Original Research Article
Exploration of Antioxidant, Antibacterial, and Alpha-glucosidase Inhibition Potential of *Cirsium verutum* (D. Don) Spreng Extracts*: In vitro* and *in silico* Approach

ABSTRACT

Certain medicinal plants have been used to address common infectious as well as non-infectious diseases. Diabetes mellitus poses a major global health challenge, leading to significant mortality and morbidity each year. Finding side-effect-free treatments for diabetes has always been difficult. Bacterial resistance is recognized as a significant medical concern, highlighting the need to identify new compounds that could serve as potential starting points for addressing or managing infectious diseases. The study focused on the phytochemical extraction of *Cirsium verutum* (D. Don) Spreng, evaluating its bioactivity and identifying potential molecules through molecular docking that could act as starting compounds for creating alternative drugs to address specific health issues. The aqueous extract demonstrated superior performance, displaying the highest phenolic content and exhibiting notable inhibitory effects on the alpha-glucosidase enzyme (31.39±0.02 mg GAE/gm and 70.46% inhibition with an IC_{50} value of 37.37±1.46 µg/mL, respectively). The free radical scavenging activity using DPPH indicated the maximum activity in the hexane extracts an IC_{50} of 442.17±0.42 µg/mL. The extracts of *C. verutum* demonstrated minimum inhibitory concentration (MIC) values ranging from 0.78 to 12.5 mg/mL and minimum bactericidal concentration (MBC) values ranging from 3.12 to 25 mg/mL in the antibacterial assay. The fully characterized compounds from *C. verutum* were searched from various papers, followed by their molecular docking. In the molecular docking study, it was discovered that among the seven top candidates, pectolinarin (01) was identified as the most promising candidate, exhibiting a docking score of -11.076 kcal/mol against intestinal alpha-glucosidase. This integrated study approach will aid in the discovery of new antibiotics and the management of diabetes.

Keywords: Bioactivity, antibacterial, alpha-glucosidase, antioxidant, molecular docking, diabetes management

1. INTRODUCTION

Plants have historically been valuable sources of medicinal compounds, with many modern medications deriving directly or indirectly from botanical origins. Medicinal plants are valued for their recognized medicinal properties [1]. The rising global use of medicinal plants is motivated by the recognition that their natural constituents provide significant therapeutic advantages without side effects, driving scientific research into bioactive molecules [2], [3].

Diabetes mellitus (DM) is a complex and persistent metabolic condition that has emerged as a major worldwide health issue. Its widespread effects affect a substantial portion of the population, posing significant health risks [4], [5]. Numerous studies have been published on the use of traditional medical approaches in the treatment of diabetes, as well as the use of naturally produced compounds from plants [6]. There is increasing demand for medicinal plants with anti-hyperglycemic properties due to their perceived advantages of fewer side effects and lower expenses [7]. Ethnobotanical research suggests that around 800 plant species are believed to possess properties that could be beneficial in managing diabetes

[8]. The haphazard use of antibiotics as well as due to genetic mutations or gene transfer in bacteria has led to an increase in antimicrobial resistance (AMR) and multidrug resistance (MDR) [9]. This resistance can result in ineffective treatments, prolonged illness, and higher healthcare costs, making it a significant global public health issue, while MDR specifically pertains to bacteria resisting multiple antibiotics, complicating treatment further. The phytochemicals, especially phenols and flavonoids, give the plant antioxidant properties that help in scavenging free radicals, thus preventing cellular damage and ageing [10].

Cirsium verutum, an herbal plant, contains various compounds such as flavonoids, polyacetylenes, acetylenes, phenolic acids, phenylpropanoids, sterols, and terpenoids [9]. *Cirsium verutum* (D. Don) Spreng stands out as one of the most prevalent and effective medicinal plants for treating diabetes, as per traditional practices in Nepal [11]. These perennial herbs grow to a height of 1–1.5 meters. They have upright, branched stems without wings, covered in long multicellular hairs. The leaves are uniformly green, smooth or slightly cobwebby underneath with long multicellular hairs, and rough on the upper surface, with sparse to dense spines [12]. It is used in traditional medicine in western Nepal for bacterial and fungal diseases [13]. Various studies have suggested that the diverse phytoconstituents present in *Cirsium* plants contribute synergistically to their multifaceted therapeutic potential, making them promising candidates for addressing various health concerns [14], [15].

This study has attempted to combine experimental laboratory work with computational techniques of molecular docking. Molecular docking is a computer-based virtual screening technique to assess the possible binding capability of a ligand at the active site of a specific target [16]. The computational program generates millions of possible poses and orientations of the ligand in a selected grid box at an active site where the most effective and scientific binding affinity between a target protein and ligand is evaluated short out in terms of docking score [17]. A larger negative binding affinity indicates a more effective interaction between protein and ligand. The result of molecular docking could yield protein-ligand adduct where interpretation of the possible type of interactions, stability of complex, responsible amino acid residues of the receptor, and distance between residues and ligand could be comprehended [18]. The molecular level analysis would help to understand the geometrical details and mechanistic pathway that could be utilized to design better candidates form plant-based resources for the management of diabetes with reduced or no adverse effects.

