Research Article

Comparative in vitro studies of antiglycemic potentials and molecular docking of *Ageratum conyzoides* L. and *Phyllanthus amarus* L. methanolic extracts

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Abstract
These in vitro studies investigated the comparative antiglycemic properties and molecular docking of the methanolic extracts of dried leaves of *Ageratum conyzoides* L. and *Phyllanthus amarus* L. in an attempt to explore natural products that could be useful in preventing secondary complications that could arise from hyperglycaemia. The methanolic crude extracts of dried leaves of *A. conyzoides* (CEA) and *P. amarus* (CEP) were partitioned into n-butanol and aqueous extracts and glycation inhibitory potentials were investigated. The result reveals that CEA and CEP exhibited highest glycemic inhibitory potential on the activities of α-amylase, α-glucosidase and sucrase investigated. The molecular docking was done on reported identified compounds in *A. conyzoides* and *P. amarus* with α-amylase (1SMD), sucrase-isomaltase (3LPO) and α-glucosidase (3WY1). Methanol crude extracts exhibited the highest inhibitory effect with the lowest IC_{50} values of (78.00 ± 1.73, 77.00 ± 1.16), (62.67 ± 1.45, 57.67 ± 0.88) and (89.67 ± 3.48, 95.33 ± 2.60) µg/mL respectively for 1SMD, 3LPO and 3WY1. The molecular docking analysis depicted that phytol had the best docking binding energy for the three enzymes and oxazolone and 9,12,15-octadecatrienoic acid showed best affinity for 1SMD and 3LPO while none for 3WY1. Crude and butanol partitioned extracts of both plants had a significant ($p < 0.05$) inhibition on glucose-induced albumin glycation, thiol oxidation and β-amyeloid aggregation. This study provides evidence suggesting that methanolic crude extracts of both plants could be used in the prevention of diabetes secondary complications.

Keywords CEA · CEP · Antiglycation · Antiglycemia · Molecular docking

1 Introduction

Diabetes mellitus (DM) is one of the most deadly diseases in the world; its rate coupled with its complications increases with time in every part of the world [1]. This is a metabolic disorder that result from excessive accumulation of glucose in the blood due to either deficiency in insulin synthesis or defects in insulin receptors. In adult, type II Diabetes mellitus is more common compared to type I Diabetes mellitus and its expected to reach 366 million cases in the year 2030 [2]. Type II Diabetes Mellitus (T2DM) is a common disorder of glucose metabolism and has been linked to insulin resistance and high calorie diets thus elevating the postprandial glucose level [3]. Different carbohydrate hydrolyzing enzymes such as α-glucosidase, amylase and sucrase in the brush border of small intestine can be inhibited through regular consumption of the antihyperglycemic drugs e.g. acarbose has been reported as one of the current management strategy for diabetes based on the reduction of glucose level [4]. This inhibition of the enzymes slow down the process of carbohydrate digestion and absorption, which in turn delay glucose absorption and hinder postprandial plasma glucose increase [5]. Nijpels et al. [6] reported that

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daily consumption of acarbose for three years reduced the risk of developing type II diabetes by 6% compared to the control.

Sometimes accumulation of amyloid, protein glycation and reduction of thiol protein could result as secondary complication of the elevated blood glucose [7]. Secondary complications in diabetic patients and failure or damage of multiple organs were due to delayed hyperglycemia [8]. Acute complications resulting from free radicals formation through glucose oxidation, non-enzymatic glycation of proteins cause lipid and protein metabolic mutation and more continual complications such as retinopathy, cataracts, atherosclerosis, neuropathy, aging and many more due to Advance Glycation End Products AGEs [9].

Systemic oxidative stress resulting from hyperglycemia due to imbalance between antioxidant and reactive oxygen specie (ROS) in favour of ROS causes an increase in insulin resistance and β-cell dysfunction, thus promoting the devlopment of type 2 diabetes mellitus [10–12]. Cellular damage caused by ROS is prevented by removing excess ROS via an endogenous antioxidant mechanisms such as catalase, superoxide dismutase, and the peroxidase-glutathione system [13]. Thiols are compounds with a free sulfhydryl (R–SH) moiety occurring in the form of proteins containing one or more free cysteine groups or low molecular-weight compounds (e.g. glutathione) in cells and extracellular fluids which is oxidized and transformed to disulfide [14]. The formed disulfide moiety is expected to be related to some secondary complications of diabetes mellitus [15]. However, the gastrointestinal side effects of known carbohydrate hydrolyzing enzyme inhibitors such as acarbose, miglitol and voglibose and inefficiency of the endogenous defense system to scavenge free radicals rest on natural exogenous antioxidants. Antioxidants from plant source are known to reduce oxidative stress thus, phytomedicines are sought as possible alternatives or adjuncts [16]. This present in vitro study investigated the carbohydrate hydrolyzing enzymes inhibitory potential, amyloid aggregation inhibition, non-enzymatic glycation inhibitory properties and thiol containing protein of different extracts of *Ageratum conyzoides* and *P. amarus* leaves for the prevention of secondary complications in DM and binding behavior of reported isolated compounds.

2 Materials and methods

2.1 Material

Absolute methanol, butanol, ethanol, congo red, dinitrosalicylic acid, para-nitrophenylglucopyranoside, trichloroacetic acid, bovine serum albumin, 5, 5′-dithiobis (2-nitrobenzoic acid) were obtained from JHD in China. Other reagents were of analytical grade and were prepared with distilled water.

2.2 Method

2.2.1 Samples preparation

Fresh leaves of *A. conyzoides* and *P. amarus* were air-dried at room temperature of 29 ± 1 °C. The samples were authenticated at the Department of Biological Sciences Herbarium, McPherson University, Nigeria with voucher numbers McUBHA0001 and McUBHP0005 for *A. conyzoides* and *P. amarus* respectively. The dried leaves of *A. conyzoides* and *P. amarus* were pulverized and 10 g of each pulverized samples were extracted with 100 mL of methanol at room temperature of 29 ± 1 °C for 24 h and later filtered. Two-third of the crude extracts were partitioned repeatedly inside a separating funnel into an aqueousextract and n-butanol extract. The crude extracts and the partitioned extracts were used as the corresponding extracts for the subsequent analyses.

