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Insight into the alpha-glucosidase inhibitory potentials of *Curcuma longa* methanolic extracts and phytochemicals: An *in vitro* and *in silico* study †

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Abstract: Diabetes is a metabolic disease of global concern, causing death due to triggered oxidative and inflammatory complications. Alpha-glucosidase has become a popular drug target for managing diabetes. This study, therefore, investigated the potential of Curcuma longa (Tumeric) rhizome methanolic extract (MECL) to inhibit Alpha-glucosidase and screened its phytochemical library through molecular biology docking for potential new drug candidates. Quantitative phytochemical analysis of MECL showed that the plant extract was abundant with phenols (790.32 ± 129.20 mg/100g), alkaloids (494.99 ± 1.27 mg/100g), flavonoids (171.08 ± 0.04 mg/100g) and terpenoids (131.99 ± 6.59 mg/100g). Moreover, in vitro inhibitory studies showed a dose-dependent increase in the inhibition of alpha-glucosidase by MECL, and the maximum inhibition (37.01%) was observed at 30 μg/ml, possibly a better inhibition with increased concentration. Further scrutiny was performed using molecular docking to screen for Turmeric phytochemicals (retrieved from PubChem) with alpha-glucosidase (PDB ID: 3W37) inhibitory potentials. Based on their binding affinity, the Top three compounds [Guaiacol (-7.422), Eriodictyol (-5.266,) and p-Tolyl-MethylCarbinol (-3.939)] were analyzed for their intermolecular interactions in the binding pocket of alpha-glucosidase and ADMET properties; and compared to the standard drug, acarbose (-9.522). Interestingly, strong and weak interactions, such as hydrogen bonding, pi-pi stacking, and charged and hydrophobic interactions, were observed with Guaiacol in the binding pocket of alpha-glucosidase. Although acarbose had a better docking score, Guaiacol showed better ADMET (including physicochemical, drug-likeness, and pharmacokinetic) properties. Future studies could evaluate those potential anti-diabetes drug candidates against other targets and analyze them through in vivo experiments.

Keywords: Diabetes; anti-alpha-glucosidase; ADMET; Guaiacol; alpha-glucosidase enzyme

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1. Introduction

Diabetes mellitus, especially Type 2 diabetes, has been a global health concern, as it affects carbohydrate metabolism and is accompanied by several debilitating comorbidities [1]. Diabetes has been ranked in the world's top 10 causes of death and premature mortality. The disease condition is characterized by elevated blood glucose caused by dysfunctional insulin production or utility. Moreover, hyperglycemic-induced oxidative stress and inflammation foster its associative comorbidities and complications [2]. Studies have shown that genetics could play a major role in the onset and development of diabetes. As for Type 1 diabetes, genetics could contribute to about 60% to 65% of individual

susceptibility. Whereas only a small percentage of Type 2 diabetes susceptibility are attributed to genetics [3].

On the other hand, our lifestyle and what we consume can also trigger the onset and progression of diabetes. Moreover, poor nutrition and eating food that contains high calories, refined carbohydrates, sweets, refined flour, and paste are high-risk predisposing factors [4,5]. Other secondary causes of diabetes include consuming food rich in cyanide, some prenatal infection, stress, physical inactivity, and many more [6]. Uncontrolled diabetes mellitus can lead to one of the most fatal malignancies, including hyperglycemia, Pancreatic Ductal Adeno Carcinoma (PDAC), and cholangiocarcinoma, a risk factor for cancer in many organs [7]. Many other complications, such as ulcers, feet, eye problems, heart diseases, kidney dysfunction, and others, can lead to death. These health problems can be prevented or delayed differently [8].

Over the last 2 to 3 decades, many scientific efforts have been put into the discovery and development of management and treatment options for diabetes, including insulin treatment, pancreatic stimulation, and others [9]. Moreover, type 2 diabetes is managed by controlling the hyperglycemia uprise through moderating the activities of alpha-glucosidase and alpha-amylase enzymes, which fosters the breakdown of starch to simple sugar and promotes the increase in the level of un-utilizable blood glucose [10]. Some phytochemicals and plant metabolites have been found to have modulating effects on alpha-glucosidase, which have been the focus of many contemporary research [11].