2. MATERIAL AND METHODS

2.1. Chemicals and Materials

Hexane, chloroform, ethanol, methanol (Fischer Scientific India), Gallic acid (Hi-media Laboratories), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and quercetin (Wako Pure Chemicals, Osaka, Japan), soxhlet apparatus (Bionics Scientific) were used.

2.2. Sample collection

The plant sample (whole plant) was collected from Bhaktapur, Nepal and their identification was confirmed as *Cirsium verutum* (D. Don) Spreng at the National Herbarium and Plant Research Centre, Botanical Garden, Godawari, Lalitpur, Nepal.

2.3. Preparation of extracts

C. verutum was macerated in methanol, and then fractionated into hexane, ethanol, chloroform, and water using a Soxhlet apparatus.

$$\text{Dry weight of extract Percentage Yield (\%)} = \frac{\text{Dry weight of extract}}{\text{Total weight of powder}} \times 100$$

2.4. Determination of total phenol content (TPC) and total flavonoid content (TFC)

The TPC of the extracts was measured using Folin-Ciocalteau reagent by ultraviolet (UV) spectrometric method, which was modified from the colorimetric method [19].

The TFC of the extracts were determined by UV spectrometric method which was modified from the colorimetric method [20].

2.5. Determination of free radical scavenging activity using DPPH

The radical scavenging activity of the plant extracts was evaluated using the spectrophotometric DPPH method [21]. The capability to scavenge the DPPH radical was measured by the reduction of absorbance and calculated by using the following equation:

$$\% \text{ scavanging} = \frac{A_o - A_t}{A_o} \times 100$$

Where, A_o is the absorbance of DPPH with 3% DMSO and A_t is the absorbance of DPPH with test sample.

2.6. Alpha-glucosidase inhibition assay

The α-glucosidase inhibitory effect of extracts was determined by the method as described by Fouotsa and team [22]. The percentage α-glucosidase inhibitory effect was calculated by the following formula:

$$\% \text{ inhibition} = \frac{A_o - A_t}{A_o} \times 100$$

Where A_o is the absorbance of enzyme substrate reaction with 50% DMSO and A_t is the absorbance of enzyme substrate from plant extract.

2.7. Antibacterial activity

Agar well diffusion method was utilized to evaluate the antibacterial activity with 5 bacteria (Gram positive organism *Staphylococcus aureus* (ATCC 25923), Methicillin-Resistant Staphylococcus aureus (MRSA), Gram negative organism *Escherichia coli* (ATCC 25922), *Salmonella Typhimurium* (ATCC 14028) and *Pseudomonas aeruginosa* (ATCC 27553)).

2.7.1. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) value

The MIC and MBC of antibacterial agents was determined using the approach of micro dilution in culture broth, as recommended by the Clinical and Laboratory Standards Institute (CLSI) of the United States [23].

2.8. Computational Procedures

2.8.1 Selection and preparation of compounds

The phytochemicals (flavonoids and phenolic compounds) found in the selected plants reported in previous work, were selected as the candidate ligands. The 3D structures of such compounds were collected from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in SDF format which was converted to pdb format for molecular docking [24]. From *C. verutum,* pectolinarin (01); CID: 168849, cirsiliol-4'-monoglucoside (02); CID: 11972311, cirsimarin (03); CID: 159460, cirsilineol-4'-monoglucosidase (04); CID: 11972310, cirsitakaogenin (05); CID: 188323, taraxasterol acetate (06); CID: 13889352, pectolinarigenin (07); CID: 5320438, cirsiumoside (08); CID: 101589677, cirsiumaldehyde (09); CID: 12366272, 5-methoxymethyl-2-furancarboxaldehyde (10); CID: 74711, luteolin (11); CID: 5280445 and 5-hydroxymethylfurfural (12); CID: 237332 were chosen as a candidate [25].

