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Antiglycaemic potentials and molecular docking studies of the extracts of *Cassia alata* L.

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Abstract

Background: This study assessed the antiglycaemic properties of the methanolic extracts of the dried leaf of *Cassia alata* L. through in vitro and in silico approaches. The methanolic crude extract (MCE) of the dried leaf was prepared and partitioned into n-butanol (BPE) and aqueous (ARE) extracts. The antiglycaemic potential was assessed in vitro by studying the inhibitory actions of the extracts with α-amylase, α-glucosidase, and sucrase. The antiglycation capacity of the extracts was evaluated through the inhibition of albumin glycation, thiol oxidation, and β-fibril formation. Previously identified compounds (emodin, quercetin, chrysoeriol, and kaempferol) were docked with α-amylase (1HNY), α-glucosidase (5ZCB), and sucrase-isomaltase (3LPO) using the Pyrex Virtual Screening tool.

Results: The results revealed that MCE had the highest inhibitory potential with the lowest IC_{50} values of 69.67 ± 0.88, 65.54 ± 0.34, and 48.35 ± 1.45 μg/mL for α-amylase, α-glucosidase, and sucrase inhibitions, respectively. The molecular docking studies showed that quercetin and kaempferol had the best docking scores with 1HNY while emodin and chrysoeriol had the best scores towards 5ZCB and 3LPO. MCE and BPE significantly ($p < 0.05$) inhibited glucose-induced albumin glycation and modification.

Conclusion: This study suggested that the extract of the leaf of *C. alata* could contain a mix of different phytochemicals that could be beneficial in reducing the absorption of glucose and preventing diabetes-induced complications.

Keywords: *Cassia alata* L., Diabetes mellitus, Hyperglycaemia, Antiglycation, Molecular docking

1 Background

Diabetes mellitus is a major metabolic disease caused by an absolute or partial lack of insulin activity mostly characterized by disorders in the regulation of carbohydrate, lipid, and protein metabolisms. The disease is usually associated with high levels of blood glucose and formations of glycated compounds known as advanced glycation end products. Pathologically, diabetes mellitus has been implicated in the pathogeneses of long-term complications such as atherosclerosis, retinopathy, nephropathy, neuropathy, and microangiopathy caused by

the elevated conversion of glucose to lipids, accumulation of sorbitol, and glycation of proteins [1, 2]. Several synthetic agents used in the treatment of diabetes mellitus include acarbose (an inhibitor of carbohydrate-digesting enzymes and brush border enzymes) and tolbutamide (an agonist of sulfonylurea receptor). While some of these agents achieve transiently controlled blood sugar levels, they exert a number of adverse effects such as induced hypoglycaemia, hepatotoxicity, cardiac failures, and cholestatic jaundice [3–5]. With the introduction of intensive insulin therapy, which is increasingly accepted as the best form of treatment for tight control of blood glucose, the clinical symptoms could only be overcome particularly in young patients. Taking

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into consideration the associated contradictions of some of the synthetic drugs, research along alternative medicine is evolving for the treatment of metabolic diseases as these plants are known to contain substantial quantities of bioactive compounds that could be responsible for the anticipated health benefits. Besides, an innumerable number of the population across the world use products derived from medicinal plants for the management of several ailments that take after the symptoms of diabetes mellitus.

Thus, this study aimed at assessing the in vitro inhibitory effects of extracts of dried leaves of *Cassia alata* L. (Leguminosae-Caesalpiniaceae) on carbohydrate-metabolizing enzymes and albumin glycation properties. This present study also analysed the binding behaviour of the bioactive components of the aerial parts of *C. alata* with amylase, glucosidase, and sucrase.

2 Methods

2.1 Sample preparation
Fresh leaves of *C. alata* were air-dried at room temperature of $29 \pm 1\,°C$. The sample was authenticated by the Technologist in Charge of Biochemistry Laboratory, Faculty of Science, Kings University, Nigeria. The dried leaves were pulverized, and 10 g of each pulverized sample was extracted with 100 mL of methanol at room temperature of $29 \pm 1\,°C$ for 24 h and later filtered. Two third of the crude extract was partitioned repeatedly inside a separating funnel into an aqueous extract and n-butanol extract. The crude extract and the partitioned extracts were used as the corresponding extracts for the subsequent analyses.