This study aims to investigate the potential of Turmeric (Curcuma longa) phytochemicals on inhibiting or modulating alpha-glucosidase enzymes with potential therapeutic options for type 2 diabetes. Although a preliminary study, we shall explore the potential of methanolic extracts of Turmeric (via in vitro inhibition studies against glucosidase) as well as the activities of the phytoconstituents (via molecular docking/computational studies to the x-ray crystal proteins structure of alpha-glucosidase (PDB ID: 3W37).

2. Materials and Methods

2.1. Materials

Dried rhizomes of Turmeric (*Curcuma longa*); analytical reagents/chemical (including methanol, hydrochloric acid, chloroform, Dragendorff's reagent, Mayer's reagent, ammonium sulphate, ferric chloride, Fehling solution (A and B), lead sub acetate, bromine water, tetraoxosulphate (IV) acid, ethyl acetate, ammonium solution, Wagner's reagent, acetic acid, concentrated ammonium hydroxide, diethyl ether, sodium chloride, potassium dichromate); equipment including [Water Bath (Gallenkamp, England), Beakers (Pyrex, England), Weighing balance (Melter HAS, U.S.A), Filter papers (Whatman), Test tubes (Pyrex, England), Spectrophotometer (Spectronic 20D Germany), Water bath (Gallenkamp, England), Refrigerator (Thermo cool, England), Centrifuge (Vickas Ltd, England)] and computational soft-wares (Schrodinger Suite v20.03).

2.2. Methods

2.2.1. Methanolic solvent extraction of dried pulverized turmeric rhizome

A weighed amount (202.33 g) of dried pulverized turmeric rhizome (DPTR) was macerated in 1000 ml analytical graded methanol under intermittent shaking for 72 h. The percentage extract yield from the DPTR was determined after the marc was separated and the solvent was air-dried.

2.2.2. Qualitative and quantitative phytochemical analysis

The presence and amount of several phytoconstituent groups from the methanolic extract *Curcuma longa* (MECL) was determined based on the method described in [1]. We investigated the presence and quantity of flavonoids, alkaloids, phenols, terpenoids, steroids, tannins, glycosides, and reducing sugar.

2.2.3. Alpha-glucosidase inhibition

The potential of the MECL (between a concentration range of 10-40 μg/mL) to inhibit or modulate the activities of Alpha-glucosidase was estimated using a method adopted from Chukwuma et al. [1]. This assay is based on the principle of relative spectrophotometry quantification of reducing sugar obtained from sucrose after the activities of the analytical graded alpha-glucosidase enzyme (Sigma-Aldrich, St. Louis, USA). All the tests were performed in triplicates, and the percentage inhibition was calculated using Equation 1.

$$\text{Percentage inhibition (Alpha-glucosidase)} = \frac{Control\ group\ abs - Sample\ abs}{Control\ group\ abs} \text{x} 100 \quad (1)$$

2.2.4. Phytochemicals and bioactive metabolite from Curcuma longa (Turmeric)

The phytochemicals of Curcuma longa rhizome were retrieved from the famous repository – Dr. Duke's phytochemical and ethnobotanical databases (https://phytochem.nal.usda.gov/). Compounds obtained from the database were further verified by checking through individual reference publications as well as other repositories such as IMMPAT (https://cb.imsc.res.in/imppat/) and LOTUS (https://lotus.naturalproducts.net/). The canonicals SMILE (a 2D structure annotation) was retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/) for each non-ubiquitous compound of Turmeric to constitute the ligand library for the molecular docking and computational analysis. The

2.2.5. Preparation and refinement of Alpha-glucosidase crystal structure for molecular docking

The structure of human alpha-glucosidase annotated with a PDB ID: 3W37 was retrieved from the protein databank (https://www.rcsb.org/). This structure was selected from several options based on the protein structure quality and its excellent resolutions (1.70A) from X-ray crystallography. The structure downloaded onto Schrodinger was prepared by eliminating all non-interacting water molecules and modeling missing side chain, loop, and disulfide bonds using Prime packages of Schrodinger (working on an OPLS3e force field). The protein structure quality was assessed using the Ramachandran plot and protein reliability report after the structural refinements.