2.2.2 Preparation of the crude α-glucosidase and sucrase solution

The mucosa of the small intestine of rats sacrificed under light anaesthesia was carefully scraped off with a glass slide, homogenized in cold sodium phosphate buffer (pH 6.8) and centrifuged at 4 °C for 20 min at 650×*g*. The clear solution was used as source of crude of α-glucosidase and sucrase solutions [17].

2.2.3 Inhibition of the α-amylase activity

The determination was carried out according to the method described by Bernfeld [18]. 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 was and 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.

2.2.4 Inhibition α-glucosidase activity

The determination was carried out based on the method described by Kim et al. [19]. 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, incubated for 90 min and the absorbance was read at 450 nm. The result was expressed as the concentration of inhibition required to inhibit 50% of α-glucosidase activity [IC_{50} (µg/mL)].

2.2.5 Assay of sucrase inhibitory activity

The determination was carried out according to the method described by Honda and Hara [20]. 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. Afterward, 0.1 mL of 60 mM sucrose solution was added and incubated for 5 min. Then, 0.5 mL of dinitrosalicylic acid reagent was transferred into the test tube and incubated in boiling water for 5 min. The test tube was cooled and the optical density at 540 nm was read. 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.

2.2.6 In vitro glycation of albumin

The preparation of glycated albumin was carried out according to the procedure defined by Safari et al. [21] 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, D-glucose (10 mg/mL) and the extract combined in ratio 3:2:1 and incubated for 72 h.

2.2.7 Estimation of anti-glycation capacity

The determination was carried out colometrically using the method described by Furth [22]. 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 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 boiledfor 10 min. The solution was centrifuged at 650×*g* and the absorbance of the supernatant was read at 443 nm. The result was reported as percentage inhibition.

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

The determination was carried out colometrically using the method described by Ellman [23]. 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 = 1.34×10^4 M^{-1} cm^{-1}. The findings were documented as a protein of nmol/mg (Figs. 1, 2, 3, 4).

2.2.9 Determination of inhibition of protein aggregation

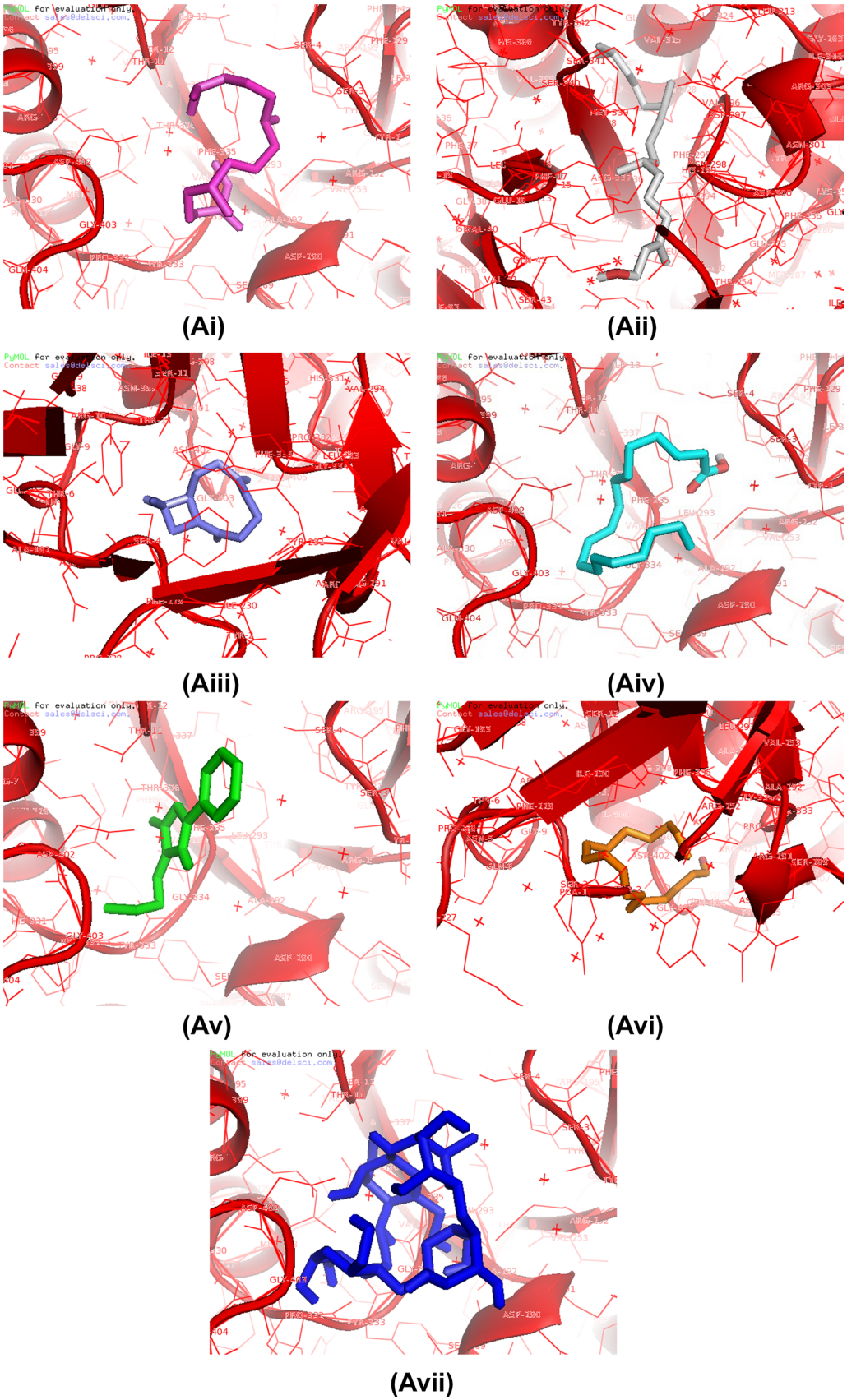
The determination was carried out colometrically using the method described by Klunk et al. [24]. Precisely, 0.1 mL of 1% Congo red prepared in phosphate buffer with 10% ethanol was added to a test tube containing 1.0 mL of glycated sample. The solution was incubated for 30 min, absorbance was measured at 530 nm and the percentage of the results was reported.