2.2 Preparation of the crude α-glucosidase and sucrase solution
The preparation of the crude α-glucosidase and sucrose solution was carried out as described by Dahlqvist [6]. The animal used was handled under the approved guidelines of the Ethical Review Committee of the College of Natural and Applied Sciences, McPherson University, Seriki Sotayo, Nigeria. The mucosa of the small intestine of rats sacrificed under light anaesthesia was carefully scraped off with a glass slide, homogenized with cold sodium phosphate buffer (pH 6.8), and centrifuged at $4\,°C$ for 20 min at $650 \times g$. The clear solution was used as a source of crude of α-glucosidase and sucrase solutions.

2.3 Inhibition of the α-amylase activity
The determination was carried out as described by Bernfeld [7]. In a test tube containing 1.0 mL of 2 mM phosphate buffer (pH 6.9), 0.1 mL of each extract was incubated with 0.05 mL of α-amylase solution for 20 min. Precisely 0.1 mL of 1.0% of freshly prepared starch solution was subsequently added and allowed to stand

for 5 min. Next, 0.5 mL of dinitrosalicylic acid reagent held in boiling water for 5 min. The solution was subsequently cooled, and the absorption was measured at 540 nm. The result was expressed in IC_{50} (μg/mL) calculated as the concentration needed for inhibition of 50% of α-amylase activity.

2.4 Inhibition α-glucosidase activity
The determination was carried out as described by Kim et al. [8]. In a test tube containing 1.0 mL of 2 mM phosphate buffer (pH 6.9), 0.1 mL of each extract was incubated with 0.1 mL of mucosa solution for 20 min. Subsequently, 0.1 mL of 3 mM of para-nitrophenylglucopyranoside prepared in 20 mM phosphate buffer (pH 6.9) was added and allowed to stand for 15 min. Then, 0.5 mL of 5.0% sodium carbonate was added and incubated for 90 min. The absorbance at 450 nm was measured, and the result was expressed as the concentration of inhibition required to inhibit 50% of α-glucosidase activity (IC_{50} (μg/mL)).

2.5 Assay of sucrase inhibitory activity
The determination was carried out as described by Honda and Hara [9]. In a test tube containing 1.0 mL of 2 mM phosphate buffer (pH 6.9), 0.1 mL of each extract was incubated with 0.1 mL of mucosal solution for 20 min. Afterwards, 0.1 mL of 60 mM sucrose solution was added and allowed to stand for 5 min. Then, 0.5 mL of dinitrosalicylic acid reagent was transferred into the test tube and allowed to incubate in boiling water for 5 min. The test tube was cooled, and the optical density was read at 540 nm. The percentage inhibition of sucrase activity was calculated, and the result was expressed in IC_{50} (μg/mL) as the inhibition concentration required to inhibit 50% of sucrase activity.

2.6 In vitro glycation of albumin
The preparation of glycated albumin was carried out according to the procedure defined by Safari et al. [10] with slight modifications. The solution contained bovine serum albumin (0.1 g/mL) prepared in 0.1 M phosphate buffer (pH 7.4) containing 0.01% sodium azide and D-glucose (10 mg/mL), and the extract was combined in a ratio of 3:2:1 and incubated for 72 h.

2.7 Estimation of antiglycation capacity
The determination was carried out as described by Furth [11]. In a test tube containing 1.0 mL of glycated sample, 0.5 mL of 10% trichloroacetic acid was added. For 5 min, the solution was centrifuged at $650 \times g$. Then, 1.0 mL of phosphate buffer and 0.5 mL of 0.3 N oxalic acids were added to the sediment and boiled for 60 min. The solution was cooled, and 0.5 mL of 10% trichloroacetic acid solution and 0.5 mL 0.05 M thiobarbituric acid were

added and boiled for 10 min. The solution was centrifuged at $650 \times g$ and the absorbance of the supernatant was read at 443 nm. The result was reported as the percentage inhibition.

2.8 Determination of inhibition of glycation-induced oxidation of protein thiol groups

The determination was carried out as described by Ellman [12]. Accurately, 1.0 mL of 0.5 mM 5,5′-dithiobis (2-nitrobenzoic acid) in 0.1 M phosphate buffer (pH 7.4) was transferred into a test tube containing 1.0 mL of glycated sample and incubated at room temperature of 29 °C for 15 min. The absorbance at 412 nm was measured. The thiol group concentration was calculated using molar extinction $= 1.34 \times 10^4 M^{-1} cm^{-1}$. The findings were documented as a protein of nmol/mg.

