from Pub Chem database (<https://pubchem.ncbi.nlm.nih.gov/>). Then it was uploaded into CORINA online tool. The generated 3D structures of ligand molecules include Pubchem ID retonecine (10198), rutin (5280805), maritimetin (5281292), 5-methoxyhydnicarpin-D (12051137), stigmatellin Y (5287028), kaempferol (5280863), quercetin 3-rhamnoside-7-glucoside (44259243), luteolin (5280445) and rhein (10168). voglibose (444020) and acarbose (41774) were considered standard drugs that were downloaded in PDB format and opened in Autodock version 4.2 for the energy optimization process. For molecular docking analysis, only the dominant nine compounds were selected, including positive and negative modes on chromatogram peaks and were used for ligand preparation.

Docking

The binding site of 5NN8 was analyzed using the information about the amino acid residues by using the literature and studies with the known, co-crystallized ligand bound with the 5NN8 PDB structure. After ligand and target preparations Auto grid part is used to determine the native ligand position on the binding site by arranging the grid coordinates (X, Y and Z). After running the auto dock, the docked results were analyzed and visualized with both Autodock 4.2 and Biovia discovery online tool.

Results and Discussion

Phytochemical analysis

The powdered leaves (13 g) were undergone soxhlet extraction with non-polar to polar solvents such as petroleum ether, chloroform, acetone, methanol and aqueous for 6 hrs and the percentage yield was 2.90%, 3.68%, 2.43%, 5.85% and 4.06% respectively. The variation in the extraction yield depends on the nature of the solvents and the chemical nature of the sample (25). The study revealed that the methanolic extract showed the maximum yield with a yield % of 5.85%.

The preliminary phytochemical analysis of the aqueous leaf extract of *S. alata* showed the presence of phenols, flavonoids, glycosides, cardiac glycosides, alkaloids, terpenoids, steroids and amino acids. The methanolic leaf extract showed the presence of phenolics, flavonoids, tannins, cardiac glycosides, alkaloids, terpenoids, saponins and proteins. The qualitative analysis of acetone leaf extract contains flavonoid, tannin, glycoside, cardiac glycoside, alkaloids, terpinoids and saponin. The chloroform leaf extract contains phenolics, flavanoids, tannin, glycosides, cardiac glycosides, alkaloids, terpenoids, steroids and amino acids depicted in Table 1.

Table 1. Preliminary phytochemical analysis of *Senna alata* crude extract of leaves.

S. No	Phytochemicals	Chloroform extract	Acetone extract	Methanolic extract	Aqueous extract
1	Phenolics	+++	---	+++	+++
2	Flavanoid	+++	+++	+++	+++
3	Tannin	+++	+++	+++	---
4	Glycoside	+++	---	---	+++
5	Cardiac glycoside	+++	+++	+++	+++
6	Alkaloids	+++	+++	+++	+++
7	Terpenoids	+++	+-	+	+
8	Saponin	---	+++	+++	---
9	Steroids	+++	---	---	+++
10	Amino acids	+++	---	---	+++
11	Proteins	---	---	+++	---
12	Carbohydrates	---	---	+-	---

Total phenolic content

The total phenolic content (TPC) was expressed as gallic acid equivalents (GAE), as shown in Table 2. The highest total phenolic content was observed in methanolic extract (41.953±1.227 mg of gallic acid equivalents (GAE)/g of extract) followed by acetone extract (37.837±1.491 mg of gallic acid equivalents (GAE)/g of extract). Aqueous extracts had a very low amount of phenolic compounds (Table 2).

Total saponin content

Total saponin content (TSC) was expressed as diosgenin (Dgn) equivalents. The saponin content showed the highest value and is observed in methanolic extract (290.167±8.642 mg of Dgn/g of extract), followed by aqueous extract (224.00±1.154 mg of Dgn/g of extract). The acetone and chloroform extracts also contain ample quantities of saponin (153.00±3.605mg of Dgn /g of extract and 126.833±14.923 mg of Dgn /g of extract respectively) (Table 2).

Total flavonoid content

Total flavonoid content (TFC) was expressed as quercetin equivalents (QEE). Methanolic extract observed the highest TFC (265.466±15.035 mg of QEE/g of extract), which was followed by acetone and chloroform extracts with 188.666±16.788mg of QEE/g of extract and 152.833±11.917mg of QEE/g of extract respectively. The

Table 2. Quantitative analysis of major secondary metabolites from the crude extracts of *S. alata* leaves.

Sl.no	Secondary metabolites (mg/g)	Chloroform extract	Acetone extract	Methanolic extract	Aquoues extract
1	TPC	28.471±2.272	37.837±1.491	41.953±1.227	2.007±0.371
2	TSC	126.833±14.923	153.00±3.605	290.167±8.642	224.00±1.154
3	TFC	152.833±11.917	188.666±16.788	265.466±15.035	127.75±7.454
4	TTC	5.583±0.166	4.083±0.083	6.166±0.333	44.266±0.145

aqueous extract also contains enough amounts of flavonoids (127.75±7.454mg of QEE/g of extract).

Total tannin content

The total tannin content (TTC) of the extracts was expressed as tannic acid equivalent (TAE) in mg/g of extract. The aqueous extract (44.266±0.145 mg of TAE/g of extract) had greater tannin content than the other extracts. The other extracts, methanol, chloroform and acetone, show nearly equal quantities of tannin (6.166±0.333 mg of TAE/g of extract, 5.583±0.166 mg of TAE/g of extract, 4.083±0.083 mg of TAE/g of extract respectively).

The quantification of metabolites showed that the methanolic extract possesses higher amounts of TPC, TSC and TFC. But the TTC content was found more in the aqueous extract, as shown in Table 2.

Evaluation of antidiabetic activity

In the present study, the antidiabetic activity of acetone, methanol and aqueous extract of *S. alata* was tested by using α -amylase inhibition and α -glucosidase inhibition assay and its IC₅₀ values were calculated.

α -Amylase inhibition assay

In the present study, different solvent extracts of *S. alata* were subjected to α -amylase inhibitory assay along with acarbose as a standard drug. As per the results obtained from the study, it has been noted that the percentage inhibition value significantly increases with an increase in the concentration, as shown in Fig. 2 and Table 3.