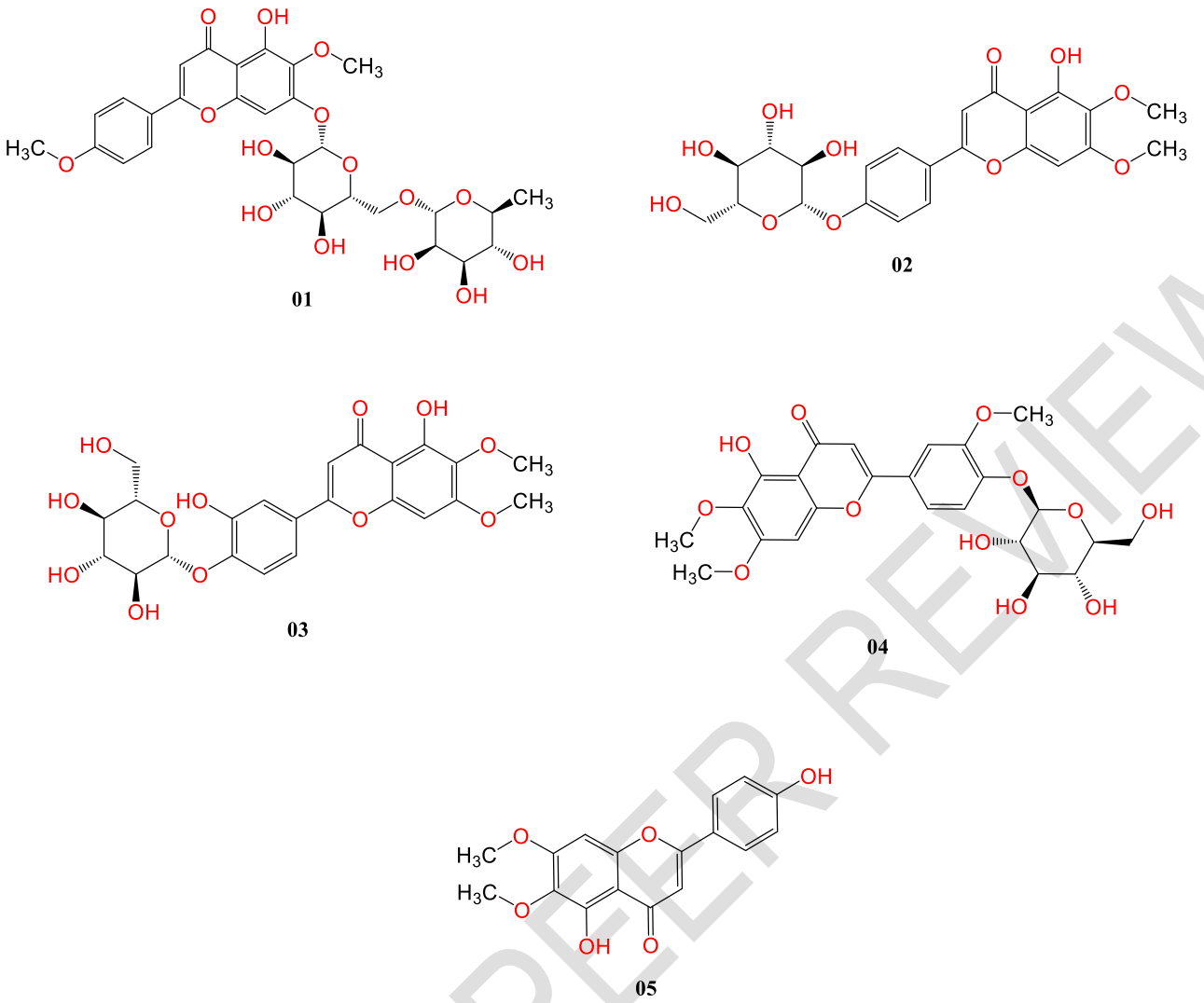


Fig. 1. Molecular structures of top hit candidates from *C. verutum* (01-05)

2.8.2. Target Protein Preparation and Molecular Docking Calculation

The 3D crystallographic structure of intestinal glucosidase (Maltase-Glucoamylase) of PDB ID: **3TOP** with native ligand alpha acarbose was retrieved from RCSB protein data bank in pdb format (https://www.rcsb.org/) [26]. The protein structure was prepared for docking by removing water molecules, and nonstandard residues. By adding polar hydrogen atoms and checking the bond order in the PyMol software, the protein structure was saved in pdb format [27]. Flexible receptor molecular docking in hydrated environment was performed by using the DockThor web server (https://dockthor.lncc.br/v2/) [28]. Interpretation of the data was done after downloading the result file. PyMol and Biovia Discovery Studio software were used for the visualization and interpretation [29].

2.8.3. Molecular docking protocol validation

The docking protocol was validated by calculating the RMSD between the pose of the ligand in the crystal structure and pose of the same ligand in the docked structure. The RMSD between the two superimposed structure of 1.70 Å indicated good selection of molecular docking algorithm in capturing the naturally occurring phenomena and possibly the local minima. Fig 2. presents the superposition of the ligand in the crystal structure (green) and docked ligand (purple). The protein structure and selected ligands were uploaded to the server and the grid box size (x=14, y=17, z=15) and center (x= -31, y=35, z= 15) at the catalytic site of the target were chosen.

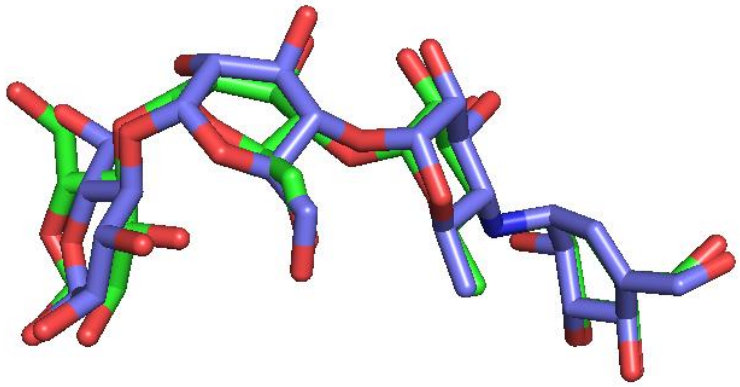


Fig. 2. Superposition of native ligand acarbose (green) in crystal structure with docked ligand (purple)

3. RESULTS AND DISCUSSION

3.1. Phytochemical Screening and Percentage Yield

Phytochemical screening showed the presence of different phytochemical compounds such as flavonoids, alkaloids, steroids, tannin, saponin, phenols, terpenoids, and glycosides (Table 1). The maximum percentage yield of *C. verutum* was observed in ethanol extract (12.38%), followed by aqueous (8.96%), hexane (7.07%), and chloroform (3.24%) (Table 2).