2.9 Determination of inhibition of protein aggregation

The determination was carried out as described by Klunk et al. [13]. Precisely, 0.1 mL of 1% Congo red prepared in phosphate buffer with 10% ethanol was added to a test tube containing 1.0 mL of glycated sample. For 30 min, the solution was incubated and the absorption was measured at 530 nm. The percentage of the results was reported.

2.10 Statistical analysis

The findings were evaluated using one-way variance analysis (ANOVA) for the mean differences between the different extracts followed by multiple comparison tests by Duncan for post hoc correlations at $p < 0.05$ and reported as means ± standard deviation of three determinations.

2.11 Molecular docking and in silico evaluation of drug-likeness of the compounds

The compounds selected for docking are the major compounds that are biologically active molecules as previously reported [14, 15]. These compounds were docked with the hydrolases. The 3D SDF format structures of the compounds were obtained from the PubChem database. The compounds were emodin (PubChem CID: 3220), quercetin (PubChem CID: 5280343), chrysoeriol (PubChem CID: 5280666), and kaempferol (PubChem CID: 5280863). The target 3D structures of the enzymes were identified and retrieved from the Protein Data Bank (PDB). The enzymes were human pancreatic α-amylase (1HNY), α-glucosidase (5ZCB), and sucrase-isomaltase (3LPO). All the compounds and the enzymes were converted into AutoDock pdbqt format. The binding scores of the compounds and the target enzymes were measured using PyRx-Python Prescription 0.8 (The Scripps Research Institute), and the interactions were visualized using PyMOL ver. 1.1eval (De Lano Scientific LLC, CA, USA). The computations were compared to those generated through the virtual screening of acarbose (PubChem CID: 41774) with the hydrolases. The in silico evaluation of the drug-like nature of the selected compounds was predicted using the SwissADME server (http://www.swissadme.ch/index.php) [16].

3 Results

The extracts exhibited varying inhibitory activities with the selected carbohydrate-digesting enzymes (Table 1). The results revealed that the extracts contained significant amounts of α-amylase, α-glucosidase, and sucrase inhibitors with the MCE having the highest inhibitory potential as revealed by the corresponding lowest IC_{50} values of 69.67 ± 0.88, 65.54 ± 0.34, and 48.35 ± 1.45 μg/mL for α-amylase, α-glucosidase, and sucrase inhibition respectively. This was followed by a significant inhibitory effect of BPE on the enzymes ($p < 0.05$).

The results of the virtual screening of the active phytocompounds with the selected hydrolases are presented in Table 2. The binding energies, estimated through docking studies of the compounds with the target enzymes, revealed that emodin, quercetin, chrysoeriol, and kaempferol had docking scores of -6.0, -6.8, -5.1, and -6.8 (kcal/mol), respectively, with α-amylase (1HNY). The best score towards α-glucosidase (5ZCB) was observed for emodin (-5.9 kcal/mol) while chrysoeriol showed the highest affinity towards sucrase-isomaltase (3LPO) (-7.7 kcal/mol). The interaction between the compounds and the enzymes is depicted in Fig. 1. It was also observed that all the compounds showed intersecting molecular interactions inside the binding site of each of the enzymes (Fig. 2). The theoretical evaluation of lipophilicity (log P, calculated octanol/water partition coefficient) revealed all the selected compounds have consensus log $P < 5$ as shown in Table 3 and are drug-like in nature.

The determination of antiglycation properties of the extracts was performed by investigating the inhibitory potential of the extracts against albumin glycation, oxidation of thiol groups, and β-amyloid aggregation (Table 4). There were significant differences in the inhibitory effect of the extracts against glucose-induced albumin glycation and oxidation of thiol groups. The albumin glycation inhibitory potential of BPE was statistically ($p < 0.05$) equivalent to that of MCE, but significantly higher than the value obtained from ARE. The results similarly revealed a significant decrease in the oxidation of thiol groups in the albumin-glycated sample when incubated in the presence of the extracts. It was observed that MCE and BPE exhibited significantly ($p < 0.05$) higher activity than ARE. Moreover, MCE caused a significant ($p < 0.05$) and the lowest inhibition of β-amyloid aggregation (73.43 ± 0.57%).