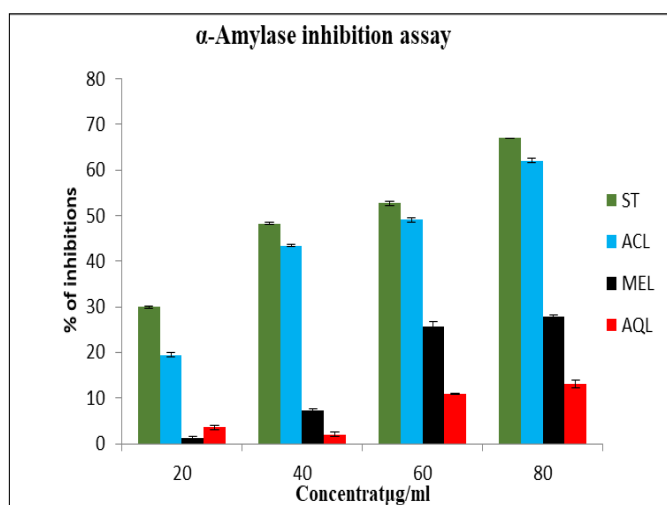


Fig. 2. The percentage of inhibition of different crude extracts of *S. alata* leaves in an α -amylase inhibition assay. **ST** is acarbose (standard drug); **ACL** is acetone extract of leaves; **MEL**: Methanolic extract of leaves; **AQL**: Aqueous extract of leaves.

Table 3. Percentage inhibition of α -amylase assay at different concentration of *Senna alata* crude leaves extracts.

Sample name	concentration of a sample (μ g/ml)	Percentage inhibition with standard error
Acetone extract	20	19.51±1.22
	40	43.77±0.35
	60	49.08±0.63
	80	59.88±1.40
Methanolic extract	20	1.245±0.33
	40	7.89±0.2
	60	25.49±1.33
	80	27.74±0.30
Aqueous extract	20	3.82±0.23
	40	2.076±0.75
	60	11.1±0.44
	80	13.53±1.23
Acarbose (standard)	20	29.95±0.12
	40	48.77±0.22
	60	52.68±1.3
	80	67.48±0.23

According to the results, the acetone extract of leaves showed 50% (IC₅₀) inhibition at the concentration of 66.00 μ g/ml, followed by methanolic and aqueous extract with 50% inhibition at the concentrations of 114.858 μ g/ml and 298.366 μ g/ml respectively.

α -Glucosidase inhibition assay

In the present study, the *S. alata* leaves fractions were evaluated for the inhibitory action of the α -glucosidase enzyme and satisfactory inhibitory activity was observed, as shown in Fig. 3 and Table 4. Based on the result ob-

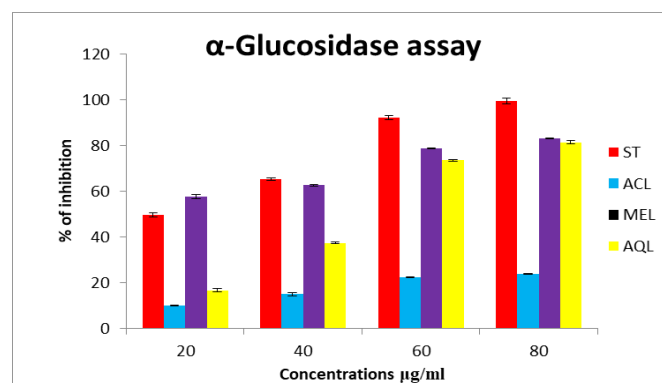


Fig. 3. The percentage of inhibition of different crude extract of *S. alata* leaves in α -glucosidase inhibition assay (**ST**: acarbose, standard used; **ACL**: acetone extract of leaves; **MEL**: methanol extract of leaves; **AQL**: aqueous extract of leaves).

Table 4. Percentage inhibition of α -glucosidase assay at different concentration of *Senna alata* crude leaves extracts.

Sample name	concentration of a sample ($\mu\text{g/ml}$)	Percentage inhibition with standard error
Acetone extract	20	9.71 \pm 0.12
	40	13.57 \pm 0.33
	60	21.9 \pm 0.36
	80	21.23 \pm 0.60
Methanolic extract	20	57.480 \pm 0.906
	40	62.586 \pm 0.378
	60	78.663 \pm 0.281
	80	82.940 \pm 0.157
Aqueous extract	20	16.681 \pm 0.717
	40	37.386 \pm 0.322
	60	73.503 \pm 0.409
	80	81.326 \pm 0.738
Acarbose (standard)	20	49.50 \pm 0.901
	40	65.274 \pm 0.528
	60	92.16 \pm 0.802
	80	99.232 \pm 1.296

tained, it is found that the methanolic extract of leaves possesses less value of IC_{50} at the concentration of 39.977 $\mu\text{g/ml}$ when compared to the standard, which has an IC_{50} value of 35.15 $\mu\text{g/ml}$. In comparison, the aqueous extract has an IC_{50} of 47.054 $\mu\text{g/ml}$. A relatively negligible amount of inhibition was observed in the acetone extract of the leaves.

HR-LCMS techniques

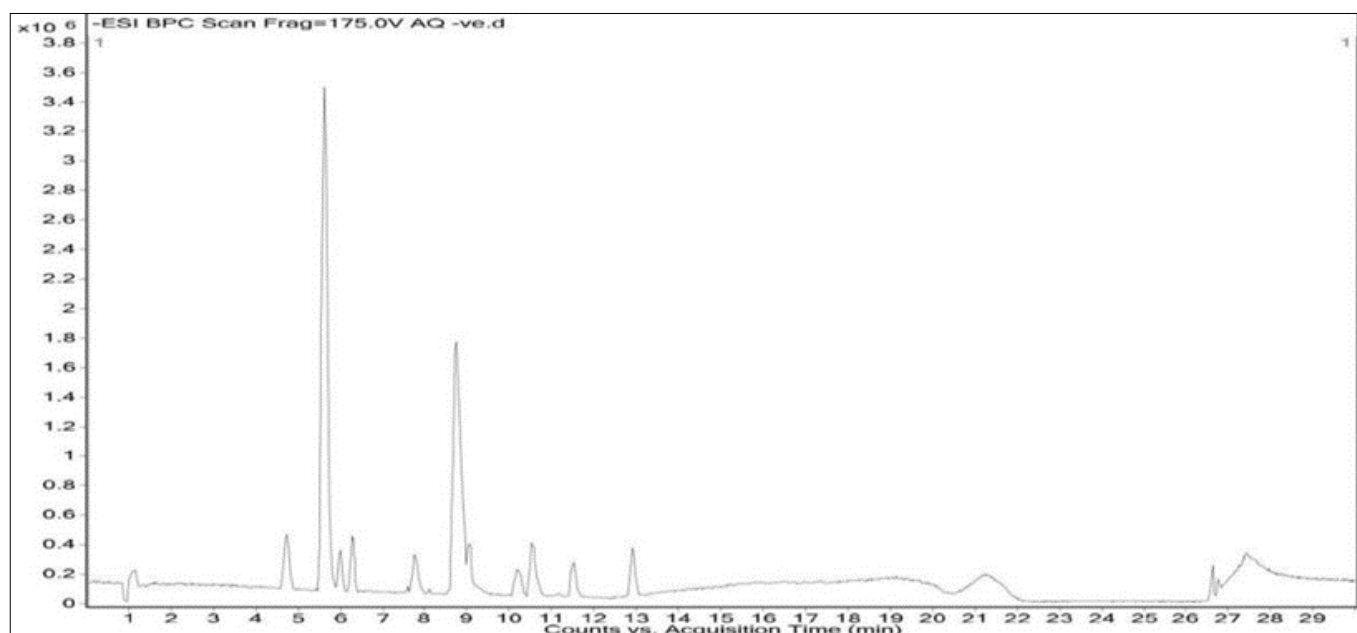
Comparing the high inhibition activity of extracts against α -amylase and α -glucosidase, the methanolic extract showed good inhibition against α -glucosidase enzyme. Therefore, they were used for further HR-LCMS analysis.