Furthermore, Schrodinger's sitemap tool was used to scan the protein structure for probable binding sites, of which the site with the best site score and volume was selected for molecular docking and phytochemical screening. The receptor grid generation package was used to specify and isolate the perimeter of selected binding pockets for docking by placing a grid that restricts the ligands to only the binding pockets.

2.2.6. Molecular docking for probable inhibitors of alpha-glucosidase and ADMET analysis

The phytochemicals library of Turmeric was docked on to refined alpha-glucosidase structure using both the standard precision (SP) and extra-precision (XP) module of the GLIDE package of Schrodinger suite. The standard precision module was used for virtual screening to select the top 20 ligands from 45 before a thorough docking analysis with the XP_GLIDE. The XP-visualizer was used to study the Interaction of the ligands in the binding pocket of Alpha-glucosidase protein both in 2D and 3D orientations. Moreover, the standard ligand acarbose was also docked to glucosidase with XP_GLIDE for references and comparison with our turmeric phytochemicals. The SWISSADME (www.swissadme.ch/) free-sourced online tools were used to predict the Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) properties of the top 3 scoring compound as well as the standard drug, acarbose, to evaluate their pharmacokinetic performance and potential druggable properties.

3. Results

3.1. Methanolic extract yield and its phytochemicals constituents

After a maceration of the DPTR (202.33 g) in 1000 ml of methanol, the percentage yield of the methanolic Extract (which weighed 12.86 g) was 6.35%. Furthermore, the phytochemical constituents of the extracts were evaluated through qualitative and quantitative analysis, indicating the abundance of phenols, alkaloids, terpenoids, and flavonoids. Whereas the amount of tannin, glycosides, and reducing sugars was insignificant (Table 1)

3.2. In vitro inhibition of alpha-glucosidase by Methanolic Extract of Turmeric Rhizome

The methanolic Extract of Turmeric showed a dose-dependent inhibition of alpha-glucosidase activities from the concentration of 10 to 30 μg/ml of the Extract. The result of the inhibition is shown in Table 2, and IC50 was calculated to be 20.92 μg/ml.

3.3. Molecular docking of Curcuma longa phytochemical library to the binding pocket of Alpha-glucosidase

The phytochemical library constitutes 50 non-ubiquitous phytochemicals retrieved from Dr. Duke's phytochemical database. These compounds' canonical smiles, which represented their 2D structure, were processed on Schrodinger using the LigPrep tools. The alpha-glucosidase protein structure retrieved from the protein databank (unprocessed – Fig 1a) was refined by removing surrounding water molecules, modeling the missing loop and chain, and creating a disulfide bond. The quality of the final refined structure (Figure 1b) was evaluated using the Ramachandran plot (Figure 1c), having all the amino acid residue in the allowed regions, except a few glycine and proline, which can does not affect the structure quality as the outlier. The entire refined structure of alpha-glucosidase was scanned for probable binding pockets using the sitemap tools, and the site score and volume (Table 3) assigned were used to select the binding site for ligand docking. The Receptor Grid generation package placed a grid around the selected binding site (Fig 2).

The ligand library was docked onto the alpha-glucosidase binding site using the standard precision and extra-precision module of GLIDE. The top-scoring compounds (Table 4) include Guaiacol Limonene and others. Interaction of the top scoring compounds (Guaiacol) in the binding pocket of the protein was examined and found to have surface-charged Interaction (negative and positive), hydrogen bonding on Glu 792 and ILE 759, as well as pi-pi stacking (from the aromatic ring to TYR 659) (Fig. 3a.). Moreover, acarbose (a standard) was docked to alpha-glucosidase. Its Interaction in the binding pocket was examined as a comparison (Fig.3b). Our results showed that acarbose expressed a better docking score than the phytochemicals of Turmeric. However, its bulky molecular structure raises some interesting issues on its pharmacokinetics and safety profile as a drug.