2.2.10 Statistical analysis

The results obtained were expressed as mean ± standard deviation of three determination and analyzed using one-way variance analysis (ANOVA) for mean differences between different extracts followed by Duncan multiple range test for post hoc correlation at $p < 0.05$.

2.2.11 Molecular docking

In association with in vitro antiglycemia activity, it is useful to carry out molecular studies to predict the binding affinity at the active site of the selected hydrolases. The docked selected compounds with carbohydrate hydrolyzing enzymes were the major compounds identified as an active agents in *Ageratum conyzoides* [25, 26] and in *P. amarus* [27] and the 3D SDF format structures were obtained from PubChem data base. The compounds were neophytadiene (PubChem CID: 10446), caryophyllene (PubChem CID: 5354499), phytol (PubChem CID: 5280435), 9,12,15-octadecatrienoic acid (PubChem CID: 860), 9,17-octadecadienal (PubChem CID: 5365667) and oxazolone (PubChem CID: 1712094) while the 3D structure targeted enzymes were obtained as from RCSB Protein Data Base (PDB) as 1SMD, 3LPO and 3WY1 for α-amylase, sucrose-isomaltase and β-glucosidase respectively. All the compounds and the enzymes were autodocked into pdbqt format and the affinity energy (kcal/mol) between compound and enzyme were measured using



(Ai) (Aii) (Aiii) (Aiv) (Av) (Avi) (Avii)

◂**Fig. 1** Molecular docking of compounds with enzyme **Ai** α-amylase (1SMD) and neophytadiene (10446), **Aii** α-amylase (1SMD) and phytol (5280435), **Aiii** α-amylase (1SMD) and caryophyllene (5354499), **Aiv** α-amylase (1SMD) and 9,12,15-octadecatrienoic acid (860), **Av** α-amylase (1SMD) and oxazolone (1712094), **Avi** α-amylase (1SMD) and 9,17-octadecadienal (5365667) and **Avii** α-amylase (1SMD) and acarbose (41774)

PyRx-Python Prescription 0.8 and visisualized using PyMOL ver. 1.leval. The affinity results of the compounds were compared to those of acarbose (PubChem CID: 41774) with the enzymes.

3 Results

The inhibitory potential of the extracts varied towards the three selected carbohydrate hydrolases (Table 1). The results depicted that no significant difference ($p < 0.05$) between the butanol extracts and the metanolic extracts of the plants but are significantly different ($p < 0.05$) from the aqueous extracts of the plants towards the α-amylase activity. Also, butanol and methanol extracts possessed higher inhibition than the aqueous extracts; although the methanolic extracts had the best inhibitory potential. There is significant difference ($p < 0.05$) among the extracts towards sucrase activity except for both aqueous and butanol extracts of *A. conyzoides* leaves with methanolic crude extract of *P. amarus* (CEP) possessing the highest inhibition (57.67 ± 0.88). Similarly no significant difference ($p < 0.05$) in inhibition towards α-glucosidase between aqueous residual extract of *P. amarus* (AREP) and butanol partitioned extract of *P. amarus* (BPEP) and between methanolic crude extract of *A. conyzoides* (CEA) and CEP which are significantly difference ($p < 0.05$) from aqueous residual extract of *A. conyzoides* (AREA) and butanol partitioned extract of *A. conyzoides* (BPEA). However, none of the extracts from both plants possessed inhibitory potential more than acrabose towards the three carbohydrate hydrolyzing enzymes.

The visual screening results of selected reported compounds present in both plants with the selected carbohydrate hydrolases revealed the binding energies (Table 2). The docking report showed that all the compounds docked towards α-amylase (1SMD) possessed higher affinity than acarbose except caryophyllene while none has higher affinity than acarbose towards sucrase-isomaltase (3LPO) and α-glucosidase (3WY1). Also, phytol (5280435) has the highest biniding energy towards the three enzymes compared to other two compounds from *A. conyzoides* and none of the compounds from *P. amarus* docked with α-glucosidase (3WY1) possessed any binding energy.

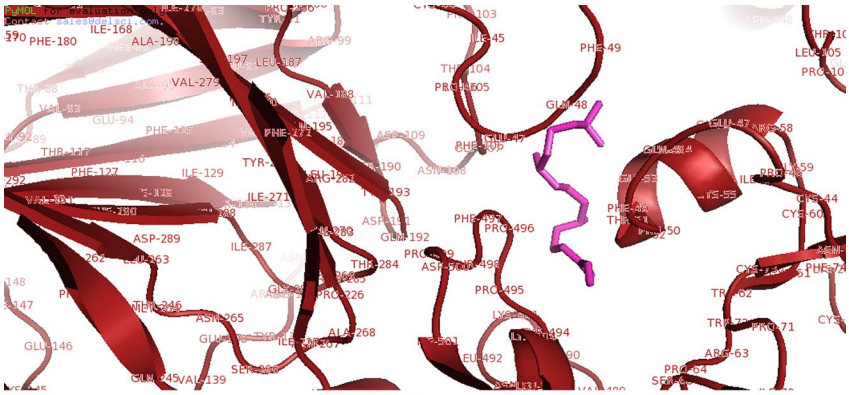
The antiglycation properties of the extracts were evaluated through the assessment of the inhibitory potential of the extracts against albumin glycation, thiol groups oxidation, and β-amyloid aggregation (Table 3). There were significant differences in the inhibitory effect of the extracts against glucose-induced albumin glycation thiol groups oxidation and β-amyloid aggregation. The albumin glycation inhibitory potential of BPEA and CEA were statistically ($p < 0.05$) different from that of BPEP and CEP respectively but with values higher than what is obtained from AREA and AREP and AREA had the least. Also, extracts of *P. amarus* were not only statistically different from their corresponding *A. conyznoides* extracts but higher than their values. There is a significant decrease in the thiol groups oxidation in the albumin-glycated sample when incubated with the extracts but no significant difference ($p < 0.05$) between BPEA and CEA and their corresponding BPEP and CEP with CEP being the highest. However the aqueous extracts exhibited less inhibitory effect with AREA being the least. It was also observed that inhibition of β-amyloid aggregation followed the same trend and CEP had the highest inhibition of β-amyloid aggregation (28.00 ± 0.58%) while AREA had the least inhibition of β-amyloid aggregation (6.67 ± 0.33).