This approach was used to separate and identify

chemical components based on their molecular weight, retention time and MS/MS fragments obtained from the Metabolite and Chemical Entity Database (METLIN) (23). From the present study, the chromatogram of both negative and positive modes observed major peaks, which are depicted with retention time (Figs. 4-5) and the individual Mass fragment data of both negative and positive modes as in Figs. 6 and 7.

A total of sixteen plant-derived compounds (negative and positive mode) were observed in the methanolic extract of *S. alata* based on METLIN database, which includes 11-o-Galloylbergenin, quercetin 3-rhamnoside-7-glucoside, cynaroside, luteolin, rhein, purpurin, retronecine, myricetin 3-galactoside, rutin, maritimetin, 6-C-galactosylluteoline, kaempferol, morindone, 5-methoxy hydnocarpin-D, stigmatellin Y and pheophorbide A. Retronecine showed the highest peak in positive mode with rt of 1.144 which is followed by 5-methoxy hydnocarpin-D with retention time of 9.669. Rutin, kaempferol and stigmatellin Y also showed the peaks with rt of 5.606, 7.748 and 16.409 respectively. In the case of the negative mode, quercetin 3-rhamnoside-7-glucoside showed a high peak with rt of 5.606, which was followed by luteolin with rt of 8.904.

Screening medicinal plants for antidiabetic activity has increased, as it is critical to find new and effective antidiabetic medicines. As a result, encouraging the urban population to adopt a healthy lifestyle by including antidiabetic and antioxidant-rich greens in their daily diet would be one of the most cost-effective strategies to manage the disease. Phytochemical analysis plays an important role in the determination of bioactive compounds of medicinal plants (26, 27). The methanolic extract of the plant leaf possesses a higher amount of secondary metabolites, especially the flavonoid class, as shown in Table 2. HR-LCMS analysis also depicted mostly flavonoids (Table 5 and Table 6), as shown in Fig. 8. Different plant-derived compounds have been reported to have versatile biological activity. Flavonoid is an important class of secondary

