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Exploring the molecular mechanism of interaction and inhibitory potential of *Capparis spinosa* L. phytoconstituents on diabetes-related targets.

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ABSTRACT

Capparis spinosa L. is a useful medicinal herb that has attracted attention of researchers over the years due to its numerous bioactive components and pharmacological activities. Prominent among the biological activities of this herb is antidiabetic and hypoglycemic effect. The current study evaluates the precise molecular interaction and inhibitory potential of *C. spinosa* phytoconstituents on human α-amylase and α-glucosidase as diabetes-related targets using *in silico* modelling and computational procedures. Among the phytoconstituents studied, we found naringin and rutin having the highest affinities to both enzymes. However, other phytoconstituents may also work together to synergistically potentiate the antidiabetic activity. Estimated binding energies of the phytochemicals, which ranged from -5.2 kcal/mol to -10.8 kcal/mol, were comparable to that of control ligand (acarbose). Varied degree of hydrophobic interaction and numbers of hydrogen bond were established by all the phytoconstituents under this study at the substrate binding site of the enzymes. Molecular interaction analysis showed α-amylase amino acid Trp59, Gln63, His101, Arg195, Asp197, Lys200, Glu233, His299, Asp300, His305 and His309 as well as α-glucosidase His105, His112, Lys156, Gln170, Asp172, Arg200, Asp202, Leu227, Gly228, Glu271, His280, Leu300, Asn301, His332, Asp333, Val335, Tyr389 and Arg400 at the active site as essential residues for hydrophilic interaction with the phytochemicals. In addition, π-π interaction was also predicted as one of the possible contributory molecular forces stabilizing the enzyme-ligand complexes observed in the study. Based on the binding affinity, it is suggested that rutin and naringin contribute immensely to the enzymes inhibition. The other phytoconstituents, which can also penetrate into the active site of the proteins, displayed similar competitive mechanism of inhibition while the target-ligand complex stability was maintained by various molecular interactions. In summary, this research provides insight into the underlying mechanism and molecular basis for the antidiabetic and hypoglycemic effects of *C. spinosa*. Such mechanism includes inhibition of α-amylase and α-glucosidase while rutin and naringin, which are found in the leaves and fruits of the herb, appear as the critical antidiabetic phytochemicals of the plant. Our study corroborates the previous reports which claim that the antidiabetic effect of the herb is conferred on it by its phytoconstituents and thus, validates the local use of *C. spinosa* in the management of diabetes.

Keywords: *Capparis spinosa*, α-amylase, molecular interaction, α-glucosidase, molecular docking.

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INTRODUCTION

The act of using locally available medicinal plants in the management of various ailments plighting human health dated back into decades. *Capparis spinosa* L. is one of the important plants whose traditional application in the treatment of diverse type of human diseases has been known since antiquity [1-3]. *C. spinosa* L. (Capparaceae) (Figure 1) is a perennial winter-deciduous plants that belongs to the genus Capparis, which is known to be made up of 250 different species [4-6]. The plant is widely found across the globe ranging from Europe, Asia, Madagascar, Australia to Africa [5]. This herb, which is commonly called Carper, is also distributed in various locations including rocky areas, in drylands, and deserts of the Mediterranean environment, Iran, Crimea, Pakistan, Armenia as well as India [4,5,7]. Peculiar characteristics of this plant include its showy/pinkish-white hermaphrodite flowers and its fleshy alternate leaves [5, 8, 9].

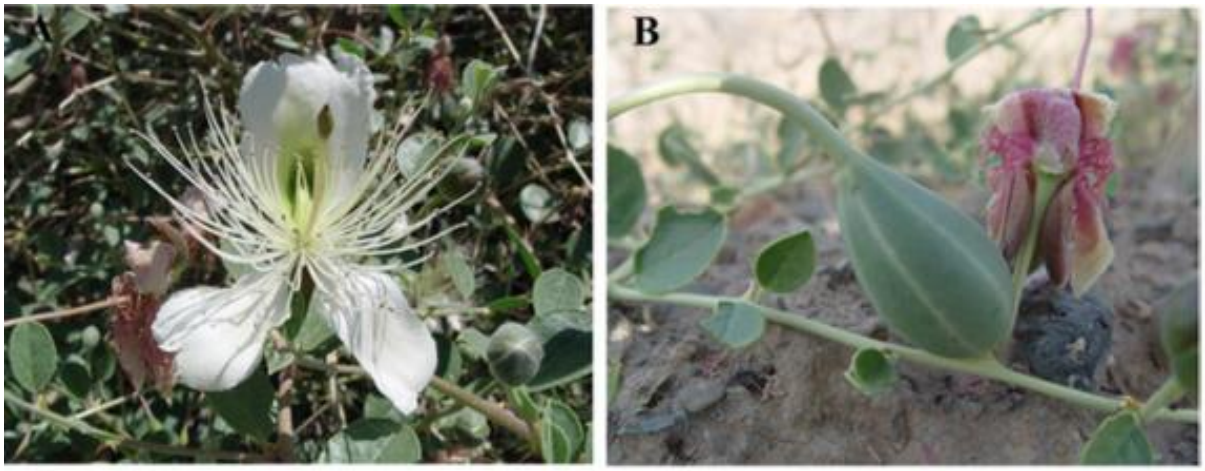

Fig. 1. Morphology of aerial parts of *Capparis spinosa*. A: The flower and leaves; B: the fruit [10].