Table 1 Carbohydrate-digesting enzyme inhibitory potentials of the extracts of dried leaves of *C. alata*

Extracts	α-Amylase (IC$_{50}$, µg/mL)	α-Glucosidase (IC$_{50}$, µg/mL)	Sucrase (IC$_{50}$, µg/mL)
BPE	192.04 ± 20.31[a]	143.33 ± 0.34[a]	69.67 ± 2.96[a]
ARE	385.66 ± 73.39[b]	338.31 ± 1.43[b]	499.33 ± 93.27[b]
MCE	69.67 ± 0.88[c]	65.54 ± 0.34[c]	48.35 ± 1.45[c]
Acarbose	24.23 ± 2.54[d]	27.17 ± 1.22[d]	31.23 ± 3.87[d]

Different letters within the column designate significant differences ($p < 0.05$)
BPE butanol partitioned extract, *ARE* aqueous residual extract, *MCE* methanol crude extract

4 Discussion

The methanolic extract of *C. alata* has been reported to have strong antioxidant and anti-inflammatory properties [17, 18]. It has also been shown to contain phenolic compounds that could be responsible for probable protection against hyperglycaemia-mediated diseases [19]. The methanolic extract was partitioned to separate the phytoconstituents into n-butanol non-polar and aqueous polar phases. A previous study had reported that kaempferol, a flavonoid, is predominant in the butanol fraction of methanolic extract of *C. alata* [20]. These antioxidant compounds had been suggested to be beneficial in the alleviation of complications that are associated with diabetes [21].

The results obtained revealed that the crude extract had the highest inhibitory potential towards α-amylase, α-glucosidase, and sucrose, hydrolases that are usually implicated in postprandial hyperglycaemia [22]. Inhibition of the activities of these enzymes could be beneficial in the control of hyperglycaemia by limiting carbohydrate digestion. However, the butanol fraction displayed higher inhibitory potential compared to the aqueous residual fraction. Although the extracts might not contain the oligosaccharides-like structure of acarbose that could aid their affinity to the glycoside hydrolases, modifications in the structural conformation of the enzymes could be induced by the phytochemical constituents of the plant. Besides, bioactive compounds from botanical sources have been reported to have hypoglycaemic effects, and these include the phenolics and the flavonoids [23, 24]. It should be noted that the activities investigated were estimated based on the weight of the dried leaves which could be higher than the activities of the respective secondary metabolites which would be accountable for the observed effects.

Further studies were carried out on the molecular mechanic assessment on the prediction of possible interactions between the bioactive components of the plant and the enzymes. The binding energies of the compound enzyme-docked complexes revealed that there could be interactions between the selected compounds and the hydrolases based on the score of the model with the least values. The enzymes, namely, human pancreatic α-amylase (1HNY), α-glucosidase (5ZCB), and sucrase-isomaltase (3LPO) were the selected molecular targets. These macromolecules were studied in detail to check the binding occurring between the 3D structure of the macromolecules and the compounds. Inhibition was measured by the binding energy. The binding poses obtained for the best binding compounds were visually inspected. Quercetin and kaempferol, of the four compounds selected for the study, had better interactions with α-amylase (1HNY) based on the scoring values as indicated by the lesser binding energy. The complex with lesser binding energy had the best docking and thus the highest affinity [25]. These compounds showed similar behaviour with α-amylase (1HNY); this could be due to the similarity in the structural conformations of these compounds in the α-amylase (1HNY). The binding energies were compared with acarbose, a known inhibitory compound, and all the selected compounds showed higher stability than acarbose in their interactions with the α-amylase (1HNY). Emodin had the highest comparative binding score with α-glucosidase (5ZCB), followed by quercetin. Kaempferol showed the least stability. A good dock score was obtained from the

Table 2 Comparison of predicted binding free energies (kcal/mol) amongst the compounds

