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Original Article

Liquid chromatography-mass spectroscopy and molecular docking analysis of Gymnema sylvestre methanol leaf extract revealed antioxidant potential of quercetin

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Abstract:

Background and aims: *Gymnema sylvestre*, commonly known as periploca of the woods, belongs to the family Asclepiadaceae. Several studies have reported that the plant possesses some medicinal properties. A wide variety of diseases such as cancer, diabetes, and cardiovascular disease in human are directly associated with oxidative stress. This study was aimed to examine the possible antioxidant activity of ethyl acetate and aqueous fractions of *G. sylvestre*.

Methods: The antioxidant property of the extract fractions was determined using *in vitro* assays, 2,2 - diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant potential (FRAP) methods. To identify compounds in the fractions with the antioxidant property, liquid chromatography-mass Spectroscopy (LC-MS) was carried out.

Results: Ethyl acetate fraction showed the highest DPPH scavenging activity (significantly higher than the standard) [(88.98±2.69)% with lowest IC_{50} at 1.01±0.04 mg/cm^3] compared to aqueous fraction [(83.86±0.64)% with highest IC_{50} at 1.04±0.01 mg/cm^3] and standard ascorbic acid [(84.71±0.60)% and IC_{50} 1.03±0.01 mg/cm^3]. However the aqueous fraction was significantly weaker than the standard for DPPH scavenging activity. The FRAP assay yielded similar result to those of DPPH assay [(0.452±0.002)%, (0.426±0.000)%, and (0.425±0.001)% for ethyl acetate extract, aqueous extract and the standard (ascorbic acid), respectively]. LC-MS of the fractions revealed different compounds including $C_{17}H_{15}O_7$, $C_{27}H_{20}O_{18}$, $C_{28}H_{24}O_{16}$ (in ethyl

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acetate extract) and $C_{15}H_{10}O_9S$, $C_{15}H_{16}O_{12}$, $C_{27}H_{26}O_{16}$ (in aqueous extract). Molecular docking analysis of the identified compounds against some useful enzymes (alpha glucosidase and alpha amylase) suggested that these compounds could be a potential inhibitors of the enzymes.

Conclusion: Therefore, *G. sylvestre* leaf extract could be recommended as a potential natural antioxidant source that could be used in preventing oxidative stress-associated diseases and in drug management.

Keywords: Antioxidant, in vitro, Gymnema sylvestre, ethyl acetate, free radical, aqueous.

INTRODUCTION

Gymnema sylvestre, commonly known as periploca of the woods, gymnema or Australian cow plant belongs to the family Asclepiadaceae (Tiwari *et al*., 2014). It is cultivated in many parts of the world particularly Africa and Asia. In Nigeria, the plant is mostly cultivated in the northern region of the country (Ibrahim *et al*., 2017). It is one of the most widely known herbs and has antidiabetic potential and other beneficial properties for health (Bone, 2007).

Vitamin C and E, carotenoids, flavonoids and tannins are some of the secondary metabolites that are present in many plants and can be used as antioxidants for both humans and animals (Rachh *et al*., 2009). However other medicinal potentials of the plants depend on their phytochemical compounds that produce a definite physiological effect on the human body (Rouhi *et al*., 2017).

Therefore, search for other plants with antioxidant properties is currently being encouraged (El-Hela *et al*., 2010). The exclusive defendant on plants as a source of medicine is up to 90% in most of the African countries (Hostettmann, *et al.,* 2000). Murtala *et al.,* (2017), suggested the potential of natural remedy of *G. sylvestre* for the management of type 2 diabetes and some other metabolic disorders. Several studies have revealed numerous important properties of various fractions of this plant extract against different illnesses (Tahir *et al*., 2017). In another study carried out by Alam *et al*. (2011), the hydroalcoholic extract of *G. sylvestre* had a good wound healing property as compared to the control group.