Gradually over the years, a high medicinal and economic value has been recognized for *C. spinosa* in diverse traditional medicines among people like the Chinese, Iranian, Ayurvedic, Greco-Arabian and Unani. In Africa, the herb is popularly known for its edible fruit and bud (caper berry) [1-5]. Meanwhile, the various parts of the plant are considered as a very essential source of medicine worldwide [8]. For instance, decoctions made from the root bark of *C. spinosa* are employed traditionally to treat anemia, arthritis, dropsy and gout while the stem bark extract is reported to be diuretic [11]. The leaves, buds and whole roots are also used in the treatment of skin diseases, kidney, liver diseases as well as gastrointestinal disorder. The fruits are particularly used for the treatment of diabetes, fever, headache and rheumatism [5, 7, 12]. The root cortex of *C. spinosa*, having bitter taste, is also known for its analgesic and carminative properties. This part is also useful as appetizer, tonic, antidiarrheic, astringent in addition to its potential to treat hemorrhoids, rheumatism and spleen disease [8]. As food source, the young shoots, buds and fruits (unripe or ripe) of *C. spinosa* are consumed as parts of soups, sauces, jam and salads condiment as well as in other foods in the Mediterranean region [5, 6]. Therefore, *C. spinosa* has many culinary uses and these discoveries altogether made this plant an interesting field for research.

The presence of phytochemicals in abundance has been documented for *C. spinosa* mostly polyphenolics such as flavonoids and phenolic acids [13, 14]. In 203, Zhou and co-workers [15] reported the presence of biflavonoids such as ginkgetin and isoginkgetin from the plant in addition to a flavonoid (sakuranetin). The plant is also a very good source of alkaloids and glucosinolates (glucocapparin, glucocleomin, glucoiberin, glucocapangulin, sinigrin and glucobrassicin) [16]. As an aromatic plant, strong flavor of *C. spinosa* has been attributed to its content of methyl isothiocyanate, a very pungent chemical which is usually released after enzymatic reaction involving the mustard oil glycoside named gluco-capparin (methyl glucosinolate) [15-18]. The phytochemicals identified in *C. spinosa* are believed to provide the health-improving benefits associated with the plant due to their various biological activities. In general, biological efficacy of a plant usually depends on the type of phytoconstituents found in such plant. The herb possesses a wide range of pharmacological activities. Its antioxidant, antifungal, anticancinogenic, anti-inflammatory,

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antihypertensive, antihepatotoxic, inhibition of NF-kappa B, antidiabetic and hyperlipidemic, anthelmintic, anti-obesity, counter-irritant, antimicrobial, antimutagenic, anti-nociceptive effects have been extensively documented [4, 5, 7-9, 13]

The predictions by International Diabetes Federation and World Health Organization (WHO) which suggest a growing burden of diabetes, with a projection that the prevalence of diabetes mellitus amongst people between 20–79 years will rise from 285million in 2010 to 439million by 2030 [19, 20], has necessitated rigorous research into newer and more affordable medications with possibly less toxic effects for diabetes management. Diabetes is a disease characterized by elevated blood glucose level. The body of a diabetic person either does not produce insulin in sufficient amount or is unable to effectively make use of its own insulin. Since there is no current, less invasive cure for diabetes, one of the strategies employed in the management of the disease is inhibition of saccharide hydrolyzing enzymes such as α-amylase and glucosidase with the aid of oral hypoglycemic drugs such as acarbose aimed at regulating blood glucose level particularly in patients with type-2 diabetes mellitus [21]. This strategy is simply based on the fact that inhibition of these enzyme hold-up digestion of carbohydrate in the body and therefore extend overall carbohydrate digestion time. The net effect is a reduction in the rate of glucose absorption and, hence, lowering the postprandial plasma glucose upsurge [22]. Over the decades, there have been frantic efforts geared towards discovery and evaluation of plant-derived compounds as antidiabetic agents. Bulk of such natural anti-hyperglycemic or hypoglycemic compounds are often phytoconstituents, mostly the polyphenolics [23, 24]. Numerous chemicals obtained from different medicinal plant have therefore been reported for their potentials to reduce high plasma glucose level in diabetic animal models. The demand on natural antidiabetic preparations/products is attracting interest due to the reported toxicity, undesirable side effects and high cost of synthetic oral hypoglycemic candidates and insulin [25-27].

C. spinosa is one of the antidiabetic plants used by local people around the World to manage diabetes, and for which *in vitro* and *in vivo* anti-hyperglycemic studies have been carried out. The current study is aimed at elucidating the precise molecular interaction of selected *C. spinosa* constituents with diabetes-related targets (α-amylase and glucosidase) and determine the inhibitory potential of these phytoconstituents on the enzymes. This study will contribute to the validation of the plant use in the management of diabetes as well as identification of the components with major contribution towards the antidiabetic activity of the various extracts of the plant.

MATERIALS AND METHODS

Selection and preparation of protein structures

The starting coordinate of alpha amylase and alpha glucosidase used in this study were retrieved from the Brookhaven protein data bank (http://www.rcsb.org/pdb) with PDB ID: 2QV4 and 3WY1 having resolution of 1.97 Å and 2.15 Å respectively. These crystal structures were deposited by Maurus *et al.* in 2007 [28], and Shen *et al*. in 2014 [29] respectively. α-amylase was co-crystallized with acarbose while α-glucosidase was found in complex with polyacrylic acid. The "FASTA" files (Accession: 2QV4_A GI:170785004 and 3WY1_A GI: 829581329) for the α-amylase and α-glucosidase respectively were retrieved from www.pubmed.org and used in homology modeling as done on the Swiss-Model Server (http://swissmodel.expasy.org). The active sites of the macromolecules were identified with reference to the co-crystallized ligands which were deleted, in addition to the crystallographic water molecules, from the proteins before molecular docking procedures.