**Fig. 4.** Chromatogram showing negative mode with retention time

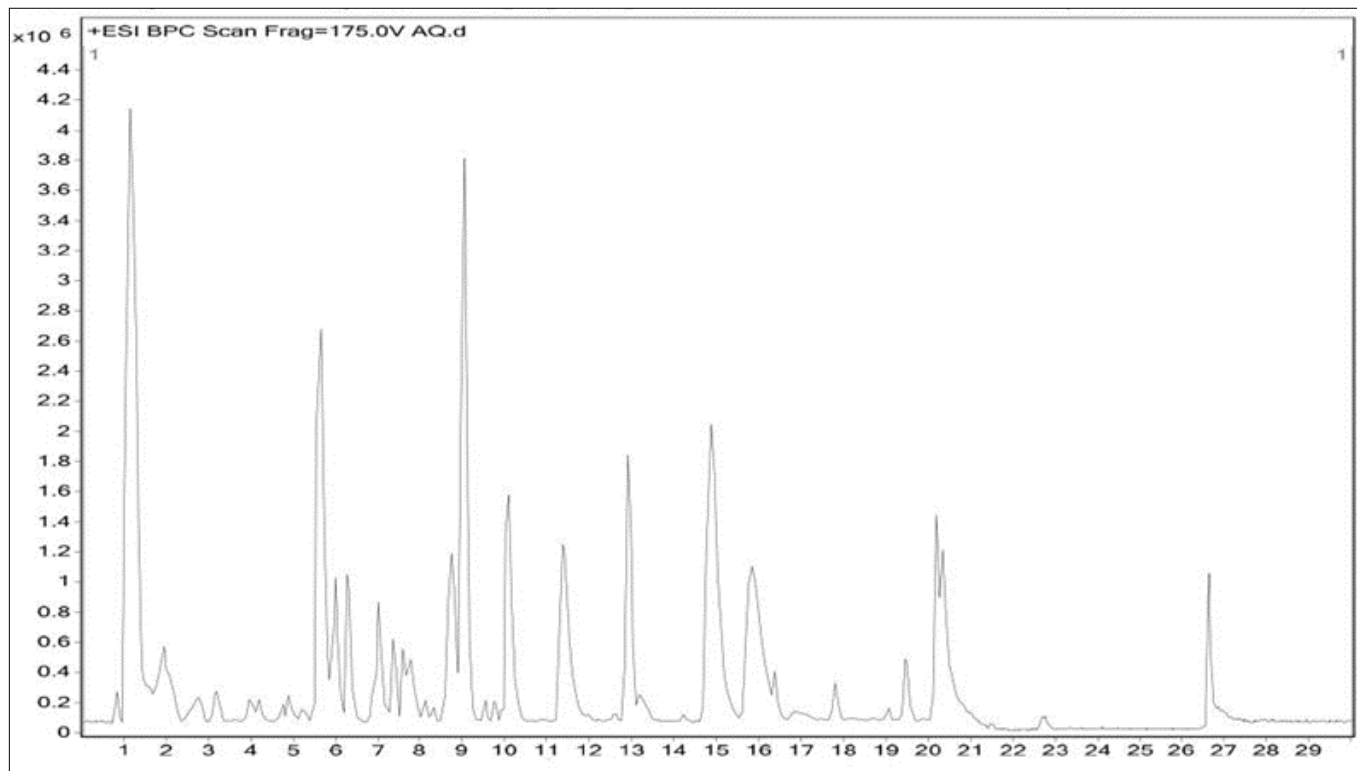


Fig. 5. Chromatogram showing positive mode with retention time

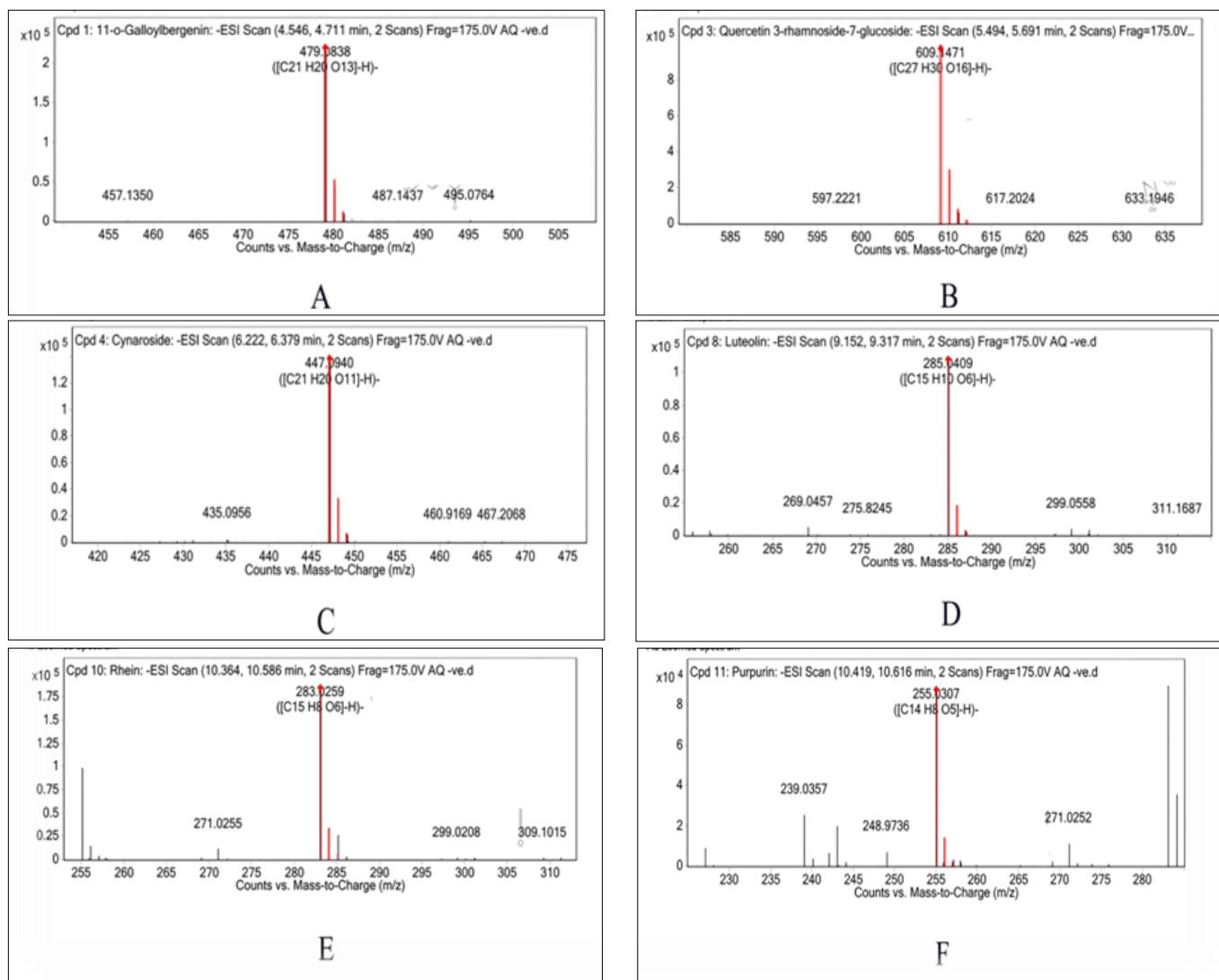


Fig. 6. The compounds with a higher peak in negative mode: (A) 11-o-galloylbergenin, (B) quercetin 3-rhamnoside-7-glucoside, (C) cynaroside, (D) luteolin, (E) rhein, (F) purpurin

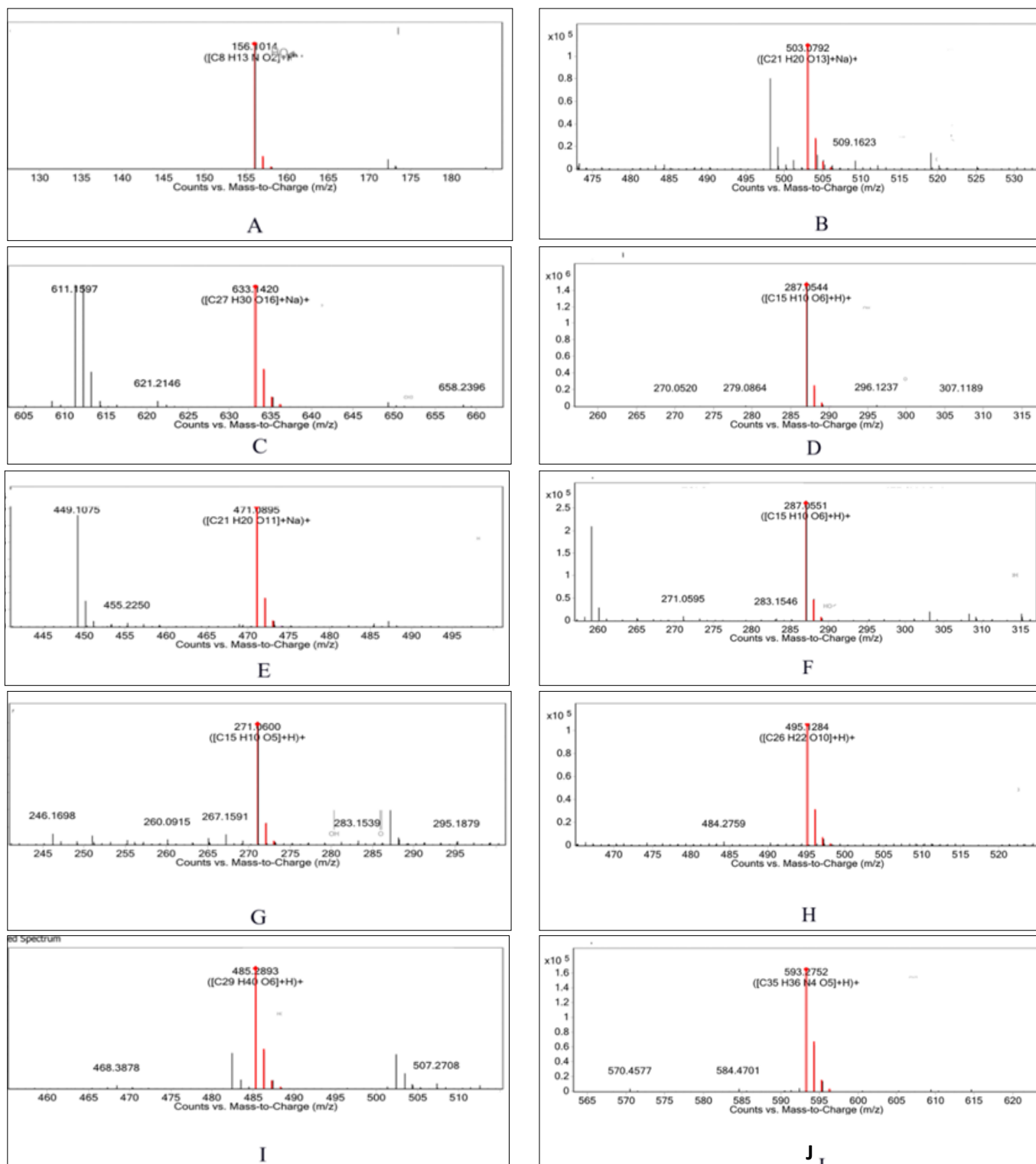


Fig.7. The compounds with MS spectrum in positive mode: (A) Retronecine, (B) myricetin 3-galactoside, (C) rutin, (D) maritimetin, (E) 6-C-galactosylluteoline, (F) kaempferol, (G) morindone, (H) 5-methoxyhydrnocarpin-D, (I) stigmasterlin Y, (J) pheophorbide A.