Many metabolic by-products in biological systems lead to the generation of reactive oxygen species (ROS) (Naverro-yepes *et al*., 2014). Such metabolic activities are dependent on appropriate free radicals production

at low concentrations (at equilibrium with the endogenous antioxidants) (Rajendran *et al*., 2014). Antioxidants are classified based on their origin and source (Hamid *et al*., 2010). Endogenous antioxidants are inbuilt defense mechanisms that help to protect the body against free radical-induced cell damage (Yadav *et al*., 2016). Exogenous antioxidants can be derived naturally from many sources, but can also be synthesized (Litescu, 2011).

The compounds obtained from natural sources with activity against free radicals show many potential on individuals which drawn concern from many researchers all over, because they are very effective and safe for consumption (Gabriele *et al*., 2017). Therefore, investigation of these potential compounds is necessary. This study is aimed to investigate the potentials of some fractions of *G. sylvestre* leaf extract against free radicals.

MATERIALS AND METHODS

Plant Sample Collection

The plant samples were obtained fom Filin Shagari Area in Bauchi State, Nigeria. They were then transferred to the Herbarium Unit of Biological Sciences Department, Bayero University, Kano, where voucher specimen no. BUKHAN0349 was deposited for them. The leaves of the samples were shade dried and then grounded into smooth powder and kept in clean polythene nylon per the procedure described by Ibrahim *et al.* (2017).

Preparation of Extract and Fractions

The leaves were extracted as reported by Ibrahim *et al.* (2017). Briefly, 40 g of the leaves was weight and dissolved in 200 ml of methanol as solvent. The mixture was left for 48 hours and then filtered using a nylon sieve. The residue was allowed to dry and re-extracted twice using a fresh solvent (methanol) for 24 hours. Five g of the dried extract was dissolved in 150 ml of water in separating funnel with addition of an equal volume of ethyl acetate, and then the resulting solution was vigorously shaken and left to stand until two clear layers were formed. The upper layer that was the ethyl acetate fraction was carefully separated from the aqueous layer at the bottom.

Sample preparation

Stock solutions of the fractions (1% w/v) were prepared per the procedure described by Ibrahim *et al*. (2017). Briefly, about 0.1g of the extract was dissolved in 10 ml of methanol. As reference, ascorbic acid was also prepared in the same manner. The stock solutions were centrifuged at 4000 rpm and the supernatant was carefully removed and stored for further analysis

2,2-diphenyl-2-picrylhydrazyl (DPPH) Assay

The antioxidant activity of the two fractions of the plant extract were evaluated using DPPH as described by Shen *et al*. (2010) with minor modifications; the DPPH solution (0.1 mM) in methanol was prepared and 2 ml of this solution was added to 1 ml of various concentrations of the extract fraction prepared by serial dilution from the stock solution (0.16, 0.3125, 0.62, 1.25 and 2.50 mg/ml). The aliquots were shaken and incubated for 30 min at room temperature. All the samples were read against blank (solvent), DPPH solution without the plant extract was used as positive control, and ascorbic acid as reference standard. All measurements were done at 517 nm using UV-Vis Spectrometer (Agilent Cary 630).

Ferric reducing antioxidant potential (FRAP) assay

The antioxidant properties of the two fractions with respect to ferric reducing power were measured using the method reported by Ferreira *et al*. (2007) with some modifications. To this end, different concentrations of the extract fractions (0.1625-2.50 mg/ ml) were prepared in different test tubes. Then, phosphate buffer (0.2 M, pH 6.6) was added (2.5 ml) was added to 2.5 ml of each extract, and 2.5 ml of 1% potassium ferric cyanide was also added to the resulting solution. The obtained solution was incubated at 50°C for 20 min and allowed to cool down. Trichloroacetic acid (10%, 2.5 ml) was added and centrifuged at 6500 rpm for 10 min. Distilled water (2.5 ml) was added to an equal volume of the supernatant and then ferric chloride (1%, 0.5 ml) was added to the resulting solution that was allowed to stand for 10 min. The absorbance was measured at 700 nm using UV-Vis Spectrometer

(Agilent Cary 630). Ascorbic acid and all reagents in distilled water were used as reference standard and blank, respectively.