3.4. Examining the ADMET properties of Acarbose and Guaiacol (Top-Scoring compound from Turmeric against alpha-glucosidase)

The adsorption, distribution, metabolism, excretion, and toxicity profile of Guaiacol and Acarbose was analyzed by the SWISSADME free-sourced online tools. The results on their physiochemical properties, drug-likeness, and pharmacokinetics are presented in Table 5. The results showed that Guaiacol possesses better ADMET properties than acarbose (despite its better docking score).

4. Discussion

Diabetes mellitus is one of the top four non-communicable diseases, requiring immediate attention from medic, paramedics, and other health professionals [3]. Its global high mortality rate as well as numerous complications/comorbidities, have raised the urgency for the development of improved diagnostics and effective therapeutic options with minimal or no side effects [12]. Many drug acts as inhibitors or moderators of enzyme activities involved in many physiological and disease states, such as diabetes. The alpha-

glucosidase enzymes, amongst others, foster the breakdown of carbohydrates to simple sugar (glucose) in the blood, possibly beyond what the body can accommodate in a diabetic state [13].

The use of medicinal plants for the treatment of different diseases has been since antiquity, and their bioactive phytochemical compositions are the foundation of the development of modern medicine [14,15]. Turmeric (Curcuma longa), among other medicinal plants, is known for its diverse biological and potential therapeutic activities against numerous disease conditions. However, its potential against diabetes has not been fully explored, especially in computational-assisted drug discovery (from its library of phytochemicals) [16]. This study, therefore, provides a preliminary investigation (*in vitro* and *in silico*) of bioactive components of the turmeric rhizome against alpha-glucosidase enzymes. We performed *in vitro* inhibition studies with the methanolic Extract of the pulverized rhizome of Turmeric against glucosidase enzyme. More so, a library of phytochemicals developed from several online repositories (Dr. Duke, IMMPAT, and LOTUS) was screened against the alpha-glucosidase crystal structure using a molecular docking approach for potent inhibitors.

On extraction of the DPTR with methanol, an excellent percentage yield of about 6.35% was achieved. Methanol has been widely used as an extractant in many studies with medicinal plants. As a polar solvent, just like ethanol and water, it extracts majorly polar phytocompounds with the potential to form transient Interaction with amino acid groups with the enzyme's binding site, thereby possibly fostering inhibition [17]. Although non-polar extractants such as n-hexane have been reported in other studies, their application for real-life applications is challenged by numerous bioavailability/accessibility of their Extract [18,19]. By adopting preliminary qualitative phytochemical analysis as well as quantitative analysis through spectrophotometric assays, we found the methanolic Extract of *Curcuma longa* (MECL) has a high abundance of phenols, flavonoids, terpenoids, and alkaloids (Table 1). Our finding was in correlation with an older study [20,21].

Furthermore, on analysis for alpha-amylase inhibition by MECL within a concentration of 10 – 40 μg/ml, a relatively dose-dependent inhibition was observed with an IC50 of 20.92 μg/ml and a maximum inhibition of 37.01%. Although the maximum inhibition was not so remarkable, there are possibilities of better inhibition at higher dosages (Table 2). More so, complete inhibition or elimination of the alpha-glucosidase enzyme may not be a recommendable treatment alternative, but rather a moderation of its activities, as the enzyme could have other physiological importance, such as prevention of Pompe disease (glycogen storage disease type II) [22]

In the same vein, we docked the library of phytochemicals of Turmeric to the binding pocket of alpha-glucosidase to screen for potent compounds with significant Interaction that possibly inhibit its activities. Based on the docking scores and binding energy estimated on the Schrodinger GLIDE software package, compounds were ranked, and the top 5 compounds are shown in Table 4. Guaiacol, amongst others, was the best compound of Turmeric, with potent inhibitory activities on alpha-glucoside. Although the docking score of the standard drug (Acarbose) was higher than Guaiacol, its pharmacokinetics and drug-likeness properties were questionable based on prediction performed on SWISS-ADME online tools (Table 5). On further examination of the Interaction with the binding pocket, using their 2D pictorial representation, it was observed that Guaiacol fitted correctly in the binding pocket of the enzyme with surfaced charged (positive and negative) Interaction, a few hydrogen bonds and pi-pi staking (Fig. 3a). However, acarbose had a more significant number of hydrogen bonds as well as other exposed hydrophilic groups which are usually not favorable biochemical interactions (Fig. 3b).