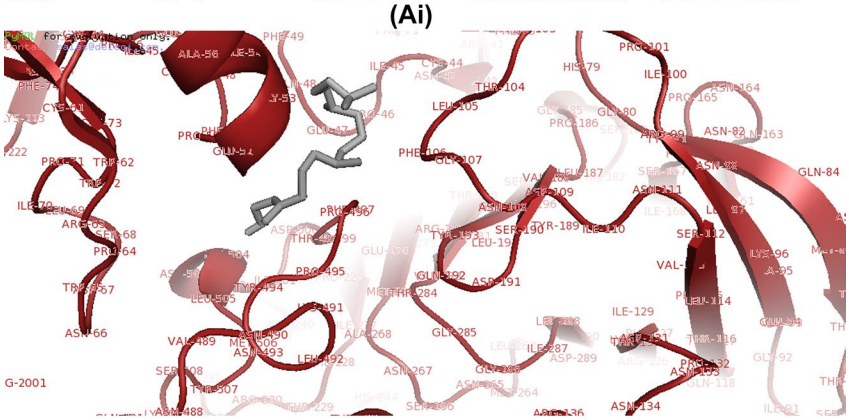
4 Discussion

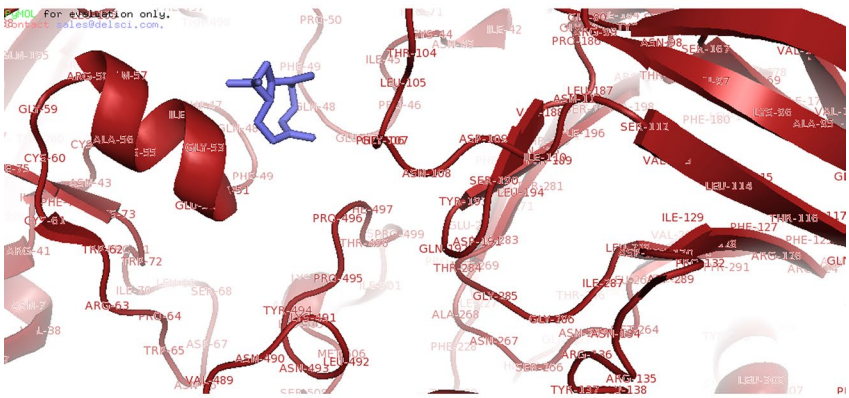
Plant species investigated for antioxidant activity are known to exhibit antidiabetic effect. It has been estimated that more than 400 herbal or plant-derived products are used for the management of T2DM across the globe [28]. The hypoglycemic effect of some plant extracts has been confirmed in human and animal models of T2DM [29]. The WHO Expert Committee on diabetes recommended that medicinal plants should be investigated further [28]. *A. conyzoides*, a family of asteraceae with an annual weed of 80–90 cm in height has been reported to exhibit antioxidant property [30]. Also, the *P. amarus* of the family Euphorbiaceae contains compounds like alkaloids, flavonoids, lignans, phenols and terpenes which have been shown to interact with most key enzymes such as amylase, glucosidase [31].

The results depicted that the crude extracts of the plants exhibited highest inhibitory potential towards the three hydrolases investigated with no significant difference ($p < 0.05$) from each other except for sucrase while the aqueous extracts had the least.

The reported percentage inhibitory potential was presumed to be the relative extracts/acarbose-induced reduction in the activities of the hydrolases with respect to the corresponding controls (without an extract or acarbose). Also, the inhibitory potential of the CE from the plants







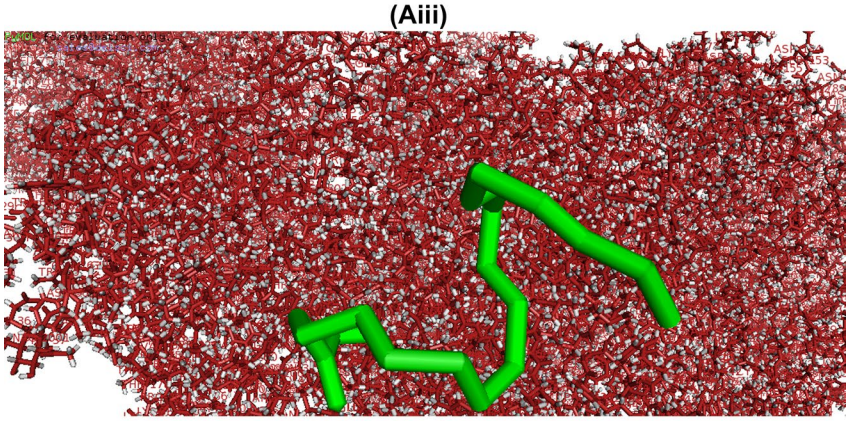
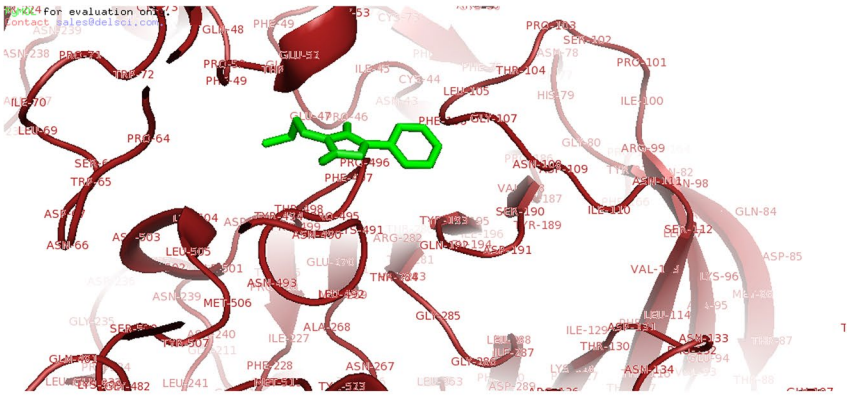
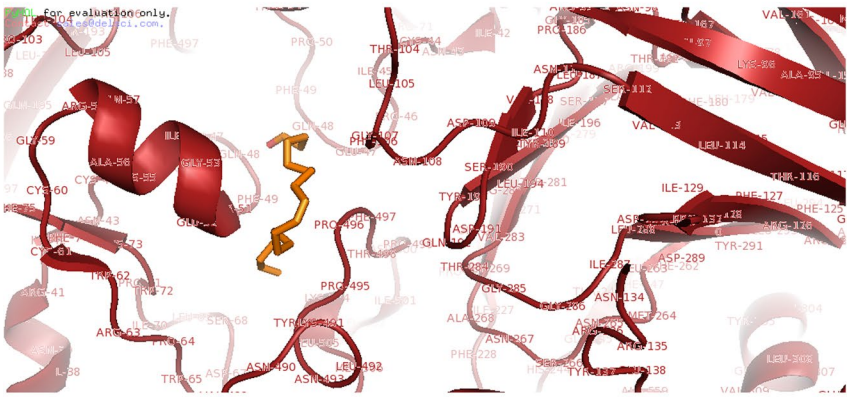