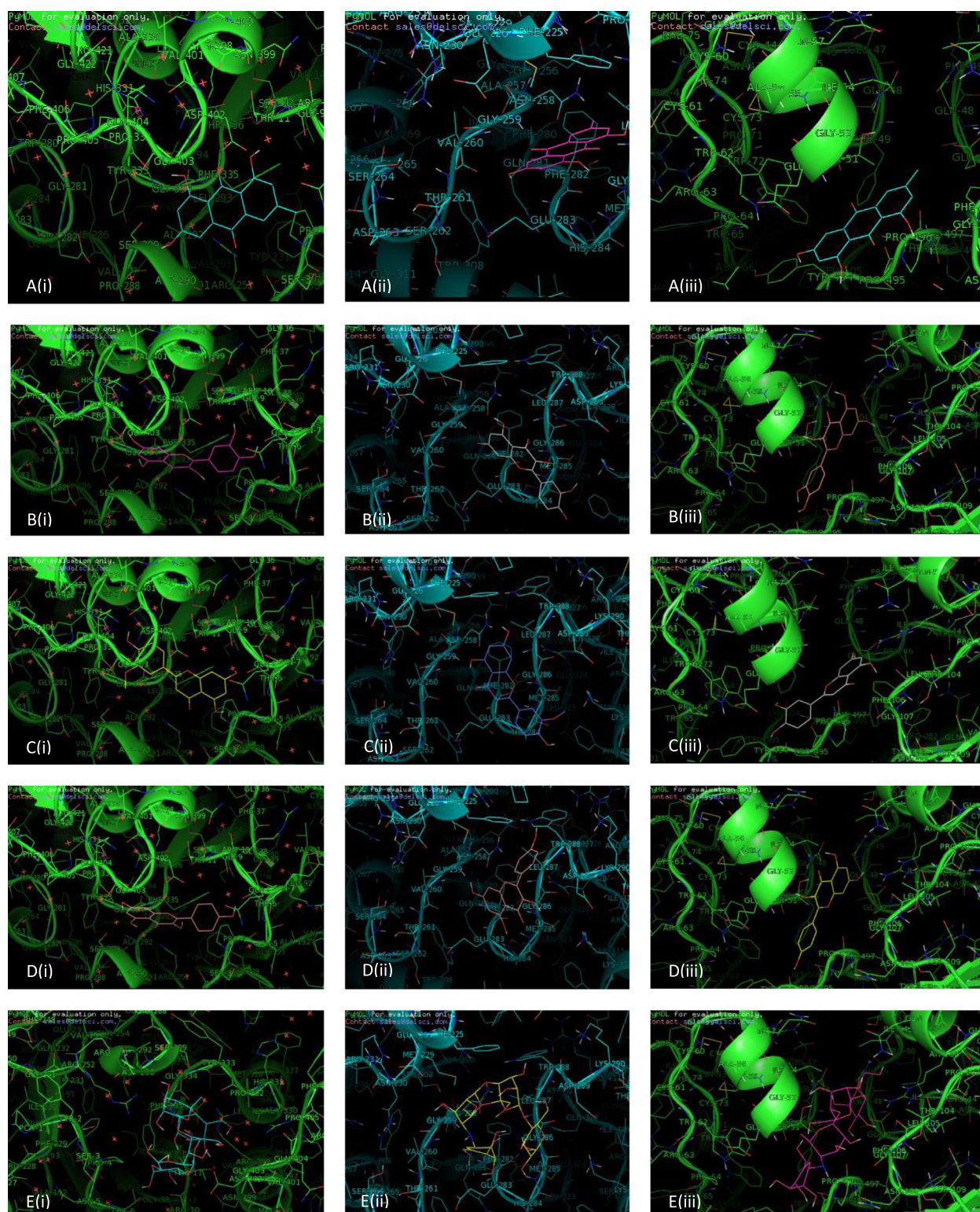| Compounds	PubChem CID	α-Amylase (1HNY)	α-Glucosidase (5ZCB)	Sucrase-isomaltase (3LPO)
Emodin	3220	− 6.0	− 5.9	− 7.0
Quercetin	5280343	− 6.8	− 5.4	− 7.3
Chrysoeriol	5280666	− 5.1	− 5.2	− 7.7
Kaempferol	5280863	− 6.8	3.4	− 7.1
Acarbose	41774	7.5	− 3.2	− 7.0