metabolites under major phenolics. Vinayakam and Xu have reported the antidiabetic activity of flavonoids such as flavanones, flavonols, flavonols, isoflavones, flavones and anthocyanidins through *in vitro* and *in vivo* models (27). The flavonoids such as Fisetin from onion (29), apigenin from *Hypericum perforatum* L., *Matricaria chamomilla* L. (30) were reported as antidiabetic compounds by lowering glucose production in blood. Here, the flavonoid class of secondary metabolites identified from HR-LCMS analysis includes rutin, kaempferol, rhein and luteolin, which were already reported as molecules with high antidiabetic activity (31). Rutin isolated from oranges, grapes and lem-

ons reported antidiabetic activity through glucose-lowering and insulin enhancing properties (32). It was reported that quercetin exhibited antidiabetic activity through increased GLUT 4 (Glucose transporter) expression (33). Kaempferol-3-O-rutinoside, quercetin, kaempferol and rutin were also reported to have antidiabetic activity through inhibition of α -glucosidase enzyme (34). Luteolin and its derivative, namely luteolin-7-O-glucoside, show antidiabetic activity by strong inhibition on α -glucosidase enzyme than α -glucosidase enzyme (35). Rhein is an anthraquinone that observed glucose uptake inhibition in the blood and also overexpressed GLUT-1 in

Table 5. Phytochemical composition of *S. alata* methanolic extract evaluated using the HR- LCMS (Negative mode).

Identified compounds	M/Z ratio	Retention time	Actual mass
11-o-Galloylbergenin	479.0838	4.644	480.091
Quercetin 3- rhamnoside-7- glucoside	609.1471	5.608	610.1545
Cynaroside	447.094	6.314	448.1012
Luteolin	285.0409	8.904	286.0482
Rhein	283.0259	10.491	284.0333
Purpurin	255.0307	10.541	256.0381

Table 6: Phytochemical composition of *S. alata* methanolic extract evaluated using the HR-LCMS.(Positive mode)

Identified compounds	M/Z ratio	Retention time	Actual mass
Retronecine	156.1014	1.144	155.0942
Myricetin 3- galactoside	503.0792	4.703	480.0885
Rutin	611.1597	5.609	610.1525
Maritimetin	287.0544	5.75	286.0472
6-C-Galactosylluteolin	471.0895	6.294	448.1002
Kaempferol	287.0551	7.748	286.0478
Morindone	271.06	8.566	270.0528
5'- Methoxyhydnocarpin-D	495.1284	9.669	494.1209
Stigmatellin Y	485.2893	16.409	484.282
Pheophorbide a	593.2752	17.832	592.2678

rats (36). In the current study, *in vitro* inhibition assays of α -amylase and α -glucosidase were carried out. It showed higher activity against α -glucosidase than α -amylase (Fig. 3). Moreover, the present study also reveals the presence of compounds such as quercetin 3-rhamnoside-7-glucoside, luteolin, rhein, rutin, kaempferol in the selected plant. The presence of such compounds may significantly contribute to the antidiabetic activity of the selected plant. The rest of the compounds identified from HR-LC MS analysis such as 11-o-galloylbergenin, purpurin, retronecine, myricetin 3-galactoside, maritimetin, 6-C-galactosylluteoline, morindone, 5-methoxy hydnocarpin-D, stigmatellin Y, pheophorbide A etc. has no reports on compound specific antidiabetic activity.

α -Amylase is an enzyme that can hydrolyze alpha bonds in alpha linked polysaccharides to produce large amounts of glucose (37). The enzyme α -glucosidase also break down dietary carbohydrate or starch and leads to intestinal absorption of a glucose molecule into the blood. This could reduce the postprandial blood glucose of hyperglycemic patients to prevent the absorption of carbohydrates after food intake. A high and good inhibition of alpha glucosidase reduces the high post prandial blood glucose level (38). Here the current study, α -glucosidase inhibitory studies performed, demonstrated that the methanolic extracts of *S. alata* had significant inhibitory potentials. The IC₅₀ value of methanol extracts (39.977 μ g/

ml) is almost similar to that of acarbose (35.151 μ g/ml), a widely used and marketed antidiabetic drug.

Molecular docking interactions of complex

Molecular docking technique was performed to evaluate the interaction of natural and drug compounds against one of the diabetic drug targets of the human lysosomal α -glucosidase enzyme. Apart from selected phytochemicals, known antidiabetic drugs such as voglibose and acarbose were also docked against 5NN8 protein.

Selected natural compounds from the leaves methanolic extract of *S. alata* shows notable affinity with diabetic drug target 5NN8 as depicted in Figs. 8-11. The docking affinity of 5-methoxyhydnocarpin-D, quercetin 3-rhamnoside-7-glucoside, kaempferol, marimetin and luteolin showed higher binding affinities to the target α -glucosidase (5NN8) with binding affinity -7.01kcal/mol, -6.64 kcal/mol, -6.60 kcal/mol, -6.60 kcal/mol, -6.52 kcal/mol respectively. The luteolin formed seven hydrogen bonds with amino acid residues Ala 284, Asp 282, Asp 518, Asp 645, Trp 613, Arg 672 and Ala 284, as shown in Fig. 8. whereas; kaempferol, 5-methoxyhydnocarpin-D, marimetin and quercetin 3-rhamnoside-7-glucoside had formed 4 hydrogen bonds with Glu 521, Met 519, Asp 616, Trp 613, 3 hydrogen bonds with Arg 411, Met 408 and Ser 379, 3 hydrogen bonds with Glu 521, Met 519, Tyr 292, 2 hydrogen bonds with Arg 281, Asn 524 respectively with 5NN8. The rest of the 4 plant derived compounds, including retronecin, stigmatellin, rhein and rutin, showed less binding affinity, which is less than 6.50 kcal/mol, like -5.50 kcal/mol, -5.60 kcal/mol, -4.96 kcal/mol and -4.34 kcal/mol respectively. Presently used antidiabetic drugs like voglibose and acarbose showed -6.55 kcal/mol and -2.38 kcal/mol binding affinity respectively. Moreover, the present molecular docking technique also revealed that 5-methoxyhydnocarpin-D, quercetin 3-rhamnoside-7-glucoside, kaempferol, marimetin and luteolin bound more strongly with the active chain residue of 5NN8 than the standard drug voglibose and acarbose which had binding affinity score -6.55 kcal/mol and -2.38 kcal/mol respectively (Fig. 11). Good binding score, hydrogen bonding and other hydrophobic interactions will lead to strong protein ligand complex (39).