Fig. 2 Molecular docking of compounds with enzyme **Ai** sucrose-isomaltase (3LPO) and neophytadiene (10446), **Aii** sucrose-isomaltase (3LPO) and phytol (5280435), **Aiii** sucrose-isomaltase (3LPO) and caryophyllene (5354499), **Aiv** sucrose-isomaltase (3LPO) and 9,12,15-octadecatrienoic acid (860), **Av** sucrose-isomaltase (3LPO) and oxazolone (1712094), **Avi** sucrose-isomaltase (3LPO) and 9,17-octadecadienal (5365667) and **Avii** sucrose-isomaltase (3LPO) and acarbose (41774)

Fig. 2 (continued)



(Av)



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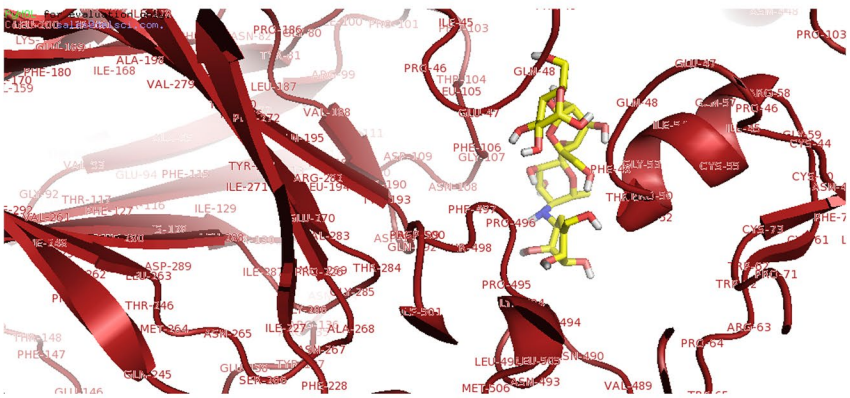
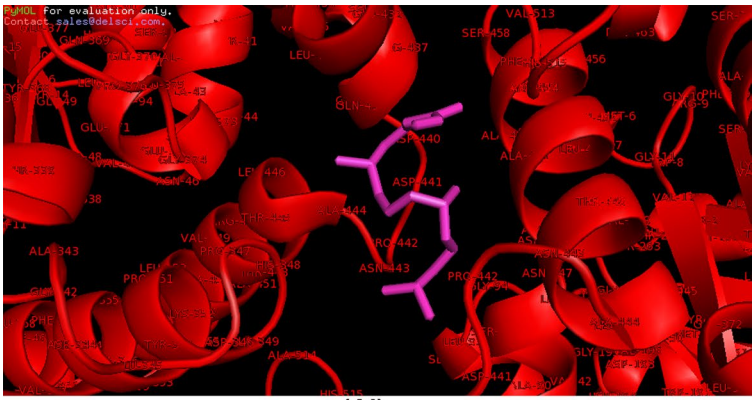


(Avii)

ranges from sucrase to α-amylase and α-glucosidase in decreasing order with CEA and CEP possessing the highest inhibition 62.67 ± 1.45 µg/mL and 57.67 ± 0.88 µg/mL respectively for sucrase. Thus, inhibiting these enzymes could play a crucial role in controlling the hyperglycemic condition by limiting glucose absorption in the blood [3, 32]. Thus lowering the postprandial hyperglycemia related responses in diabetes and complements the already established claim by [19, 33]. However, none of the extracts from plants possessed inhibitory potential than acrabose against the three assessed carbohydrate hydrolyzing enzymes.

Also, molecular mechanistic evaluation of reported compounds of methanol extracts (crude extract of both plants) against the three selected studied enzymes (α-amylase, sucrase-isomaltase and α-glucosidase) carried out revealed the possible interactions between the enzymes and the compounds. 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 least values having the highest affinity [34]. All the compounds docked against α-amylase (1SMD) possessed higher affinity than acarbose except caryophyllene while



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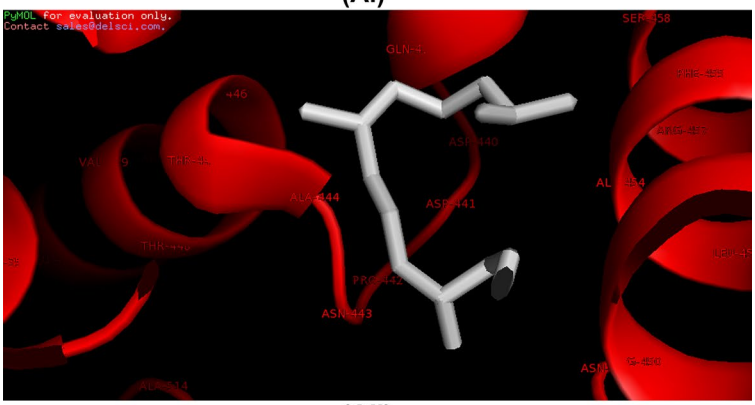