Table 1. Phytochemical screening of various extracts of *C. verutum*

Class of phytochemicals	Extracts			
	Hexane	Chloroform	Ethanol	Aqueous
Flavonoid	+	+	+	+
Alkaloids	-	+	+	+
Tannin	-	+	+	+
Terpenoids	+	+	+	+
Saponin	-	-	-	-
Phenol	+	+	+	+
Glycoside	+	+	-	+
Steroids	+	+	+	+

Note: (+) indicates the presence and (-) indicates the absence of the given phytochemical

3.2. Total Phenolic and Flavonoid Content

Higher amount of total phenolics was observed in the aqueous extract compared to other extracts of *C. verutum* (31.39±0.02 mg GAE/gm). The chloroform extracts of the plants exhibited the lowest TPC (0.25±0.04 mg GAE/gm) (Table 2).

Among all the extracts, the hexane extract of *C. verutum* exhibited the highest total flavonoid content of 0.26±0.03 mg QE/gm. The plant possesses a comprehensive array of flavonoid types [9]. *Cirsium* species exhibit a wide range of pharmacological activities owing to their rich phytochemical composition. These activities encompass antimicrobial, antioxidant, analgesic, anticancer, anti-diabetic, and anti-inflammatory properties.

Table 2. Total phenolic and flavonoid content in the various extracts

Contents	Extracts			
	Hexane	Chloroform	Ethanol	Aqueous
% yield	7.07	3.24	12.38	89.68
TPC*	1.49±0.07	0.25±0.04	10.87±0.01	31.39±0.02
TFC**	0.26±0.03	0.25±0.02	0.211±0.01	0.2±0.1

*TPC is expressed in mg gallic acid equivalent per gram (mg GAE/gm) and **TFC values is expressed in mg quercetin equivalent per gram (mg QE/gm).

3.3. Radical scavenging activity

All the extracts from the plant were subjected to the DPPH assay, and the results are presented in Table 3. All the extracts scavenged DPPH radicals, with the hexane extracts demonstrating superior activity at 68.39% and an IC_{50} of 442.17±0.42 µg/mL.

Table 3. Radical scavenging activity of various extracts

Plant extracts	% Inhibition at 500 µg/mL	IC_{50} SEM* (µg/mL)
Hexane	68.39	442.17± 0.42
Chloroform	24.98	_
Methanol	24.49	_
Aqueous	42.51	_
Quercetin**	87.01	2.3±0.01

*SEM (standard error of mean), and **Quercetin (standard)

3.4. Alpha-glucosidase Inhibition Assay

The findings regarding the α-glucosidase activities demonstrated by various extracts from the plant are presented in Table 4. The aqueous extract of *C. verutum* exhibited noteworthy inhibition of the α-glucosidase enzyme (70.46%) with an IC_{50} value of 37.37±1.46 µg/mL, comparable to the reference standard acarbose, which demonstrated 83% α-glucosidase inhibitory activity with an IC_{50} value of 387.36±0.05 µg/mL. Research suggests that phytochemicals, such as phenolics, can alleviate diabetes and obesity problems and inhibit α-glucosidase [30], [31], [32]. Polyphenols are recognized for their ability to engage with enzymes through non-specific interactions, leading to the inhibition of enzyme activity [33]. The outcomes indicated a positive correlation between the inhibitory activity against α-glucosidase and the total phenolic content. Likewise, previous studies have suggested a connection between the inhibitory activities of plant-based extracts or products against α-glucosidase and their total phenolic compounds and flavonoid contents [34], [35]. The correlation between antioxidant activity and TPC was notably strong in the plant examined, suggesting that phenolic compounds play a significant role in the antioxidant activity of these medicinal plants as described by Molan and Mahdy [36]. Our results regarding the antioxidant and anti-diabetic effects correspond with those of a study conducted by Famuyiwa and colleagues in 2019. They observed a direct association between the antidiabetic effects and redox potential data of the two compounds, implying a possible linear connection between these properties [37].

Table 4. Alpha-glucosidase inhibitory activity of various extracts of *C. verutum*

Extract	Enzyme Inhibition (%) at 500 µg/mL	IC_{50} SEM* (µg/mL)
Hexane	23.52	-
Chloroform	49.06	-
Methanol	32.02	-

Aqueous	70.46	37.37±1.46
Acarbose**	83	387.36±0.05

*SEM (standard error of mean), and **Acarbose (standard)

3.5. Antibacterial Activity

Only the chloroform extract of *C. verutum* was found to be active against Gram-positive bacteria (*S. typhi*) as described by Nazaruk and Jakoniuk [14]. Similarly, the methanolic extract of *C. verutum* showed the antibacterial activity against *E. coli* (11.3 mm) and *S. typhi* (10.8 mm). These findings suggested that different solvent extracts of *C. verutum* exhibited varying degrees of antibacterial potential, with chloroform and methanol extracts showing more promising results compared to hexane and aqueous extracts (Table 5).