Liquid Chromatography-Mass Spectroscopy (LC-MS) profile

The LC-MS profile was conducted using LC-MS (LC Water e2695 separation module with W2998 PDA and couple to ACQ-QDA MS) per the procedure described by Piovesana *et al*. (2018) with some modifications. The extracted samples were dissolved in solvent and filtered through a 0.45-μm membrane filter. Ten μl of the filtrate was injected into the column (Sunfire C18 5.0 μm 4.6 mm × 150 mm). The run was carried out at a flow rate of 1.0 mL/min, with sample and column temperature at 25°C. The mobile phase consisted of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B) with a gradient as shown in Table 1. The PDA detector was set at 210-400 nm at a resolution of 1.2 nm and sampling rate of 10 points/sec. The mass spectra were obtained using the following settings: Scan ranging from 100 m/z to 1250 m/z, ESI source in both positive and negative ion modes, capillary voltage of 0.8 kV (positive) and 0.8 kV (negative); probe temperature 600°C; flow rate of 10 cm^3/min; nebulizer gas of 45 psi, fragmentation voltage of 125 V. At the completion of analysis, a software (Empower 3) was used to process all the data. The fragmentation pattern of individual chromatogram was obtained and used to identify all the compounds.

Molecular Docking Protocol

Ligand Structures

All ligand structures (obtained from pubchem site https://pubchem.ncbi.nhm.gov) were optimized using Merck Molecular Force Field (MMFF) and the Chemistry at Harvard Macromolecular Mechanics (CHARMm) force field, both of which are implemented in Discovery Studio Visualizer (version 3.5, BIOVIA Software, http://www.3dsbiovia.com/product/collaborative-science/biovia-discovery-studio/), to remove all strains from the molecular structure. This will also ensure a well-defined conformer relationship among compounds of the study (Viswanadhan *et. al.*, 1989). From the setup calculation option on

Discovery Studio Visualizer 3.5, the calculation was set to equilibrium geometry at the ground state using density functional theory at B3LYP (Becke88 three-parameter hybrid exchange potentials with Lee-Yang-Parr correlation potential) level of theory and 6-311G (d) basis set for the geometrical optimization of the cleansed structures, i.e., B3LYP/6-311G (d) level of theory. After optimization, Discovery Studio Visualizer descriptors were obtained from the display-output and display-properties option on Discovery Studio Visualizer 3.5. The fully optimized 3D structure without symmetry restrictions was saved as PDB (Protein Data Bank) file through the file option on the Discovery Studio Visualizer 3.5.

Enzyme Structure

The X-ray crystal structure of alpha-amylase with PDB ID: 4w93 and resolution of 1.9Å, and alpha-glucosidase with PDB ID: 3wy2 and resolution of 1.47Å were downloaded from RCSB Database (http://www.rcsb.org/pdb). Receptors were optimised, the energy was minimised, and water was removed using discovery studio visualise 3.5 and Pymol version 2.2.0, respectively, and the receptors were saved as PDB file.

Docking Simulations

All protein preparation and minimization was done using tools and protocols in the Discovery Studio Visualizer 3.5. Chemistry at Harvard Macromolecular Mechanics (CHARMm) force field was used to optimize the structure. While using the protein preparation protocol, hydrogen atoms were added to the complex, after which water molecules were removed and the pH of the protein was set to almost neutral value. A sphere binding site with a nine Armstrong A° radius was defined around the bonded ligand to identify the binding site of the protein structure. The PDB files of the ligands were then imported into PyRx-virtual screening tool and were used to dock the prepared receptors. The ligands are scored based on the biased probability Monte Carlo (BPMC) procedure, which randomly selects a conformation in the internal coordinate space and then makes a step to a new random position independent of the previous one but according to a predefined continuous probability distribution. The best score

and the results on the binding energy of all the ligands were reported on a table.