Ligands preparation and optimization

A total of twenty-one (21) ligands used in this docking study were selected from the literature. Out of these compounds, twenty (20) were phytochemicals isolated from *Capparis spinosa* while the other (1) compound served as control ligand. The chemical structures of *C. spinosa* phytoconstituents: cinnamic acid (CID: 155169), rutin (CID: 5464454), naringin (CID: 161087), catechin (CID: 161259), sinapic acid (CID: 155169), vanillic acid (CID: 155169), quercetin (CID: 155169), epicatechin (CID: 155169), coumaric acid (CID: 155169), chlorogenic acid (CID: 155169), kaempferol (CID: 5280865), gallic acid (CID: 155169), *p*-hydroxylbenzoic acid (CID: 155169), 3-hydroxy, 4methyoxybenzoic acid (CID: 155169), 2,3 dimethylbenzoic acid (CID: 155169),

luteolin (CID: 5280445), 3 hydroxybenzoic acid (CID: 155169), isorhamnetin (CID: 5281654), sakuranetin (CID: 73571) and protocatechuic acid (CID: 72) were obtained from NCBI PubChem compound database (http://www.ncbi.nlm.nih.gov/pccompound) and prepared using Marvinsketch. 2D-coordinates of the ligands were sketched using ChemAxon software (https://www.chemaxon.com) and, using the Conformers suit of Marvin-Sketch, the 2D structures were converted to 3D geometry. The Merck molecular force field (MMFF94) was employed. The .sdf format of the compounds were docked into the targets using AutoDock 4.2.

Molecular docking and scoring

For ligand docking and target-ligand complex analysis, Autodock vina suite on PYMOL was used [30, 31]. First, based on the already present co-crystallized ligand in the pdb file, the inhibitor binding site was defined with grid parameters set at x=100, y=100 and z=100 while the coordinate of origin (x, y and z) was set at 12.38, 48.14 and 26.21 for α-amylase and, 19.73, -8.67 and 21.85 for α-glucosidase to include all the amino acid residues at the active site. This gives enough space to enhance adequate ligand rotation and translation. The spacing between grid points was maintained at 0.375 angstroms. All optimized ligands were docked to the active site of the proteins. While the rotatable bonds of the ligands were set to be free, the protein molecules were treated as rigid structures [32]. Throughout this *in silico* investigation, ten (10) docking runs were performed for each ligand with the number of modes set to 10 so as to achieve more accurate and reliable results.

Data analysis

The protein-ligand complexes as well as the molecular interaction were all visualized using PYMOL and snapshots were taken. Ligplot was used to depict details of protein-ligand interactions [33].

RESULTS AND DISCUSSION

This study features *in silico* experimental approach to explore the inhibitory potential, binding mode and precise molecular interaction of *C. spinosa* phytochemicals on α-amylase and α-glucosidase as diabetes-related targets. Inhibition of these enzymes, that are directly involved in dietary carbohydrate digestion, offers a unique approach for controlling postprandial glycemic reaction [11]. Acarbose, a well-known inhibitor of these enzymes, is often used in type-2 diabetes treatment to reduce postprandial hyperglycemia. It is known to delay carbohydrate digestion resulting in a reduced rate of glucose uptake and absorption [34]. Figure 2 and 3 show acarbose docked to the active site of α-amylase and α-glucosidase respectively. Acarbose, which was employed as the control ligand in this study, was found after docking experiment buried within the active site of α-amylase and α-glucosidase with energy value -7.5 kcal/mol and -7.2 kcal/mol respectively (Table 1) and established hydrogen bond interaction with catalytically-essential residues at the substrate binding pockets of the targets. Hence, acarbose possibly prevents substrate access to the binding sites. In a similar pattern, all the ligands docked in this research showed potential to bind at the active site of both α-amylase and α-glucosidase, however with varied affinities as depicted by their binding energy values (Table 1). The binding energy value (-7.5 kcal/mol) obtained for acarbose in this study is compatible with the report of Metibemu *et al*. [20]. Among the amino acid residues found within the 4 Å of α-amylase active site (Figure 4), His299, Trp59, Trp58, Glu233, Ala198, Asp197, Arg195, Tyr62, His101, Leu165, Asp300 and Gln63 were found to be significant for the inhibition of human α-amylase. For glucosidase-acarbose complex stability, amino acid residues Asn301, Leu300, Asp333, Asp202, Gln170, Arg200 and Arg400 appear to be essential. Hence, they possibly play key role in the inhibition of the enzyme. Although acarbose is effective in management of type-2 diabetes, it has been reported to exhibit adverse effects such as diarrhea, severe stomach pain and constipation [11, 20, 34]. Thus, natural product components which are expected to be relatively cheap, less harmful and pharmaceutically effective are still sought to replace this drug. Since *C. spinosa* is an herb with antidiabetic efficacy, the inhibitory potential and molecular interaction of its phytoconstituents on α-amylase and α-glucosidase were evaluated.