Table 5. Antibacterial activity of plants extracts (50 mg/mL) on five different bacteria

Plant extracts	Zone of Inhibition (ZOI) shown by bacterial strains (Diameter of well, Ø = 6 mm)				
	E. coli	*S. aureus*	*S. typhi*	*P. aeruginosa*	MRSA
Hexane	-	-	-	-	-
Chloroform	-	11	-	-	-
Methanol	11.3		10.8	-	-
Aqueous	-	-	-	-	-
Neomycin*	17	24	20	16	14

*Neomycin (positive control)

3.5.1. Minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) Value

The extracts of *C. verutum* exhibited MIC and MBC value ranging from 0.78 to 12.5 mg/mL and 3.12 to 25 mg/mL, respectively (Table 6).

Table 6. MIC and MBC of plant extract against various bacterial culture (mg/mL)

Plant extracts	*S. aureus*		*P. aeruginosa*		*E. coli*		*S. typhi*		*MRSA*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Hexane	-	-	-	-	-	-	-	-	-	-
Chloroform	0.78	3.12	-	-	-	-	-	-	-	-
Methanol	-	-	-	-	6.25	25	12.5	25	-	-
Aqueous	-	-	-	-	-	-	-	-	-	-

Note: (-) indicates no observed inhibitory activity

3.6. Molecular level details of the adducts

The intestinal α-glucosidase (PDB ID: 3TOP) was selected as the target to assess the inhibitory potential of compounds found in the plant *C. verutum* and screen for potential α-glucosidase inhibitory properties. Comprehensive information on molecular docking results, including docking scores and interactions with amino acid residues at the catalytic site of the protein, is presented in Table 7. Compounds (01-07) stood as potential hit candidates for the α-glucosidase enzyme, as they achieved comparable docking scores (< -9.5 kcal/mol) and demonstrated binding affinity comparable to that of the native ligand (-10.567 kcal/mol). Among all the candidates, pectolinarin (01) exhibited the highest binding affinity with a score of -11.076 kcal/mol. It showed significant interaction with the key active site residue ASP1279 along with others of the α-glucosidase enzyme.

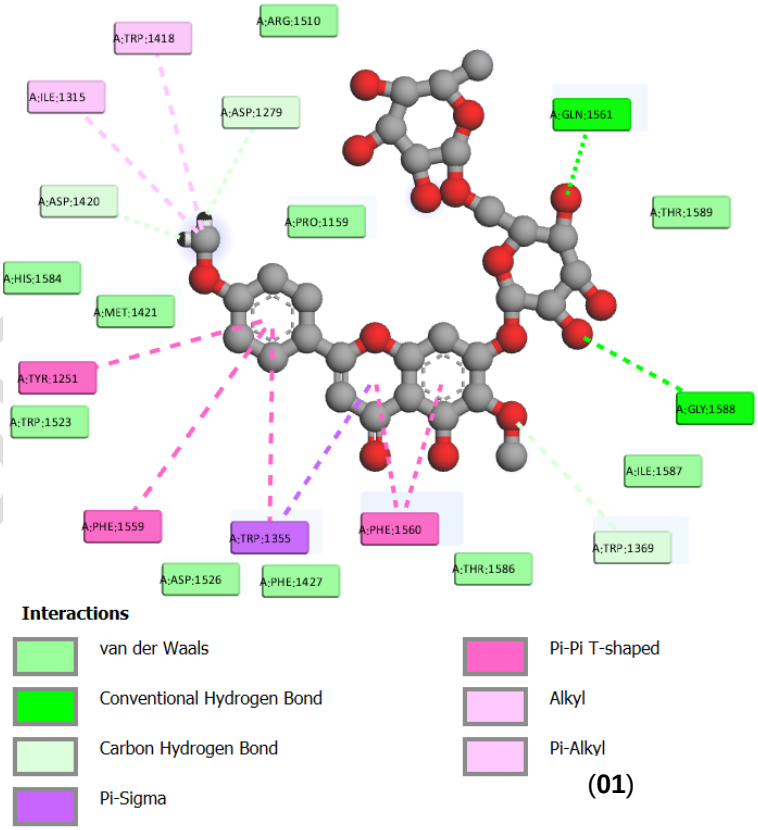
Table 7. Collective information about ligands, docking score and types of interactions on adduct