Moreover, Due to the lower molecular weight of Guaiacol compared to acarbose, a better gastrointestinal absorption and bioavailability score (0.55, more significant than the 0.25 threshold) was observed. Guaiacol showed no Lipinski violation and verbal violation, unlike acarbose, which had 3 Lipinski violations and a poor bioavailability score of 0.17. Based on the bioavailability score, a person will likely get at least 10% oral

bioavailability; the score should not be less than < 0.25. Other parameters, such as a blood-brain barrier (B.B.B.) permeant, were "yes" for Guaiacol and "No" for acarbose. The P-gp substrate of Guaiacol is "No, " meaning that it cannot be pumped out of the cell, while that of Acarbose is "Yes," meaning it can be pumped out of the cell by P-glycoprotein. To the best of our knowledge, there are no studies on the antidiabetic properties of Guaiacol. However, an older study by Yang, Le 2013 [23] showed the catalytic conversion of Guaiacol to catechol, and many catechol-containing compounds such as curcumin and zingerone, on the other hand, have been demonstrated to possess moderating activities on glucose metabolism [24].

5. Conclusion and Recommendations

In conclusion, the phytochemicals of turmeric rhizome have been shown to have some measure of moderation of alpha-glucosidase, which has the potential to manage diabetics. Guaiacol is a phytochemical with the best docking score and remarkable pharmacokinetic properties. Although acarbose (the standard drug) had a better docking interaction than Guaiacol (a phytochemical of Turmeric), Guaiacol has better ADMET properties and is less toxic than acarbose, which makes it an excellent compound for further alpha-glucosidase inhibition studies. Hence, we recommend a more thorough investigation into the activities of Guaiacol (both *ex vivo* and *in vivo*)

Table 1. Qualitative and quantitative phytochemical constituents of methanolic Extract of dried pulverized turmeric (*Curcuma longa*) rhizome.

S/N	Phytochemical Classes	Qualitative Analysis	Quantitative Analysis
1.	Phenols	+++	790.32 ± 129.20
2.	Flavonoids	++	171.08 ± 0.04
3.	Alkaloid	+++	494.99 ± 1.27
4.	Tannin	+	9.52 ± 6.59
5.	Reducing Sugar	-	23.40 ± 7.74
6.	Glycosides	-	0.00 ± 0.00
7.	Steroids	+	3.40 ± 0.00
8.	Terpenoid	+++	131.99 ± 6.59

Abundant (+++); Moderate (++); Trace (+); Absent (-).

Table 2. Mean inhibition of alpha-glucosidase activity by *Curcuma longa* Extract.

Concentration (µg/ml)	% Inhibition ± SEM
10	19.14± 0.46
20	22.29± 0.09
30	37.01± 0.28
40	31.23± 0.16
1C50 - 20.9184	

Table 3. Prediction alpha-glucosidase crystal strucure (PDB: 3W37) binding sites and their resepective site scores and volume.

Site Number	Site Score	D-score	Volume
Site 1	1.031	1.031	144.746
Site 2	0.937	0.886	166.698
Site 3	0.853	0.796	192.08
Site 4	0.692	0.643	149.548

Table 4. Top Scoring phytochemicals of *Curcuma longa* and the standard repurposed Drug (Acarbose).