Out of the *C. spinosa* phytochemicals selected for this study, naringin, rutin, luteolin, quercetin, isorhamnetin, sakurantin, kaempferol and catechins displayed relatively higher affinity for α-amylase (Table 1) and showed reliable potential to block substrate binding at the active site whereas, chlorogenic acid, luteolin,

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naringin, rutin, and kaempferol displayed strong potential to penetrate α-glucosidase active site and occupy the substrate binding pocket (Figure 4 - 9).

Table 1. Molecular docking results of screened ligands.

S/No	Ligands	Diabetes-related targets	Binding energy value (kcal/mol)	Residues involved in hydrogen bonding
1	Vanillic acid	α-amylase	-5.6	Glu233, Arg195, Asp300
		α-glucosidase	-6.0	Asp202, Gly228, Arg400
2	Sinapic acid	α-amylase	-6.0	Glu233, His299, Asp300
		α-glucosidase	-6.8	His332, Gly228, Asp333
3	Rutin	α-amylase	-9.3	Glu233, Arg195, Lys200, His299, Gln63, Asp197, His305
		α-glucosidase	-9.4	Asn301, Leu300, Val335, Tyr389, Asp202, Arg400, His105
4	Quercetin	α-amylase	-9.1	Gln63, Asp197, Asp300, Tyr62
		α-glucosidase	-7.8	Arg400, Asn301, Leu300
5	*p*-hydroxylbenzoic acid	α-amylase	-5.6	Arg195, Asp197, His 299
		α-glucosidase	-6.0	Gln170, Asp202, His105
6	Naringin	α-amylase	-10.2	Lys200, His305, His309, Arg195
		α-glucosidase	-10.8	Thr310, His280, Lys156, His112
7	Gallic acid	α-amylase	-6.4	His101, Asp197, His299, Arg195
		α-glucosidase	-6.0	Arg400, Asp333, Glu271
8	Epicatechin	α-amylase	-8.6	Asp197, His299
		α-glucosidase	-7.8	Leu300, Asn301, Glu271
9	Coumaric acid	α-amylase	-6.5	Gln63, Arg195, His299
		α-glucosidase	-6.7	Gly228, Arg200, His332
10	Cinnamic acid	α-amylase	-6.0	Trp59, Gln63
		α-glucosidase	-6.6	His332, Arg200
11	Chlorogenic acid	α-amylase	-7.6	Asp197, Gln63, His299, Arg195
		α-glucosidase	-8.5	Asn301, Leu227, Glu271, Arg400, Asp333
12	Catechin	α-amylase	-8.9	Glu233, Gln63, Asp197, Arg195
		α-glucosidase	-7.4	Asn301, Arg400, Leu300
13	3-hydroxy, 4methyoxybenzoic acid	α-amylase	-5.2	His299
		α-glucosidase	-5.7	Gly170, His105
14	3-hydroxybenzoic acid	α-amylase	-5.7	Arg195, His299, Asp197
		α-glucosidase	-6.1	Arg400, His332, Asp333, Gln170, Asp172
15	2,3 dimethylbenzoic acid	α-amylase	-5.3	His299
		α-glucosidase	-5.8	Arg400
16	Kaempferol	α-amylase	-8.6	Gln63, Asp197, Arg195
		α-glucosidase	-7.6	Arg400
17	Isorhamnetin	α-amylase	-8.8	Asp197, His305, Gln63, Arg195
		α-glucosidase	-7.5	Arg400
18	Protocatechuic acid	α-amylase	-5.9	His101, Asp197, Glu233, Arg195, Tyr62, His299
		α-glucosidase	-5.8	Asp333, Glu271, Arg400
19	Sakuranetin	α-amylase	-8.9	His299, Asp300, Gln63, Arg195
		α-glucosidase	-7.5	Arg400
20	Luteolin	α-amylase	-9.1	Asp197, Gln63, Arg195, Asp300
		α-glucosidase	-7.8	Asn301, Arg400
21	Acarbose	α-amylase	-7.5	Thr163, His101, Ala106, Gly164, Gln63, Arg195, Asp300, Glu233, His201, His299
		α-glucosidase	-7.2	Arg400, Leu300, Asp202, Gln170, Arg200, Asp333, Asn301

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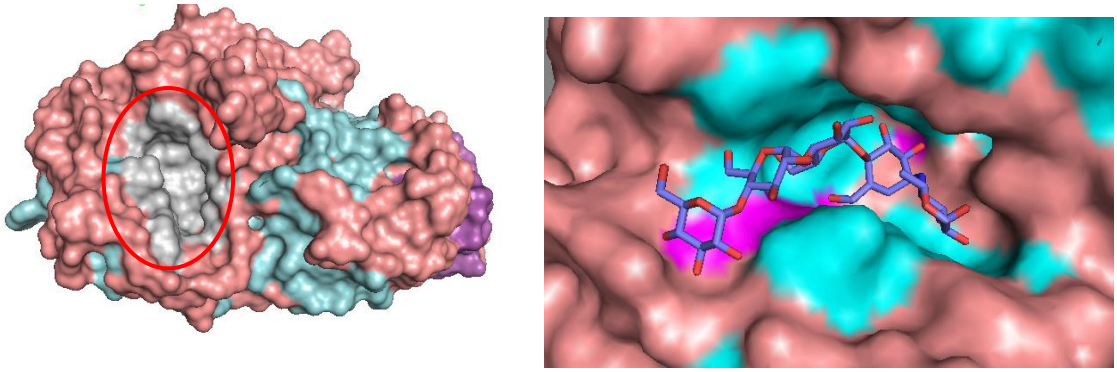

Fig. 2. Active site in surface representation of amylase (PDB: 2QV4) and stick representation of acarbose on the active site of human α-amylase.