The docking analysis scores for the selected ligands indicated that the ligand was able to attach to a target protein pocket. These selected ligands have a binding affinity of less than -6.50 kcal/mol, which can be considered to be a good inhibitor of enzymatic reactions (40). In the present study, among nine plant derived compounds, 5 natural compounds revealed a binding affinity value greater than -6.5 kcal/mol with 5NN8. Therefore, the molecular docking technique helped to narrow down the plant derived compounds on the basis of desired binding affinity and molecular interaction between ligand and 5NN8 complex, which can be later used for the development of novel alternative drugs or complementary

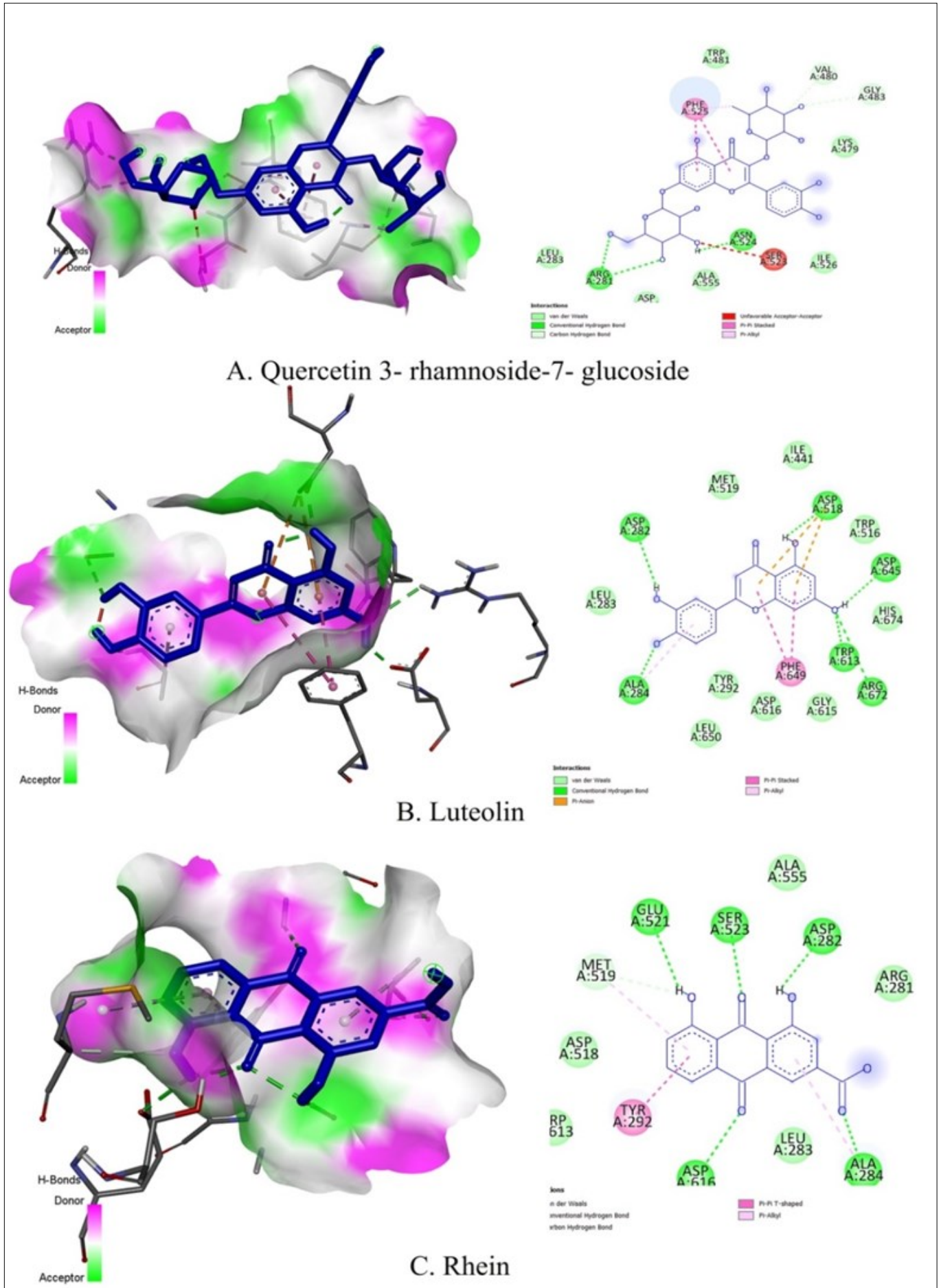


Fig.8. Molecular docking analysis of dominant compound identified through HR-LC MS analysis and docked against human α -glucosidase enzymes.