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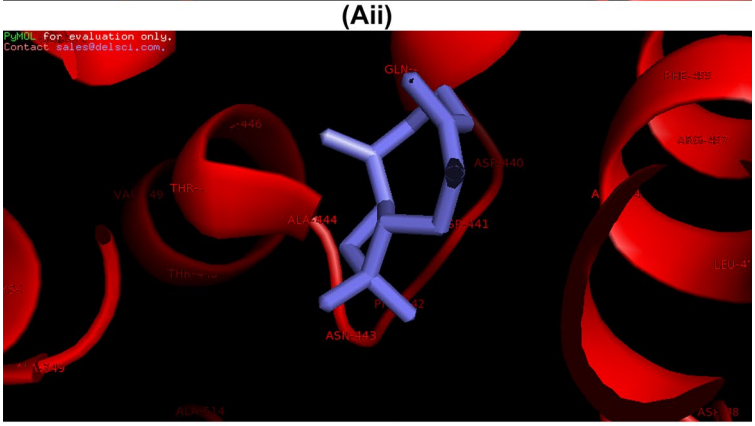

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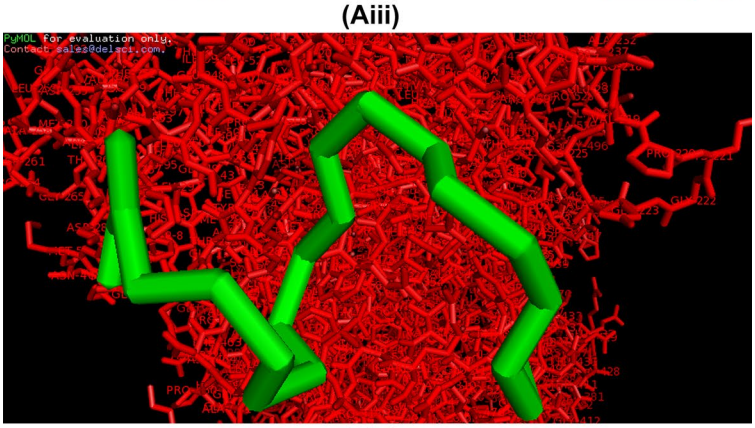
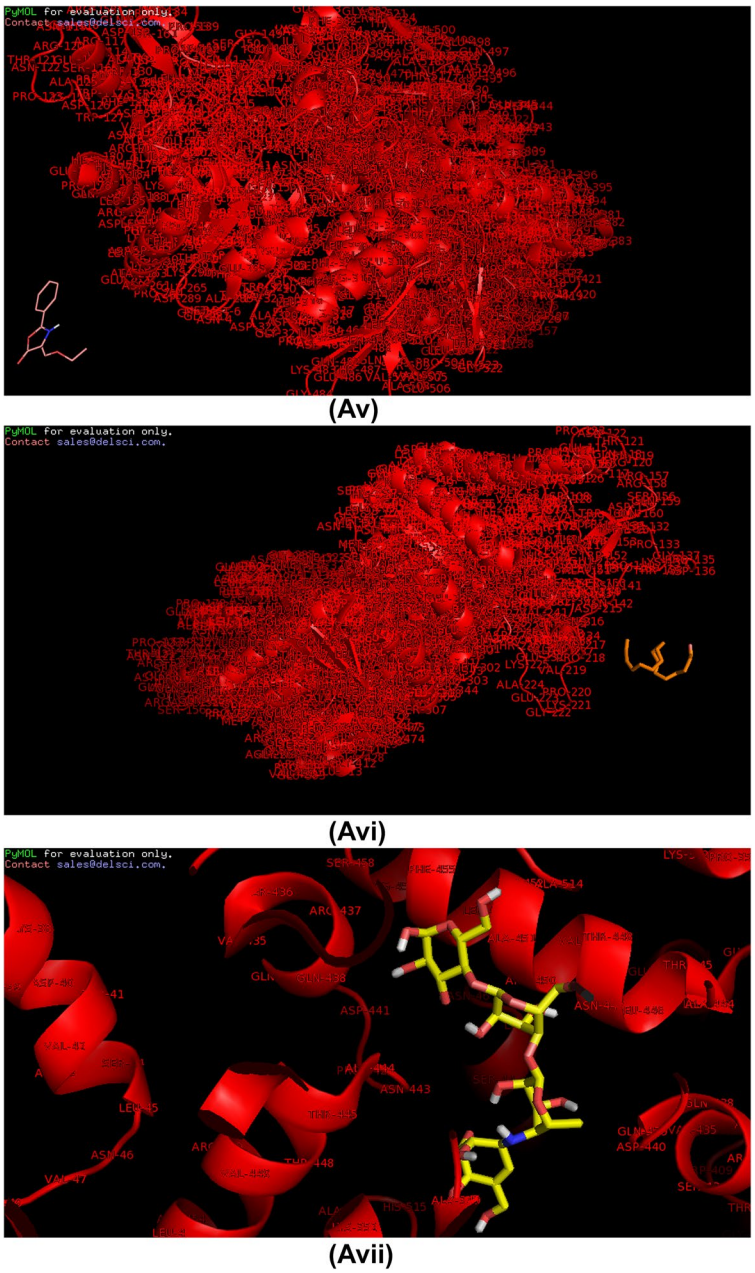


(Aiv)

Fig. 3 Molecular docking of compounds with enzyme **Ai** α-glucosidase (3WY1) and neophytadiene (10446), **Aii** α-glucosidase (3WY1) and phytol (5280435), **Aiii** α-glucosidase (3WY1) and caryophyllene (5354499), **Aiv** α-glucosidase (3WY1) and 9,12,15-octadecatrienoic acid (860), **Av** α-glucosidase (3WY1) and oxazolone (1712094), **Avi** α-glucosidase (3WY1) and 9,17-octadecadienal (5365667) and **Avii** α-glucosidase (3WY1) and acarbose (41774)