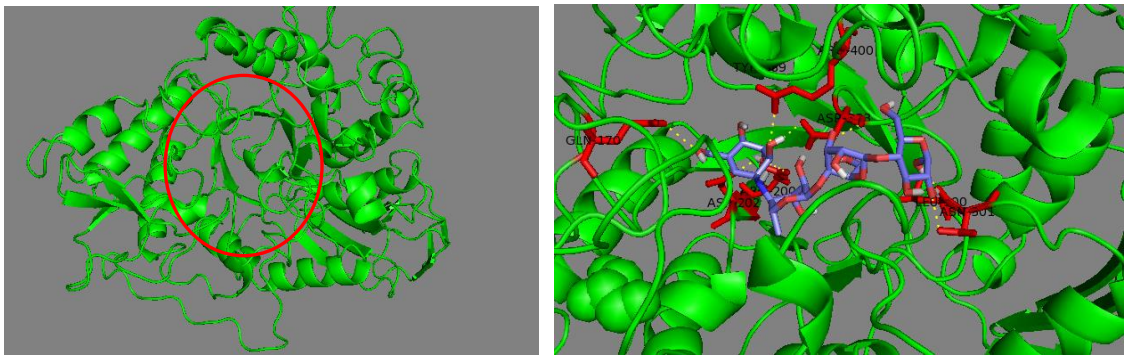

Fig. 3. Active site in cartoon representation of α-glucosidase (PDB: 3WY1) and stick representation of acarbose on the active site. Amino acid residues forming hydrogen bond with acarbose are shown as red sticks.

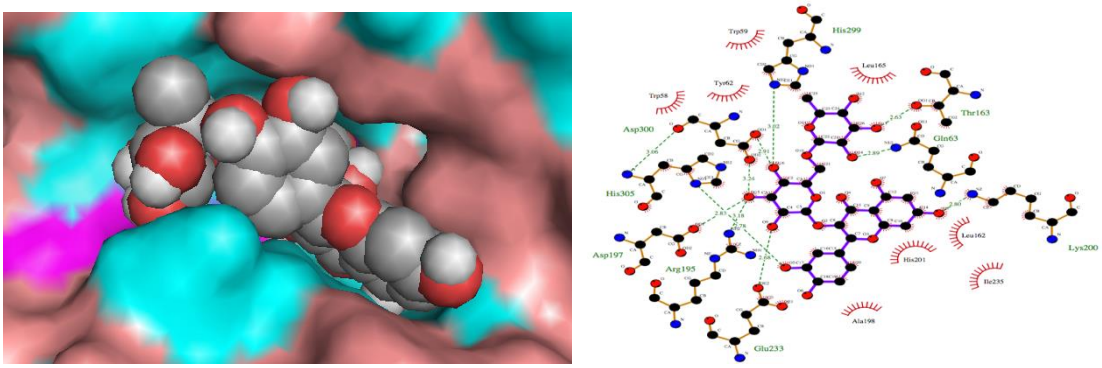

Fig. 4. Rutin, shown as sphere, occupying active site of human α-amylase and its molecular interaction analysis

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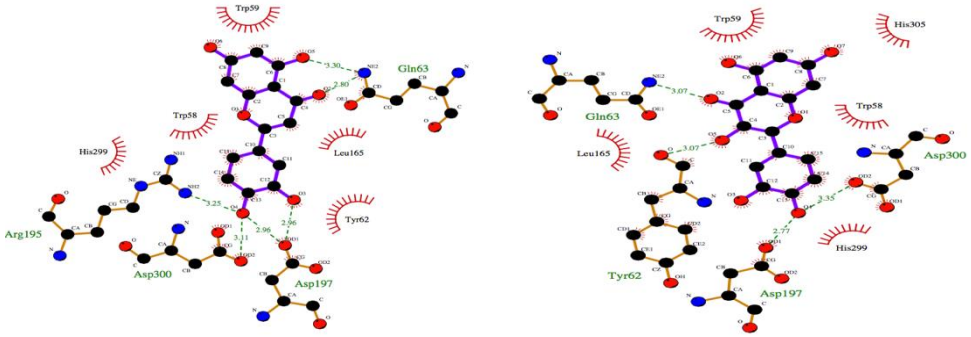

Fig. 5. Molecular interaction analysis of luteolin and quercetin with human α-amylase at the active site. Molecular interactions, as depicted by LigPlot presentations, are mediated by hydrogen bonds and by hydrophobic interactions. Hydrogen bonds are represented by green dashed lines between the atoms in figures and hydrophobic contacts are shown in red arcs with spokes radiating towards the ligand atoms [33].