Fig. 1 Molecular docking of the compounds with the enzymes **A(i)** emodin and α-amylase (1HNY), **A(ii)** emodin and α-glucosidase (5ZCB), **A(iii)** emodin and sucrase-isomaltase (3LPO), **B(i)** quercetin and amylase, **B(ii)** quercetin and α-glucosidase (5ZCB), **B(iii)** quercetin and sucrase-isomaltase (3LPO), **C(i)** chrysoeriol and amylase (1HNY), **C(ii)** chrysoeriol and α-glucosidase (5ZCB), **C(iii)** chrysoeriol and sucrase-isomaltase (3LPO), **D(i)** kaempferol and amylase (1HNY), **D(ii)** kaempferol and α-glucosidase (5ZCB), **D(iii)** kaempferol and sucrase-isomaltase (3LPO), **E(i)** acarbose and amylase (1HNY), **E(ii)** acarbose and α-glucosidase (5ZCB), and **E(iii)** acarbose and sucrase-isomaltase (3LPO)
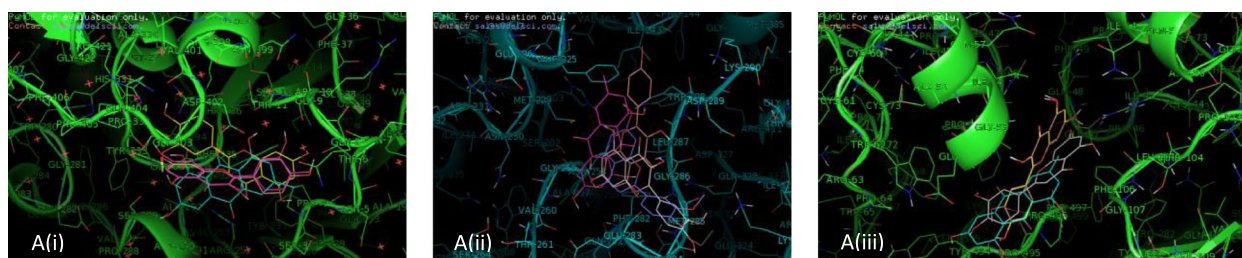
Fig. 2 Superimposition of 3D structures of emodin, quercetin, chrysoeriol, and kaempferol inside the binding site of **A(i)** α-amylase (1HNY), **A(ii)** α-glucosidase (5ZCB), and **A(iii)** sucrase-isomaltase (3LPO)

interactions of all the compounds and acarbose with sucrase-isomaltase (3LPO), showing the compounds inhibit the enzyme effectively. However, by comparing the computed binding affinities, it was observed that chrysoeriol exhibited the highest binding energy with sucrase-isomaltase (3LPO). This could substantiate the reported hyperglycaemia-ameliorative effects of chrysoeriol in diabetic rats [26].

The docking studies of the compounds with the target enzymes related to hyperglycaemia showed that the methanolic extract of the leaf of *C. alata* could contain good inhibitors that could interfere with the target hydrolases compared to acarbose and limit the rate of glucose absorption in the gut.

There are thousands of active compounds in plant-based foods; the roles they play in reducing the risk of chronic diseases and maintenance of wellness could result from the integration and synergistic action of these secondary metabolites. Thus, interactions between these compounds could result in synergistic effects and increase the observed pharmacological activity, and probably the shrub's therapeutic effects as depicted through the superimposition of the docked compounds inside the binding sites of the enzymes.

The drug-likeness of the selected compounds evaluated by the molecular weight and logarithm of the partition coefficient (log P) showed that all the compounds are drug-like molecules with values within the acceptance range (log $P \leq 5.0$, molecular weight ≤ 500 g/mol) [27]. Moreover, the evaluation through the Ghose rules also showed each of the compounds has a molecular weight that is less than 480 and lipophilicity (log P) within the range of − 0.4 and 5.6 (Table 3) [28].