S/N	Phytochemical compounds	Entry ID	Canonical SMILE	Docking Score
1.	Guaiacol	CID 460	COC1=CC=CC=C1O	-5.266
2.	P-Tolyl - Methyl	CID 110953	CC1=CC=CC=C1C(C)O	-3.939
3.	Limonene	CID 22311	CC1=CCC(CC1)C(=C)C	-3.702
4.	Quercetine	CID 5280343	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O	-3. 256
5.	Azulene	CID 9231	C1=CC=C2C=CC=C2C=C1	-3.215
6.	***Acarbose**	CID 41774	CC1C(C(C(C(O1)OC2C(OC(C(C2O)O)OC3C(OC(C(C3O)O)O)CO)CO)O)O)NC4C=C(C(C(C4O)O)O)CO	-9.522

* - Standard drug.

Table 5. ADMET prediction guaiacol and acarbose.

PARAMETERS	GUAIACOL	ACARBOSE (STANDARD DRUG)
Physiochemical Properties		
Mol. Weight (g/mol)	124.14	645.60
Num. Rotatable bond	1	9
Num. H – bond acceptors	2	19
Num. H – bond donor	1	14
Molar refractivity	34.96	136.69
TPSA (A^2)	29.46	321.17
Drug – Likeness		
Lipinski violations	0	3
Verber violations	No	Yes
Bioavailabilty score	0.55	0.17
Pharmacokinetics		
GI absorption	High	Low
BBB permeant	Yes	No
P – gp substrate	No	Yes
Log K_p (cm/s)(skin permeation)	-6.12	-16.29

ADMET - Absorption, Distribution, Metabolism, Excretion, and Toxicity; **BBB** - Blood Brain Barrier; **GI** - Gastrointestinal; **TPSA** - Topological polar Surface Area; **P – gp** - P – glycoprotein.