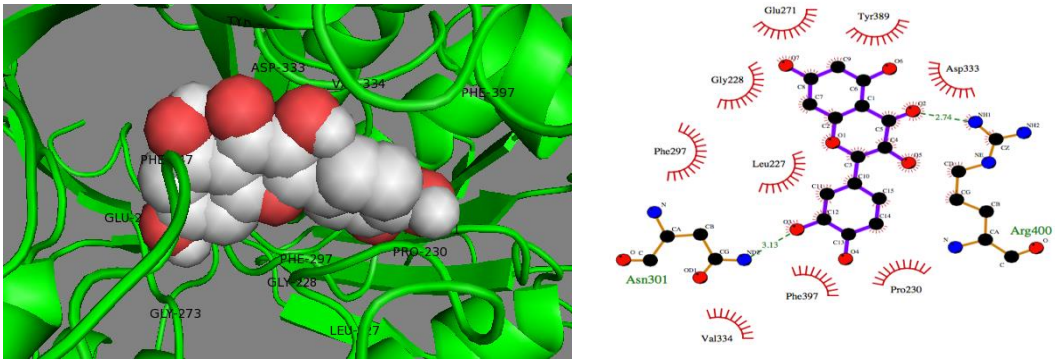

Fig. 6. Quercetin binding and its molecular interaction analysis on active site of human α-glucosidase

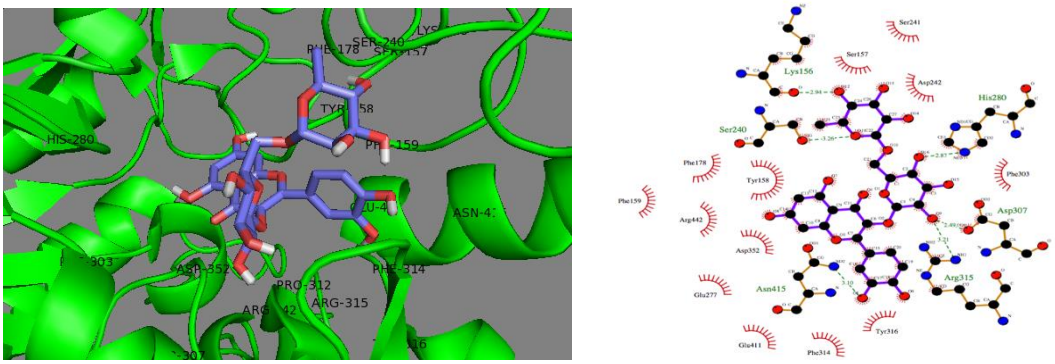

Fig. 7. Rutin binding and its molecular interaction analysis on active site of human α-glucosidase.

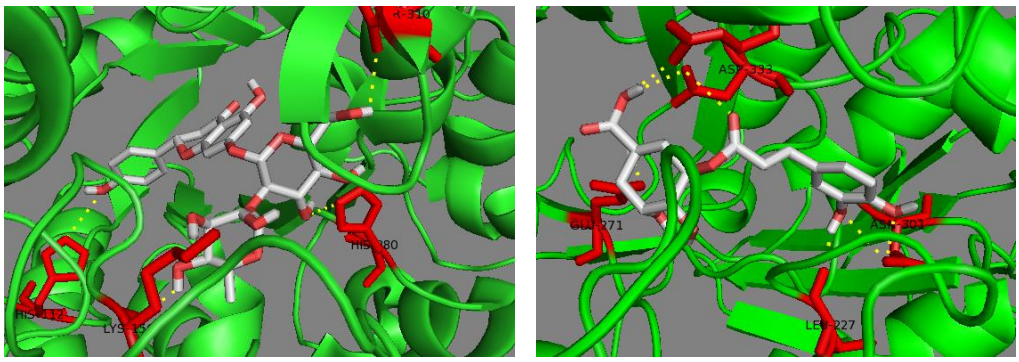

Fig. 8. Naringin and chlorogenic acid binding analysis and interaction on α-glucosidase.
Amino acid residues forming hydrogen bond with the phytochemicals are presented as red sticks.

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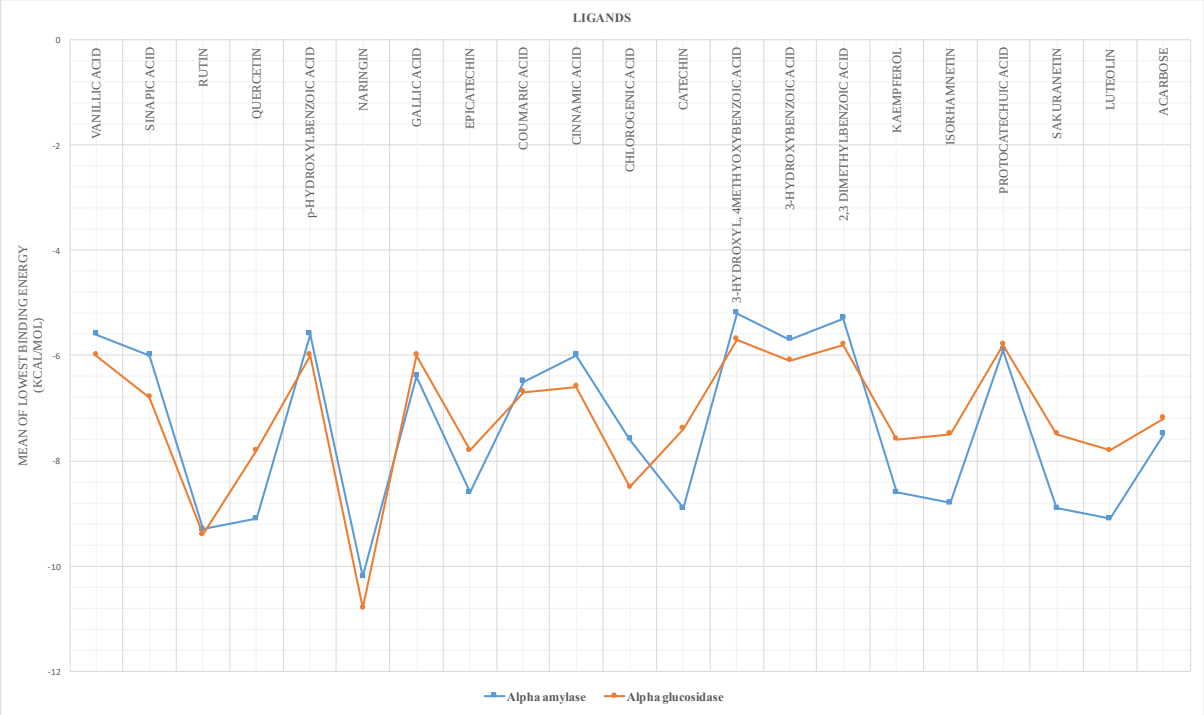