Additionally, many of the secondary complications that are associated with diabetics are stemmed from the elevation in blood glucose concentrations. Typically, the antioxidant potential of plant extracts has been considered being positively associated with inhibition of diabetes-induced complications [29]. Glycation is a non-enzymatic reaction between reducing sugars and free amino groups in biomolecules. It generates unstable Schiff base structures and Amadori products that could go through a series of transformations and form advanced glycation end products (AGEs) [30]. The investigation of the attenuation of glycation-induced protein modifications revealed that MCE could cause a significant reduction in the glycation of albumin at the selected concentration. Moreover, oxidative stress has been shown to be worsened in chronic diabetic condition. Generation of AGEs could be associated with the generation of intracellular reactive oxygen species and the release of pro-inflammatory molecules [31]. Hence, prevention of oxidative damage during the glycation process may indicate other mechanisms for the inhibition of generations of AGEs [32]. The evaluation of the effects of the extracts on free thiol groups as an index of protein oxidation in the glycated albumin samples revealed that MCE and BPE significantly suppressed and limit the oxidation of thiol group of albumin. Thus *C. alata* could counteract oxidative modifications that could be induced by glycation.

The antiglycation potential of the extracts was also substantiated through the inhibition of β-amyloid fibril formation in glycated albumin. Glycation increases the level of amyloid cross β-structure, thus aggravating the cytotoxicities of protein aggregation and in general hyperglycaemia [33]. These results plainly demonstrated that MCE and

Table 3 Drug-like natures of emodin, quercetin, chrysoeriol, kaempferol, and acarbose

Properties	Emodin	Quercetin	Chrysoeriol	Kaempferol	Acarbose
Molecular weight (g/mol)	270.24	302.24	300.26	286.24	645.60
Log P	1.87	1.23	2.18	1.58	− 6.22
Lipinski	Yes	Yes	Yes	Yes	No
Ghose	Yes	Yes	Yes	Yes	No

Table 4 Antiglycation properties of the aqueous extracts of dried leaves of C. alata

Extracts	Inhibition of albumin glycation (%)	Inhibition of oxidation of thiol groups (nmol/mg protein)	Inhibition of β-amyloid aggregation (%)
BPE	71.33 ± 2.45[a]	2.32 ± 0.01[a]	109.67 ± 1.45[a]
ARE	54.22 ± 1.58[b]	1.95 ± 0.02[b]	228.67 ± 7.51[b]
MCE	81.65 ± 3.60[a]	3.17 ± 0.06[c]	73.43 ± 0.57[c]

Different letters within the column designate significant differences ($p < 0.05$)
BPE butanol partitioned extract, *ARE* aqueous residual extract, *MCE* methanol crude extract

BPE significantly decreased the level of amyloid β-structure of albumin compared to ARE. There is an association between the carbohydrate-digesting enzyme inhibitory potentials and the ability of the extracts to inhibit the formation of AGEs. Thus, the antiglycaemic and antiglycation properties of the plant could interrelate. Although the biological functionality of C. alata has been claimed in the traditional system of medicine and had been shown by experimental studies [34, 35], the investigated biological properties could be responsible for the acclaimed medicinal values of this botanical. The study suggests the methanol extracts of C. alata could inhibit amyloid fibril formation thereby decreasing the tendency of hyperglycaemia to induce fibrillar aggregation. This shows that the plant could protect against cross-β-structure formation of albumin from α-helix possibly by concealing the glycation sites. The extracts could be considered to have a significant inhibitory activity that might contribute to their claimed medicinal activities. The phenolics and flavonoids contents of plant extracts correlate with the antiglycation properties; thus, these phytochemicals could be assumed to protect against the development of AGEs [36]. BPE has shown more biological activity than ARE in general. This observation was in agreement with the previous report that n-butanol fractions of an extract of C. alata had the antidiabetic property [20]. The study also showed that the butanol fraction of the methanolic extract of the leaves of C. alata could comprise inhibitors of hydrolases thus aiding a possibility of developing alternative therapy against secondary complications that could be associated with diabetic conditions.

5 Conclusion

The dried leaf of the methanolic extract of C. alata contains good anti-hyperglycaemic and preventive agents for albumin glycation. Compared to the aqueous residual extract, the antiglycaemic and antiglycation potential were more significant in the butanol fraction. Studies through the in silico docking showed that amylase, glucosidase, and sucrase inhibition by the verified phytochemicals expected to be found in the plant occurred with the highest bonding efficiency and hydrophobic interaction. The in silico study revealed that phytochemical components of C. alata have a high affinity to the selected hydrolases; thus, the plant could be suggested as one of the possible sources of therapeutic agents to reduce the activities of the enzymes and hyperglycaemia.

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