Fig. 3 (continued)



none had higher stability than acarbose against sucrase-isomaltase (3LPO) and α-glucosidase (3WY1). Also, phytol (5280435) has the highest biniding energy against the three enzymes compared to other two compounds from *A. conyzoides* and none of the compounds from *P. amarus* docked with α-glucosidase (3WY1) possessed any binding energy. In addition, the amino acid residues at 1SMD active site include Gly-334, Pro-332, Leu-293, Phe-335 while the residue at 3LPO acive site include Glu-47, Gln-48, Phe-49, Pro-495 and 3WY1 amino acid residues at the active site include Ala-349, Asp-440, Pro-442, Asn-443. The docking and the hyperglycemia studies showed that the methanolic extracts of the plants leaves could contain good inhibitors that could interfere with the selected hydrolases compared to acarbose and limit the rate of glucose absorption in the gut. Though, *P. amarus* leaf methanol extract posed to be more effective than *A. conyzoides* leaf methanol extract as seen in the docking studies which was not obvious in the in vitro hyperglycemia inhibition assays. In addition, the active compounds in plant-based foods are numerous in number and could exhibit synergistic properties in reducing the risk of chronic diseases and maintenance of cell safety. Therefore, interactions between these compounds could result to the increase observed pharmacological activity, and probably the shrub's therapeutic effects as depicted through the superimposition

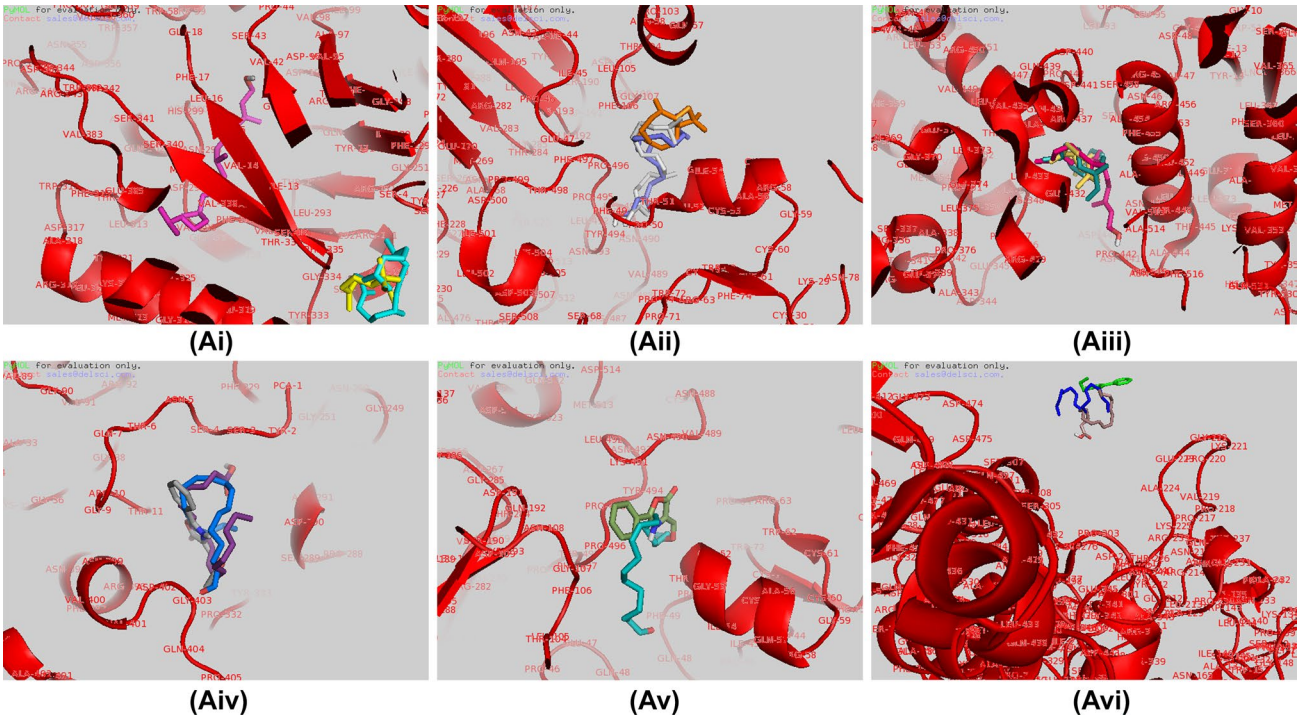


Fig. 4 Superimposition of 3-D structures of neophytadiene (10446), phytol (5280435) and caryophyllene (5354499) from *A. conyzoides* in the binding site of **Ai** α-amylase (1SMD), **Aii** sucrose-isomaltase (3LPO) and **Aiii** α-glucosidase (3WY1) and superimposition of 3-D structures of 9,12,15-octadecatrienoic acid (860), oxazolone (1712094) and 9,17-octadecadienal (5365667) from *P. amarus* in the binding site of **Aiv** α-amylase (1SMD), **Av** sucrose-isomaltase (3LPO) and **Avi** α-glucosidase (3WY1)

Table 1 Inhibitory potentials of dried leaves of *A. conyzoides* and *P. amarus* on carbohydrate hydrolyzing enzymes

Extracts	α-Amylase (IC50, µg/mL)	Sucrase (IC50, µg/mL)	α-Glucosidase (IC50, µg/mL)
AREA	734.33 ± 76.02^{a}	115.10 ± 18.98^{a}	177.33 ± 16.83^{a}
BPEA	244.67 ± 40.91^{b}	115.72 ± 6.14^{a}	136.00 ± 11.02^{b}
CEA	78.00 ± 1.73^{c}	62.67 ± 1.45^{b}	89.67 ± 3.48^{c}
AREP	373.00 ± 24.00^{d}	2075.33 ± 1474.52^{c}	158.33 ± 0.33^{d}
BPEP	257.33 ± 11.57^{b}	87.67 ± 6.23^{d}	157.00 ± 2.89^{d}
CEP	77.00 ± 1.16^{c}	57.67 ± 0.88^{e}	95.33 ± 2.60^{c}
Acarbose	32.29 ± 6.82^{f}	30.72 ± 1.42^{f}	33.11 ± 7.12^{d}

Values are expressed as mean of 3 replicates ± standard deviation of mean. Values with different superscripts within a column are significantly different ($p < 0.05$)

ARE aqueous residual extract, *BPE* butanol partitioned extract, *CE* methanol crude extract (*A A. conyzoides*, *P P. amarus*)

of the docked compounds inside the binding sites of the enzymes except 3WY1.