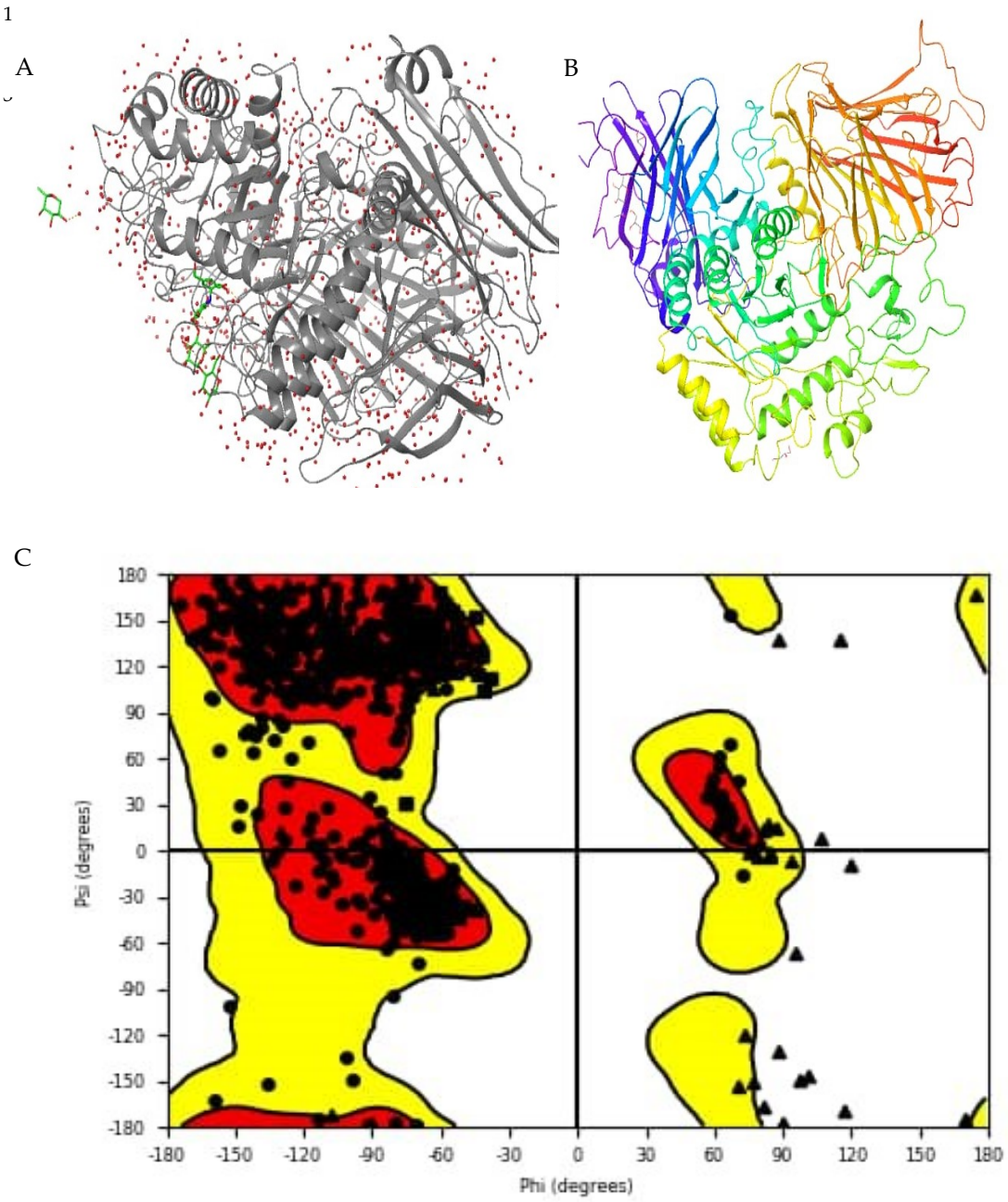

Figure 1. – Processing the PDB crystal structure of alpha-glucosidase enzyme. A) The figure of an unprocessed protein structure, with the red dots representing the non-interacting water molecule. B) The figure of the processed structure of the Human AGCS from the Protein Data Bank (ID – 3W37). The non-interacting water molecule was removed in this processed structure, and the missing hydrogen was added. C) Ramachandran plot for the processed structure of Human alpha-glucosidase (PDB ID – 3W37). Each black dot represents an amino acid of the protein (3W37). The few dots in the yellow segment are good Ramachandran points, the black point in the red segment is best Ramachandran favored, while those on the white segment are the outliers.

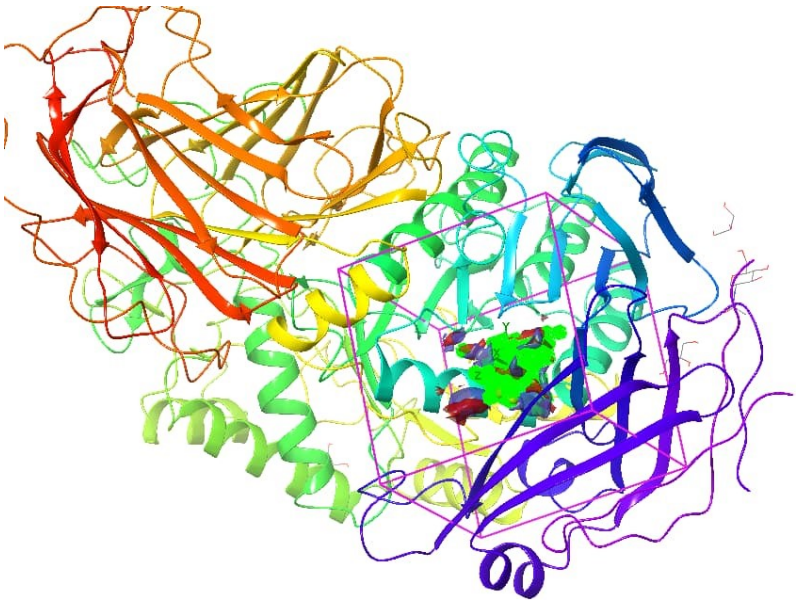

Figure 2. Cartoon representation of the Human alpha-glucosidase, with a purple grid box around the perimeter of the binding site. The properties of the binding site are represented by the red, blue, green, and yellow patches. The red patch represents the Hydrogen bond acceptor group. The blue patch represents the Hydrogen bond donor group, the green patch represents the hydrophilic region, and the yellow patch represents the hydrophobic region.