Selected ligand	Docking score (kcal/mol)	Responsible amino acid residues of target protein for		
		H-bonds	Hydrophobic interactions	Other interactions
Pectolinarin (01)	-11.076	GLY1588, **ASP1279**, ASP1420, TRP1369	TRP1418, ASP1420, TYR1251, PHE1559, TRP1355, PHE1560	PRO1159, ASP1526, THR1586, HIS1584, MET1421, ARG1510, TRP1523, ILE1587
Cirsiliol-4'-monoglucoside (02)	-10.336	ARG1510, ASP1526, **HIS1584**, **ASP1279**	TRP1355, TRP1359, PHE1560, PRO1159, TRP1251	ILE1280, ASP1157, MET1421, ILE1587, TRP1523, PHE1559
Cirsimarin (03)	-10.221	**ARG1510**, **ASP1279**, ASP1420, ASP1526	TRP1369	ASP1157, MET1421, PHE1427, ILE1280, PHE1559, TRP1355, LYS1460, HIS1584
Cirsilineol-4'-monoglucosidase (04)	-9.866	**ASP1526**, **ARG1510,** ASP1157, ASP1279, HIS1584	TRP1369, ILE1587, TRP1355	ILE1280, ARG1582, ASP1555, PHE1427, PHE1559, TRP1523
Cirsitakaogenin (05)	-9.603	ASP1279	PHE1560, ILE1587, PHE1559, TRO1355, TRP1369	ASP1420, ILE1280, THR1586, MET1421, PRO1159, ARG1510
Taraxasterol acetate (06)	-9.548	THR1586	PRO1159, MET1421, PHE1559, TYR1251, TRP1355, PHE1560	ASP1526, ILE1587, HIS1584, ILE1280, PHE1427
Pectolinarigenin (07)	-9.508	**ARG1510**, ASP1420, **ASP1279**	PHE1560, PHE1559, TYR1369, TRP1355, ILE1315, MET1421, TRP1418, TYR1251	TJR1586, HIS1584, PHE1427, ASP1157, TRP1523, ILE1280
Cirsiumoside (08)	-9.260	**ASP1279**, **ASP1526**, ASP1157, TRP1369	PHE1559, PHE1560, TRP1355	ILE1280, THR1586, LYS1460, PHE1427, MET1421
Cirsiumaldehyde (09)	-7.895	THR1586, **ASP1157**, MET1421, **ARG1510**, ASP1526	–	TRP1355, MET1421, PHE1559
5-Methoxymethyl-2-furancarboxaldehyde (10)	-7.443	**ARG1510**, GLN1372, ARG1377, TYR1251	PHE1559, TRP1355	ASP1526, ILE1587, THR1586, PHE1560, MET1421
Luteolin (11)	-7.376	**ARG1510**, **HIS1584**, ASP1526	PHE1559, TYR1251, MET1421	ASP1157, ASP1420, ASP1555, ASP1279,
5-Hydroxymethylfurfural (12)	-7.013	**HIS1584**, **ASP1279**, **ARG1510**, **ASP1526**	TYR1251, PHE1559	ASP1526, PHE1560, THR1586, ASP1157, ASP1420, TRP1323
Acarbose	-10.567	ASP1279, **HIS1584**, ASP1526, **ARG1510**, ASP1157,	TYR1415, PHE1559, PHE1560	MET1421, LYS1460, SER1425, TYR1355, PRO1159, THR1586, ILE1280, TRP 1523