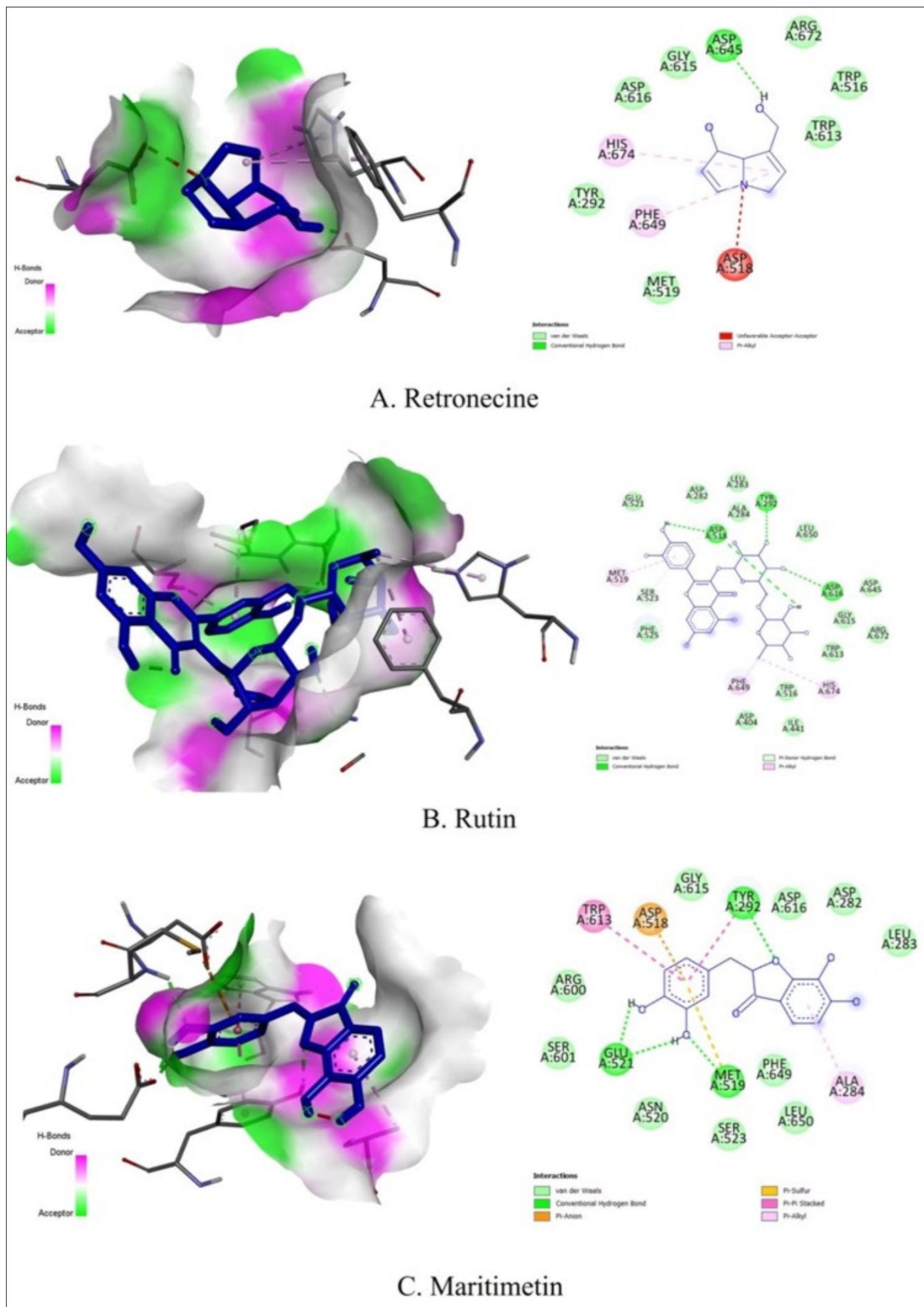


Fig. 9. Molecular docking analysis of dominant compound identified through HR-LC MS analysis and docked against human α -glycosidase enzymes.

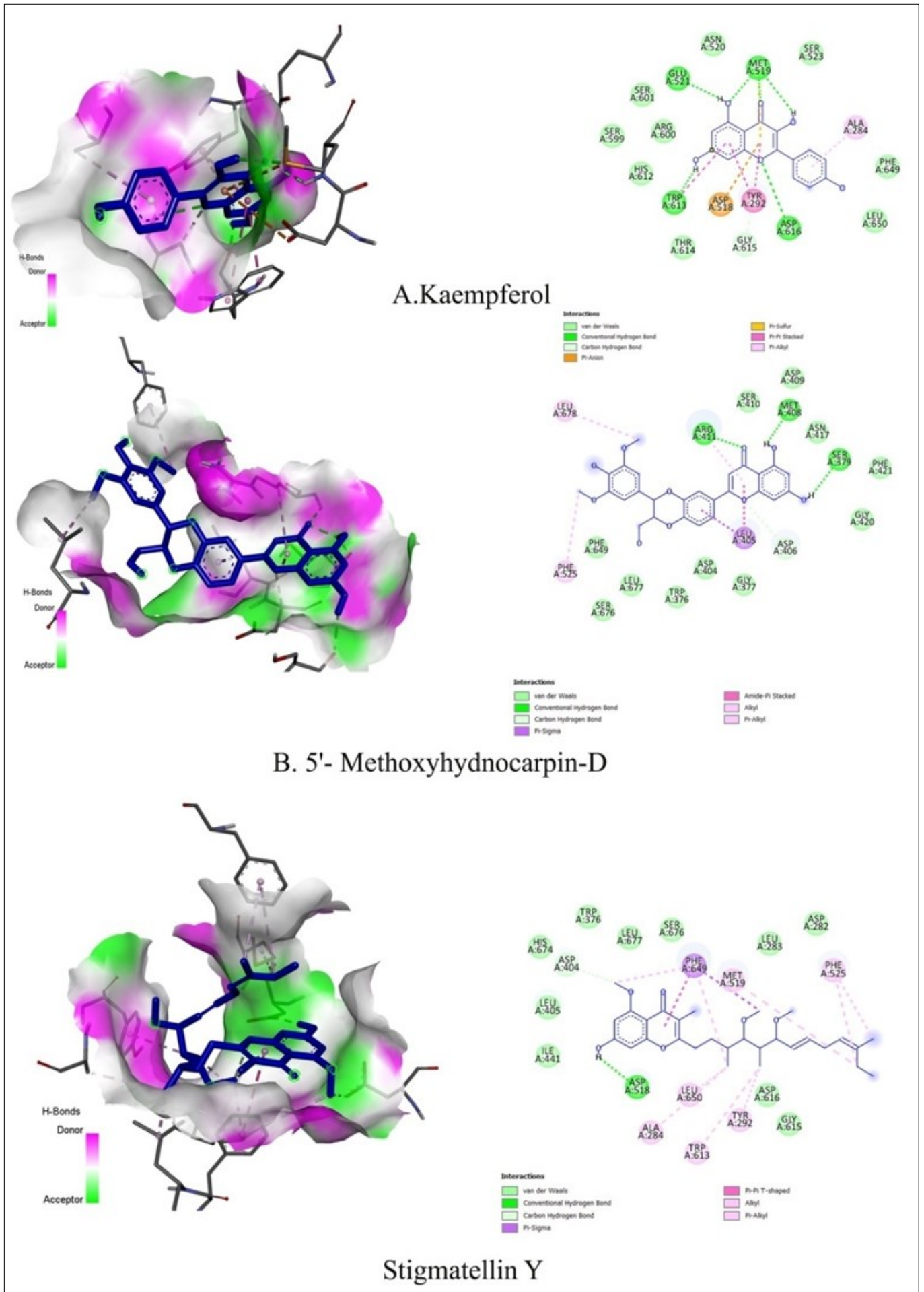


Fig.10. Molecular docking analysis of dominant compound identified through HR-LC MS analysis and docked against human α -glycosidase enzymes