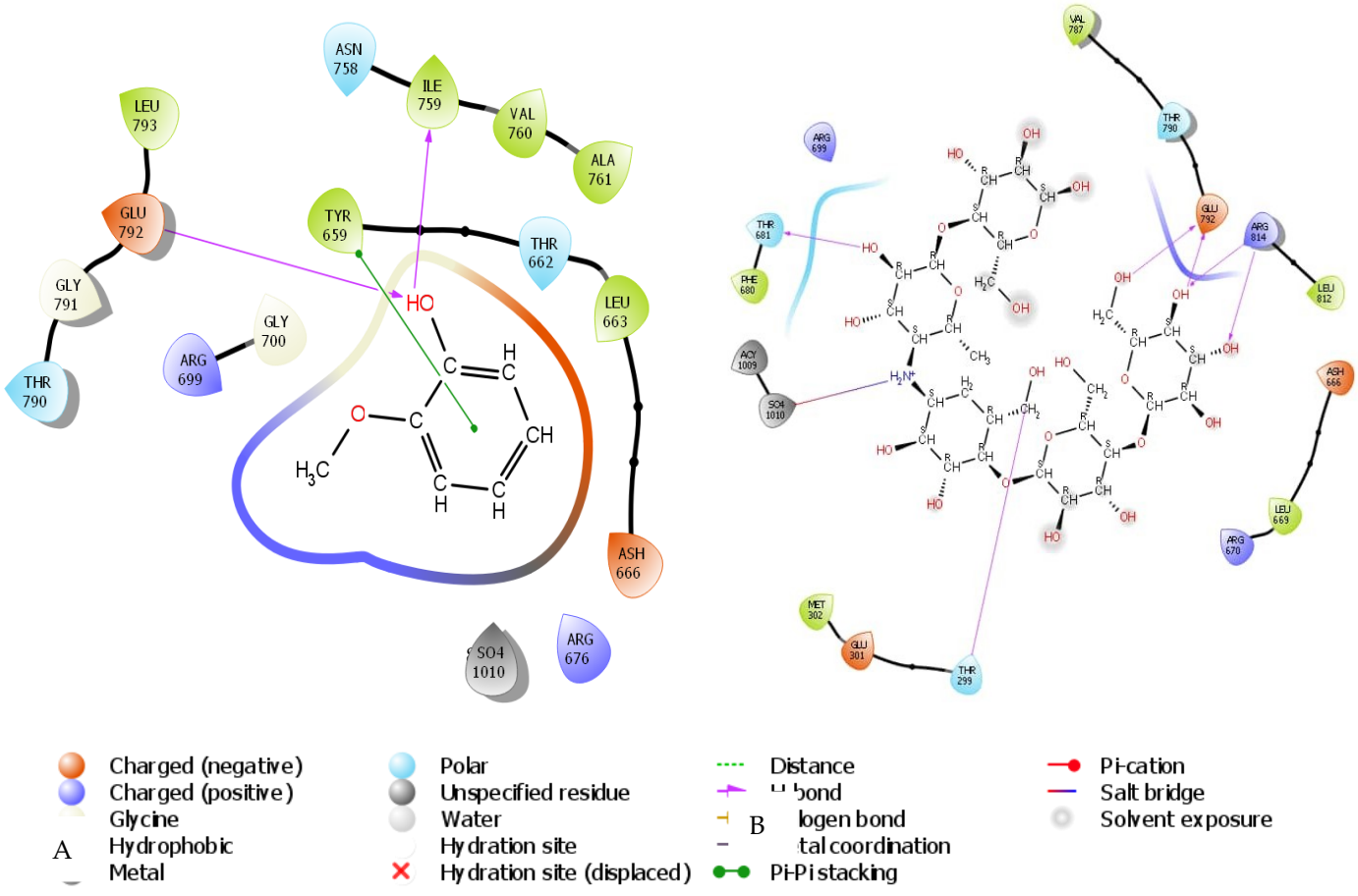

Figure 3. - Two-dimensional (2D) representation of the Interaction between Guaiacol (A) and acarbose (B) with the amino acid residues in the binding pocket alpha-glucosidase (PDB ID – 3W37).

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