Albumin, an abundant plasma proteins, its glycation form glycated albumin (GA) that is ten times more than the glycation of hemoglobin in type II Diabetes mellitus [35]. Human albumin presents 50% of the normal individual's plasma protein and it is a marker reflects a short-term glycemic control [36]. In serum, the concentration of all thiols added together is lower than albumin being the most abundant thiol [37]. Glycation is one of the major disruptive spontaneous/non-spontaneous reactions occurring between proteins and reducing sugars that result in secondary complications in diabetic patients. *P. amarus* and *A. conyzoides* methanol extracts were significantly different ($p < 0.05$) from aqueous and butanol extracts on antiglycation property. However, the methanol extract of *P. amarus* exhibited better inhibitory protein glycation. Thus, preventing the deposit of long chain fatty acids and promote drug binding at various stages of diabetes [38, 39]. Also, the methanol extracts of both herbs

Table 2 Evaluation of binding energies (kcal/mol) for the selected molecules from dried leaves of *A. conyznoides* and *P. amarus*

Methanolic extracts	Molecules	PubChem CID	MW (g/mol)	α-Amylase (1SMD)	Sucrase-iso-maltase (3LPO)	α-Glucosidase (3WY1)
A. conyzoides	Neophytadiene	10446	278.5	−3.9	−5.6	−5.3
	Caryophyllene	5354499	204.35	13.1	−5.3	−6.2
	Phytol	5280435	296.5	−5.2	−5.8	−6.8
P. amarus	9,12,15-Octadecatrienoate	860	278.4	−4.3	−6.4	0.0
	Oxazolone	1712094	217.22	−5.7	−6.1	0.0
	9,17-Octadecadienal	5365667	264.4	−4.0	−5.2	0.0
Control	Acarbose	41774	645.6	0.7	−7.0	−9.8

Table 3 Oxidation of thiol, anti-glycation and β-amyloid aggregation inhibitory properties of dried leaves of *A. conyznoides* and *P. amarus*

Extracts	Inhibition of albumin glycation (%)	Inhibition of thiol groups oxidation (nmol/mg protein)	Inhibition of β-amyloid aggregation (%)
AREA	48.67 ± 0.88^{a}	1.76 ± 0.06^{a}	6.67 ± 0.33^{a}
BPEA	64.33 ± 0.88^{b}	2.02 ± 0.09^{b}	16.00 ± 0.58^{b}
CEA	74.67 ± 1.45^{c}	2.92 ± 0.04^{c}	26.33 ± 0.33^{c}
AREP	53.67 ± 0.33^{d}	1.87 ± 0.02^{d}	9.00 ± 0.58^{d}
BPEP	69.67 ± 0.33^{e}	2.17 ± 0.06^{b}	16.67 ± 0.88^{b}
CEP	77.67 ± 0.33^{f}	2.89 ± 0.13^{c}	28.00 ± 0.58^{c}

Values are expressed as mean of 3 replicates ± standard deviation of mean. Values with different superscripts within a column are significantly different ($p < 0.05$)

ARE aqueous residual extract, *BPE* butanol partitioned extract, *CE* methanol crude extract (*A A. conyzoides*, *P P. amarus*)

could probably inhibit the activation and aggregation of platelet and promote glucose uptake [40, 41].

Furthermore, oxidation of macromolecules such as lipids, DNA and proteins by ROS plays an important role in diabetes, cardiovascular disease and other diseases relating to aging like inflammation, cancer. Findings from epidemiology and experiment as well as clinic provided evidence on supportive role of reactive oxygen species (ROS) such as singlet oxygen, superoxide anions (O_2), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH) in the etiology of diabetes, cardiovascular diseases, aging etc. [42]. No significant difference between the methanol extracts of the plants as well as the butanol extracts while a significant difference is observed among the three solvents with aqueous extract possessing poor thiol protein protection activity and a fair active of thiol protein protection is observed for butanol extracts of both plants. The high concentration of thiol proteins methanol extracts indicates high potency for the methanol extracts of the plants and could help to reduce the effect of oxidative damage on the sulphydryl group thus protecting the human body from lipid peroxidation that causes cardiovascular disease, a secondary complication of diabetes.

To substantiate the glycation inhibition findings, the inhibition of β-amyloid fibril formation in glycated albumin was carried out since glycation has been reported by Emendato et al. [43] to increase the level of amyloid cross β structure, thus aggravating the cytotoxicities of protein aggregation and in general hyperglycaemia.

5 Conclusion

In an attempt to search for a novel phytomedicine for the prevention of secondary complications such as neuropathy, nephropathy, retinopathy etc. arising from diabetes, anti-amyloid aggregation potential of *P. amarus* and *A. conyzoides* extracts using different solvents were evaluated. The result of the present study showed that *P. amarus* exhibited stronger inhibitory potential in all three solvents against amyloid formation when compared with *A. conyzoides* fibrillation-inhibiting potential except with butanol extracts where there is no significant difference. Although *P. amarus* and *A. conyzoides* may be suggested as a potential therapeutic drug for prevention and treatment of secondary complications arising from DM, bioactive compounds of methanol extract of *P. amarus* could probably prevent secondary complications in diabetes compared to *A. conyzoides* methanol extract based on the results obtained from the docking studies and

the biochemical estimations carried out by the present researchers. However, there is a need to carry out further investigations on other reported compounds in the plant with the help of the in silico approach to generate more effective and potential drug through ligand-based drug designing approaches.

Compliance with ethical standards

Conflict of interest The authors declared that they have no conflict of interest.

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