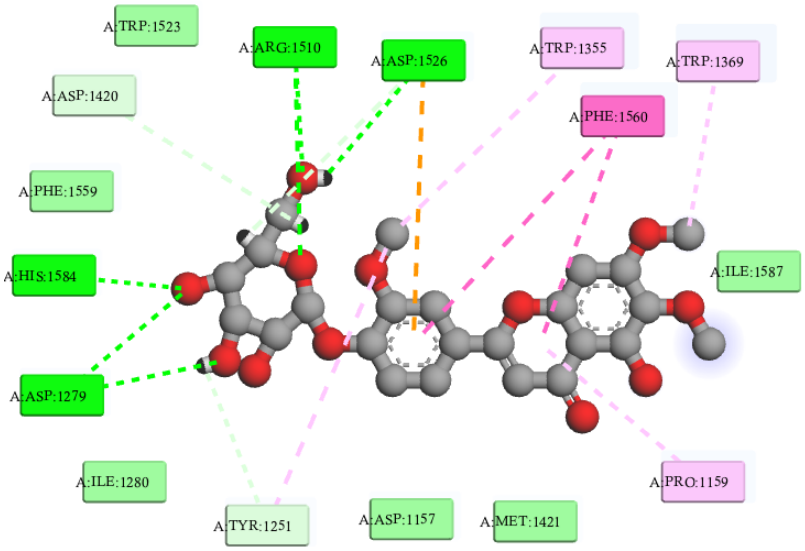
ASP1420

Bold letters indicate the active site residues of the enzyme

The native ligand (acarbose) was found to have H-bonding interaction with amino acid residues HIS1584, ASP1279, ASP1226, ASP1157, ARG1510 and ASP1420 with a binding affinity of -10.567 kcal/mol. Residues TYR1415, PHE1559, and PHE1560 interacted with Pi-alkyl hydrophobic interactions with native acarbose [38], [39]. Fig 3. illustrates the types of interaction between ligands and active site residues. The top candidate, **ligand (01)** interacted with amino acids ASP1279, GLY1420, GLY1588, and TRP1369 through H-bonding while TRP1355, PHE1559, TYR1251, PHE1560, ILE1418, and TRP1420 interacted through hydrophobic interactions containing Pi-sigma, Pi-alkyl, alkyl, and Pi-Pi stacked interactions. H-donor site of amino acids, glutamine 1561 and glycine 1588 interacted with acceptor oxygen atom of ligand (01) and formed strong hydrogen bonds (<2.8 Å). The aromatic benzene ring of amino acids, phenylalanine (1560, 1559) and tyrosine 1251 formed Pi-Pi T interactions with the ring structures of the ligand in the best orientation. Additionally, it was found that most of the ligands formed complexes with protein, interacting through hydrogen bonding with active site amino acid residues ASP1279, HIS1584, ASP1526, ARG1510 and ASP1157 along with hydrophobic interactions with residues PHE1559, PHE1560 and TYR1251, which claims the scientific approach of the current research work [38], [40].
The effective interaction and orientation of the ligand at the binding site of the target makes the adduct more stable [41], [42]. More number of hydrogen bond formations, reasonable hydrophobic interactions, other electrostatic interactions (Pi-cation, Pi-anion) and van der Waal forces are the factors determining the stability of the complex geometrically at physiological temperature [41].
In this research, the binding capability of the ligands found in *C. verutum* to the α-glucosidase enzyme was screened through molecular docking method to support the *in vitro* data. Through *in silico* method it was found that most of the selected ligand showed the effective binding affinity with the target protein. Ligands **01**-**07** could be concluded as hit binding candidates with the α-glucosidase enzyme as they exhibited comparable binding affinities to that of the native ligand. The *in vitro* glucosidase inhibition assay showed that the aqueous extract was potential inhibitor of the glucosidase (IC_{50}<39 µg/mL) and was found to be lower inhibitory concentration than that of standard drug acarbose (Table 4). The *in vitro* and docking calculation result suggested that the compounds found in aqueous extract of the plant could be potential inhibitor of intestinal glucosidase on the evidence of $IC_{50,}$ binding affinity and effective interactions. Furthermore, the effective interactions between the catalytic residues of enzyme with selected ligands may reveal the stability of the adduct and consequently the probable inhibition of the enzyme activity.



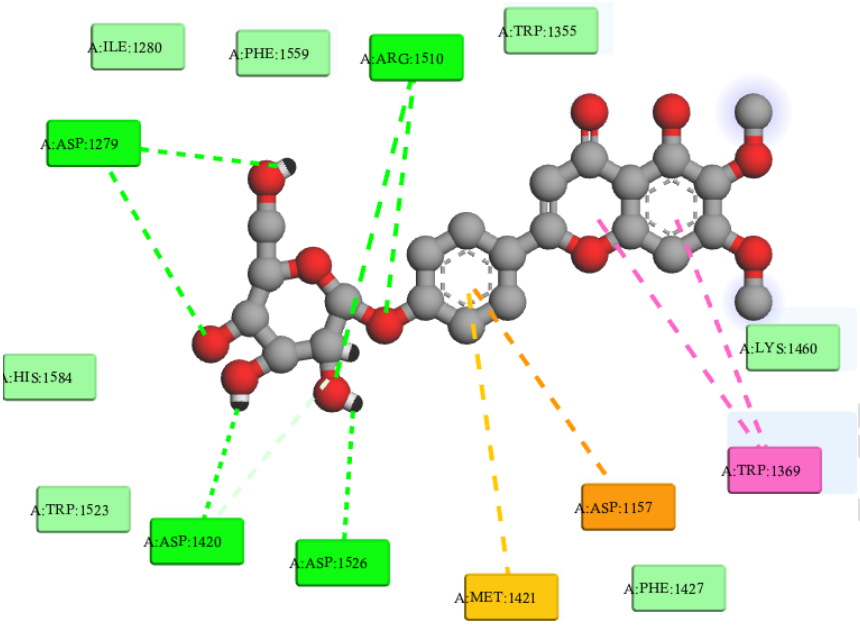
(**01**)



Interactions

- van der Waals
- Conventional Hydrogen Bond
- Carbon Hydrogen Bond
- Pi-Cation
- Pi-Donor Hydrogen Bond
- Pi-Pi T-shaped
- Pi-Alkyl

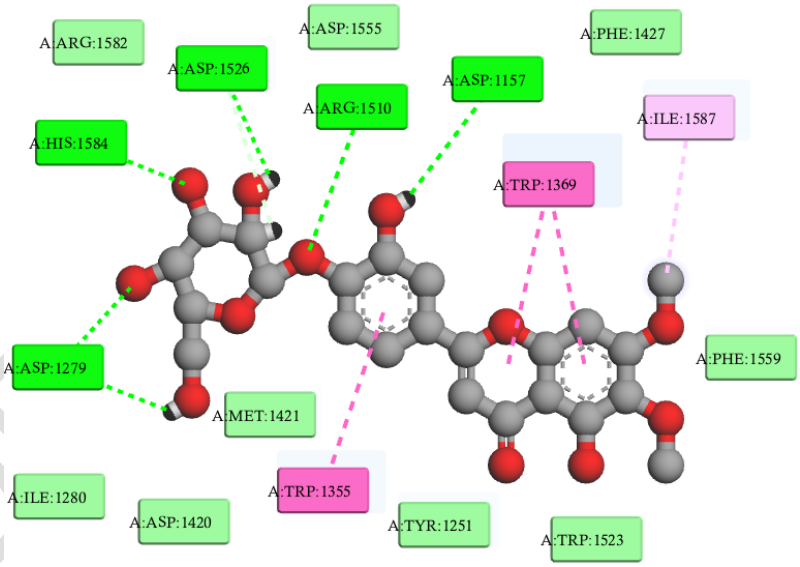
(02)