Fig. 9. Comparing the mean of lowest binding energy of ligands against α-amylase and α-glucosidase.

Earlier, Wei and colleague reported the inhibitory potential of naringin on α-amylase *in vitro* [35]. In this study, we report the lowest binding energy value (-10.2 kcal/mol) for naringin, one of the known *Citrus* flavonoids, against human α-amylase. This phytochemical is reportedly present in the leaves, bud and fruits of *C. spinosa* [36]. Evidence abound to show the potency of various extracts of these parts of the plant in management of diabetes. The possible key role of naringin in the antidiabetic activities of *C. spinosa* is hence suggested in this study. Interestingly, our result is in agreement with the report of Liu and coworkers who observed that naringin (as well as other Citrus flavonoids) can bind with α-amylase to form a new stable complex with relatively strong binding affinity [37]. According to their reports, the interaction for the Citrus flavonoids and α-amylase was measured by surface plasmon resonance.

Rutin showed the tendency to completely occupy α-amylase active site (Figure 4) and possessed relatively low binding energy (-9.3 kcal/mol), thus high affinity for the active site. The bioflavonoid displayed complex hydrophilic interaction with the protein (Figure 4). These bonds may account for the strong interaction observed between amylase-rutin complex in this study. The great amount of rutin in the fruits, leaves and bud of *C. spinosa* was recently reported by Mollica *et al*. [36]. Hence, it is suggested from our results that rutin contributes in an immense way to the antidiabetic effects of the plant. Luteolin, quercetin and kaempferol are also good inhibitors of the enzyme with binding energy -9.1 kcal/mol, -9.1 kcal/mol and -8.6 kcal/mol respectively. This observation is in agreement with the reports of Tadera *et al*. [38] and Ming *et al*. [39]. As shown in Figure 5, luteolin which is another active component of *C. spinosa* also interacted via hydrogen bond formation with one or more of the three important residues Asp197, Glu233 and Asp300 on human α-amylase active site, thereby interrupting substrate binding with a resulting inhibition of enzyme catalysis. Amino acid residues Asp197, Glu233 and Asp300 have earlier been identified as the essential three residues found in putative amylase active site located in conserved domain A of the enzyme [41, 42]. In addition, both ligands established hydrogen bond with amino acid residue Gln63. It is also worthy to note the contribution of hydrophobic interaction towards the stability of enzyme-ligand complex as seen in the present study. Few residues of the targets such as His201, Trp59 and Asp333 of amylase appear to involve in hydrophobic bonds. The low binding energy values for the ligands, as obtained in this work, indicate the strong binding affinity of the phytoconstituents for the target proteins [20, 39]. This molecular docking scores clearly suggest that these Caper-derived components possess inhibitory activity that is relatively similar to that of the

control ligand on the enzymes. The results of the current investigation are consistent with previously reported experimental results [11, 36, 41]. Metibemu *et al.* [20] had reported similar findings for isorhamnetin obtained from *Corchorus olitorus* leaves and compared its potential to inhibit the enzymatic activity of α-amylase with acarbose *in silico*. This indicates the possible efficacy of these phytoconstituents in diabetes management.

The inhibitory potential of the *C. spinosa* phytochemicals against α-amylase in decreasing order, can be summarized as naringin > rutin > luteolin = quercetin > isorhamnetin > kaempferol > sakuranetin = catechin > isorhamnetin > epicatechin > chlorogenic acid. With a competitive mode of inhibition, the phytochemicals inhibit the catalytic activity of the enzymes with a net effect of reduction in glucose absorption; a desirable effect in hyperglycemia management [38]. Observations made from current study are compatible with claims from previous studies that various preparation of *C. spinosa* showed antidiabetic activities. Selfayan and Namjooyan [11] reported that the ethanolic extract of root and leaves of *C. spinosa* possessed inhibitory activity on pancreatic α-amylase *in vitro*. Eddouks and colleagues [42, 43] observed the antidiabetic properties of the aqueous extract of *C. spinosa* powdered fruits *in vivo* while Hashemnia and co-workers [44] demonstrated the beneficial effect of the plant alcoholic extract for treating hyperglycemia in experimental animal models.