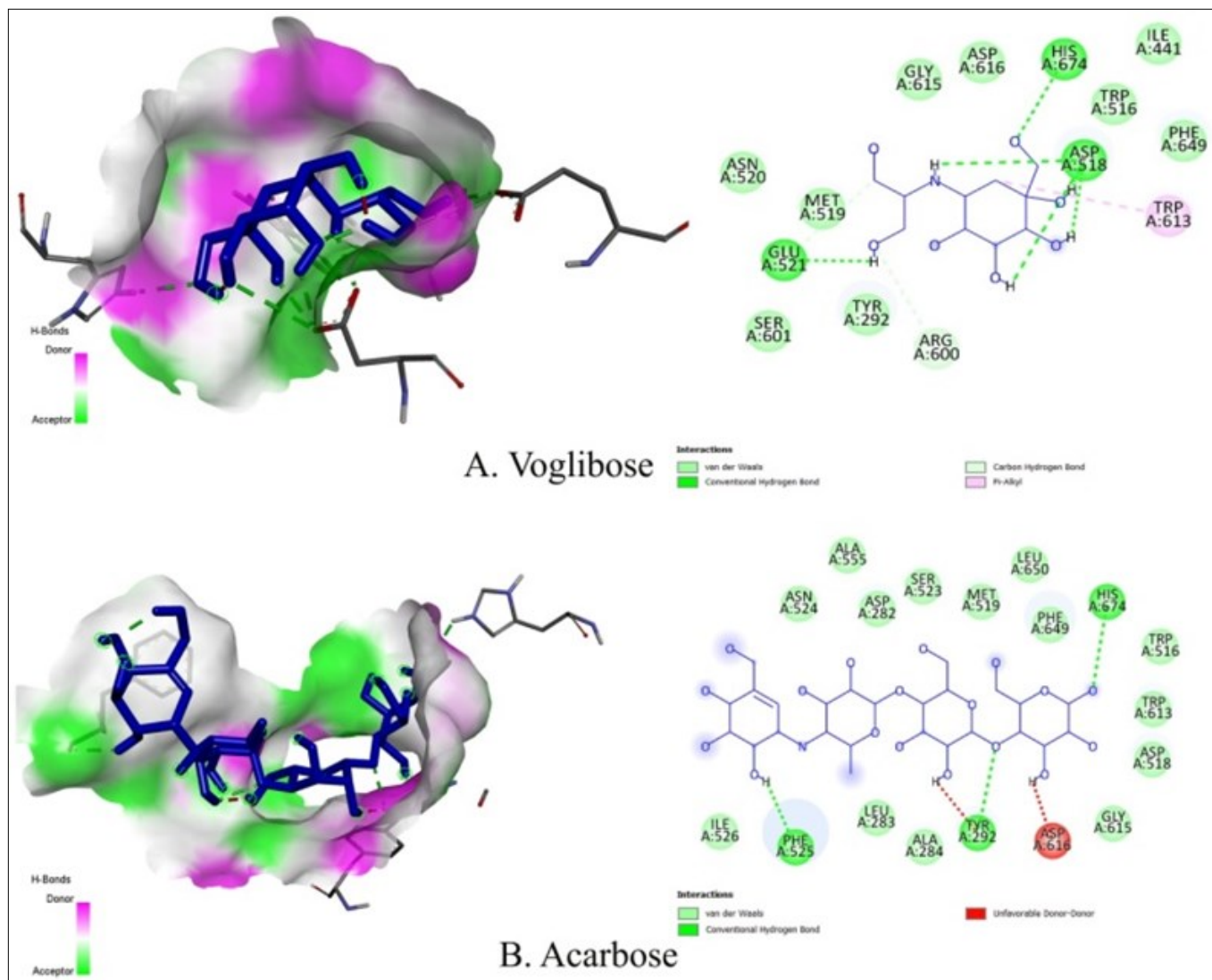


Fig.11. Molecular docking analysis standard drug used against diabetic docked against human α -glycosidase enzymes.

medicine against diabetic diseases. The residues Trp-516 and Asp-518 are demonstrated to be critical for the catalytic function of 5NN8 (38). The catalytic pocket of the 5NN8 also contains amino acid residues such as Trp 376, Trp 481, Phe 525, Asp 282, Asp 616, Arg 600, Leu 677, Leu 678 (41).

The present molecular docking study also revealed that the flavonoids identified through HR-LCMS, namely quercetin 3-rhamnoside-7-glucoside, luteolin (Fig. 8),

marimetin (Fig. 9), kaempferol, 5-methoxyhydrocarpin-D (Fig. 10) more strongly bound with the hydrogen bonding (length of hydrogen bonding between ligand 5NN8 were also depicted in the Table 7) and as well as other hydrophobic interactions to the active site of 5NN8 than the standard drugs. This may occur due to the strong hydrogen bond and hydrophobic interactions of the protein-ligand complex found in the catalytic pocket of the 5NN8.

Table 7. Ligands with the number of hydrogen bonds and respective bond length

Sl.no	Ligand name	Number of hydrogen bonds with target (5NN8)	Hydrogen bond length between ligand and target (\AA)
1	Quercetin 3- rhamnoside-7- glucoside	2 bonds with Arg-281, Asn-524	2.36 and 2.96 respectively
2	Luteolin	7 bonds with Ala-284, Asp-282, Asp-518, Asp-645, Trp -613, Arg -672, Ala -284	2.34, 2.69, 2.89, 3.96, 3.58, 3.54, 3.85 respectively
3	Rhein	5 bonds with Glu-521, Ser-523, Asp-212, 616, Ala-284	3.25, 3.04, 2.95, 3.64 and 2.67
4	Retronecine	1 bond with Asp-645	3.69
5	Rutin	4 bonds with 2 with Asp-518, remains with Asp -616 and Tyr-292	4.62 and 3.47, 2.92 and 3.45 respectively
6	Maritimetin	3 bonds with Glu-521, Met-519 and Tyr-292,	2.94, 2.68 and 3.68
7	Kaempferol	1 bonds with Glu 521, Met -519, Asp- 616, Trp 613	2.35, 2.39, 3.23 and 2.58 respectively
8	5-Methoxyhydrocarpin-D	3 bonds with Arg -411, Met-408 and Ser -379,	2.63, 2.49 and 2.99 respectively
9	Stigmatellin Y	1 bond with Asp-518	2.67
10	Voglibose (standard drug)	5 bonds, 3 with Asp-518, one with His-674, and Glu-521.	3.98, 3.72, 2.99, 3.21, 2.68 respectively
11	Acarbose (standard drug)	3 bonds with Phe-525, Tyr-292 and His-674	2.68, 3.12 and 3.45 respectively

Here, *in vitro* study of methanolic extracts against α -glucosidase was correlated with the molecular docking technique

Conclusion

S. alata was analyzed for the antidiabetic potential and the result revealed that the methanolic extract of the plant contained a significant amount of secondary metabolites. In the quantification of secondary metabolites, it has been found that methanolic extract contains a high quantity of TPC, TSC and TFC. TTC was found to have more in the chloroform extract. Among all analyzed antidiabetic assays, methanolic and acetone extracts were found to effectively inhibit α -glucosidase and α -amylase respectively. Methanolic extract showed an inhibition value similar to standard acarbose. HR-LCMS analysis also identified compounds such as quercetin 3-rhamnoside-7-glucoside, kaempferol, rutin and rhein that exhibited antidiabetic activity. Furthermore, the molecular docking study revealed that 5-methoxyhydnocarpin-D, quercetin 3-rhamnoside-7-glucoside, kaempferol, maritimetin and luteolin established interactions with amino acid residues found in the catalytic pocket of 5NN8. Among these docked compounds, kaempferol, luteolin and quercetin 3-rhamnoside-7-glucoside showed significant antidiabetic activity. Hence, further studies are needed for the identification and authentication through *in vivo* and clinical background of the mode of action of such bioactive molecules against diabetics.

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Authors contributions

BT put the project idea and worked with RC on all procedures, also writing the article, ST helped to design the experiments and write the article.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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