Interactions

van der Waals	Pi-Cation
Conventional Hydrogen Bond	Pi-Sulfur
Carbon Hydrogen Bond	Pi-Pi Stack

(03)



Interactions

van der Waals	Pi-Pi Stacked
Conventional Hydrogen Bond	Pi-Pi T-shaped
Carbon Hydrogen Bond	Alkyl

(04)

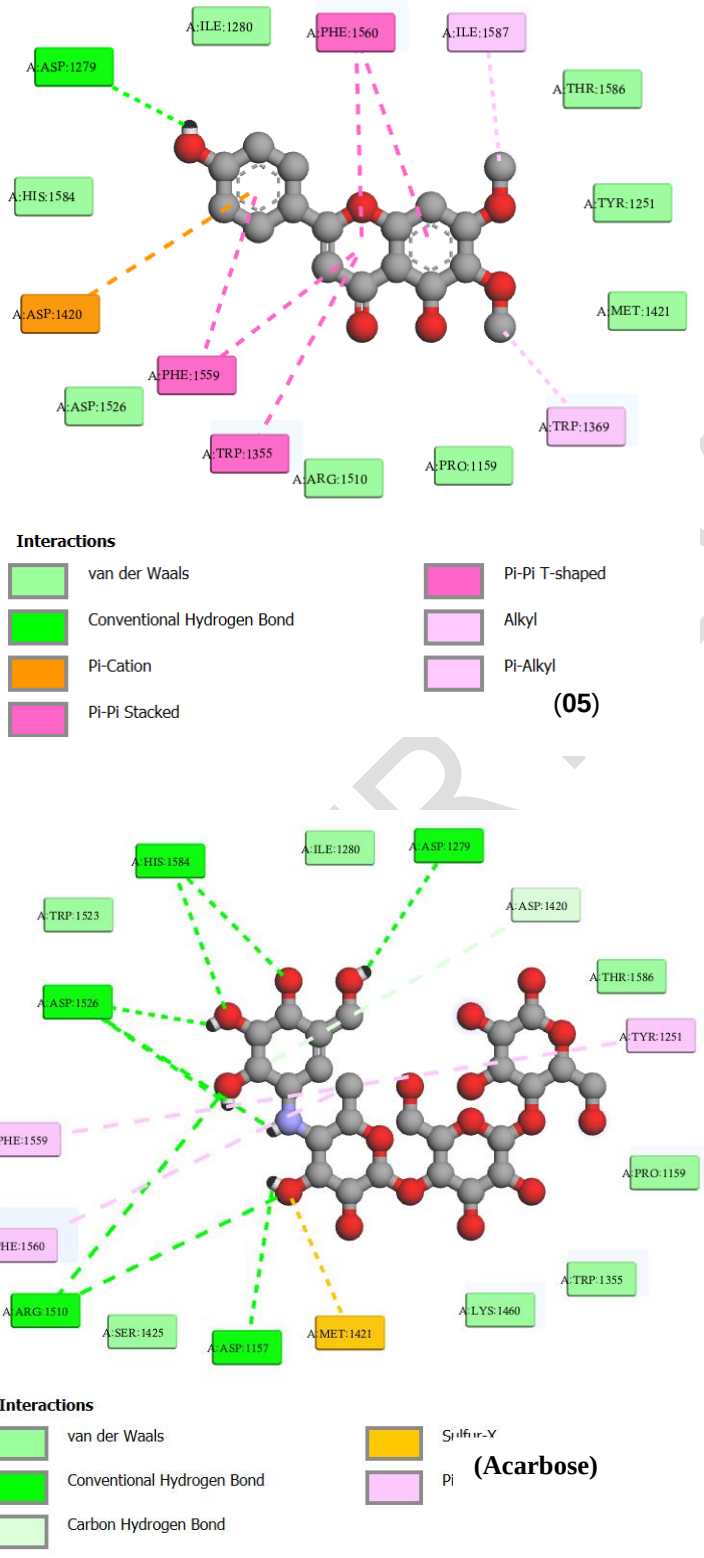


Fig. 1. 2D interactive presentation of the protein-ligand complex with the hydrophobic surface of the protein (shadowed reason on amino acid residues) for compounds from *C. verutum* (**01**-**05**) and **acarbose**

4. CONCLUSION

Through various experimental assays and computational docking analysis, the study showed the effectiveness of *C. verutum* extracts in addressing both infectious and non-infectious diseases. This was verified by evaluating its phytoconstituents, antibacterial activity, antioxidant potential, and alpha-glucosidase inhibition capability. It highlighted good antibacterial properties in the methanolic extracts, while the aqueous extracts displayed alpha-glucosidase inhibitory potential. However, the antioxidant potential of the plant was found to be weak. Additionally, the computational approach identified a potential candidate for future in silico studies. Given that pectolinarin (01) demonstrated the highest binding affinity with the alpha-glucosidase enzyme with a score of -11.076 kcal/mol. These findings not only highlight the therapeutic potential of *C. verutum* in the development of new antibiotics and anti-diabetic agents but also encourage further exploration of its bioactive compounds to unlock their full pharmacological potential. Overall, this research lays a solid foundation for future studies aimed at harnessing the medicinal properties of this plant for therapeutic applications.

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