The relative consistency of interaction with residue Gln63 by the phytochemicals is worth noting. Structurally, amino acid Gln63 is located on one of the two segments that form domain A of α-amylase. This domain is significant for enzymatic activity of human alpha amylase [39, 40]. The glutamine displays its amide at the end of its side chain and this amide could form chains of H-bonds that might provide stabilization between the enzyme-ligand complex. According to this study, this amine group of glutamine readily interacts with the carbonyl group the rutin, luteolin, sakuranetin, isorhamnetin, kaempferol and quercetin. The hydrogen bond between Gln63 and the carbonyl oxygen of the aromatic ring of the ligands may significantly contribute to the high affinity and hence, inhibitory potential of the phytoconstituents. In this evaluation, hydrogen bond formed between this glutamine residue and phytoconstituents was observed playing a key role in their intermolecular association [45]. This indicates the importance of hydrogen bond in stabilizing the protein-ligand interactions. Hydrogen bond is known to play key role in structural stability of many biological molecules as well as in enzyme catalysis [45]. Taken together, the stability of the amylase-ligand complex was achieved by the contribution of amino acid residues found within the 4 Å region. The aromatic part of the flavonoid in the α-amylase active site also permit π-π interaction with Trp59 [20, 46, 47]. This interactions, coupled with electrostatic attractions occurring between the active site amino acid residues of the enzyme might account for the high binding affinity and greater inhibitory effect of the flavonoids on the enzyme [20, 39, 40, 45-48].

Results for the molecular interaction of *C. spinosa* phytochemicals with α-glucosidase is presented in Table 1. Quercetin displayed binding energy -7.8 kcal/mol and formed hydrogen bond with residues Leu300, Asn301 and Arg400 while the estimated binding energy for rutin was -9.4 kcal/mol. In addition to the hydrogen bond seen with glucosidase-quercetin complex, rutin established hydrophilic interaction with residues His105, Tyr389, Val335 and Asp202. For luteolin, hydrogen bond was found with residues Asn301 and Arg400. The consistency of these residues in the glucosidase-ligand interaction suggests their key role in the enzyme inhibition. Compared to acarbose with energy value of -7.2 kcal/mol, these phytochemicals may be considered as potent inhibitor of the enzyme. Previous reports have suggested that acarbose is an alpha-glucosidase inhibitor with inherent potential to delay or even prevent development of diabetic complications [49, 50]. Figure 6-8 revealed that quercetin (-7.8 kcal/mol), rutin (-9.4 kcal/mol) and luteolin (-7.8 kcal/mol) possess the capacity to bind at the active pocket of the enzyme, thereby inhibiting the activities of the protein. Although naringin was predicted to possess highest binding affinity to the enzyme (-10.8 kcal/mol), its binding location appears tilted compared to that of rutin and acarbose as it engaged residues His280, Lys156, His112 and Thr310 in hydrophilic interaction. The ability of naringin to alter glucose-regulating enzyme activities has been reported to play a crucial role in the glucose-lowering effect of this compound in experimental animals [51, 52]. Ability of *C. spinosa* phytoconstituents to penetrate the hollow passage that leads to the active pocket of α-glucosidase may have enhanced their inhibitory effect on the enzyme. These results support the hypothesis that α-glucosidase enzymatic activity is altered by *C. spinosa* constituents; a contributing factor in the anti-hyperglycemic effect of the herb. Our analysis is thus compatible with previous wet experiment results [53-55]

According to this study, rutin and naringin were observed to interact with both enzymes with strong affinity (Figure 9). However, previous reports had claimed a high quantity of rutin in the herb compared to naringin. This suggests that these phytochemicals, especially rutin, might contribute majorly to the antidiabetic effects of *C. spinosa* via inhibitory activities on α-amylase and α-glucosidase. Since naringin, rutin and other phytochemicals have been identified at the leaves, flower and bud extracts of *C. spinosa*, the use of these parts of the plant for diabetes treatment is therefore validated. This study, therefore, corroborates recently reported findings obtained from *in vitro* and *in vivo* experiments [39].

CONCLUSION

Management and prevention of human diseases such as diabetes with the use of natural products and medicinal plants like *C. spinosa* may be relatively beneficial with less adverse effects compared to synthetic pharmacological agents. According to this study, the selected phytoconstituents of *C. spinosa* are effective inhibitors of human α-amylase and α-glucosidase which may be explored in the treatment of type-2 diabetes. Detailed observation of the ligand-receptor interaction exposed the fact that the ligands obviously entered into the putative binding site of the enzymes required for conversion of substrate to products and exert their inhibitory effects competitively thereby preventing substrate binding which, of course, may result in lowering of the elevated blood glucose level in diabetes. The formation of hydrogen bonds and other important interactions between the residues and the ligands at the active site possibly contribute to the inactivation of the enzymes, hence justifying the widely acclaimed blood glucose lowering effect of *C. spinosa*. Among the compounds, rutin and naringin are very potent inhibitors compared with the synthetic drug (acarbose) currently used in control of hyperglycemia associated with type 2 diabetes mellitus. These phytochemicals are predicted to work synergistically to give *C. spinosa* its antidiabetic properties. Understanding the precise interaction and enzymatic inhibition on α-glucosidase and α-amylase thus provide a possible explanation for the molecular basis and mechanism of the antidiabetic activity of the herb, which is shown to be related to the phytoconstituent content. Hence, the use of the plant in the management of type-2 diabetes is thereby justified.

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Authors contribution:
All authors contribute equally to this work.

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