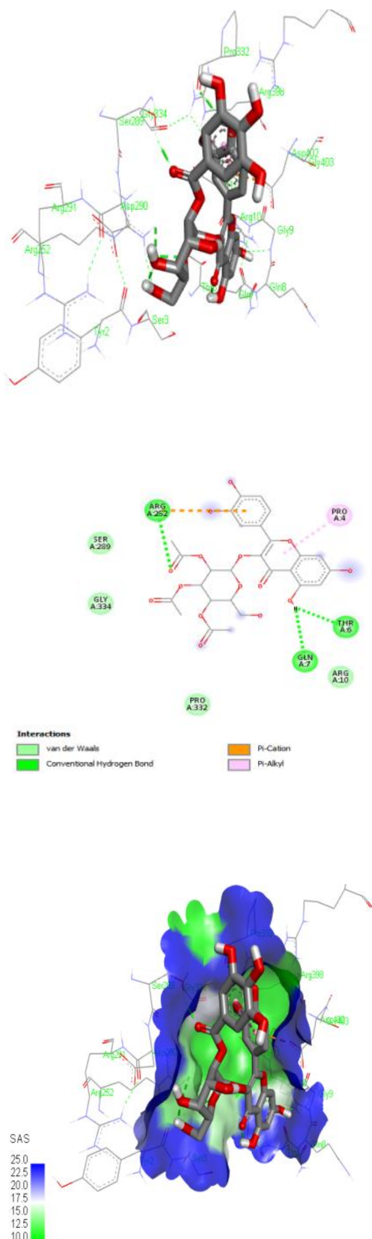

Figure 1: BIOVIA docking for predicted pose for (Quercetin 3-(2-galloylglucoside) in human pancreatic alpha-amylase (PDB ID: 4W93) showing: (Panel A and B) interactions with amino acid residues of target protein in 3D and 2D respectively; (panel C) the compound docked in the binding pocket of the protein

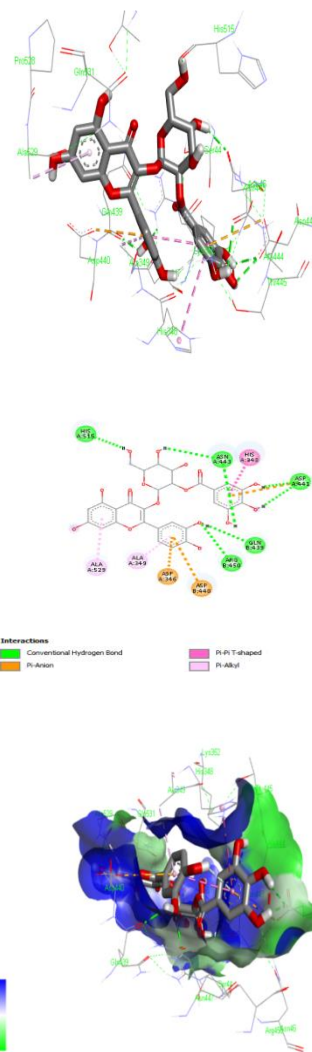

Figure2: BIOVIA docking for predicted pose for (Quercetin 3-(2-galloylglucoside) in alpha-glucosidase (PDB ID: 4wy2) showing: (Panel A and B) interactions with amino acid residues of target protein in 3D and 2D respectively; (panel C) the compound docked in the binding pocket of the protein.

Statistical Analysis

Data were analyzed using SPSS Minitab Package. The results were expressed as mean± standard deviation

(SD) of the three experiments and IC_{50} was calculated as inhibition percentage.

Table 1: LC-MS Solvent gradient

Time	%A	%B
0	95	5
1	95	5
13	5	95
15	5	95
17	95	5
19	95	5
20	95	5

Table 2: 1 2, 2-diphenyl-1-picrylhydrazyl (DPPH) Scavenging Assay

concentration (mg/mL)	% Inhibition		
	Aqueous faction	ethyl acetate fraction	ascorbic acid
0.15	75.44±0.75	63.79±0.75	69.85±14.27
0.31	78.87±0.56	77.90±0.72	78.75±3.49
0.62	81.09±0.24	81.52±9.64	80.4±2.56
1.25	82.90±0.32	84.40±4.82	82.47±1.36
2.50	83.86±0.64	88.98±2.69	84.71±0.60
IC_{50} (mg/mL)	1.04±0.00	1.01±0.04	1.03±0.01

Values are expressed as mean ± standard deviation, (n=3). Ascorbic acid was used as standard. IC50 is the 50% inhibitory concentration in mg/mL

Table 3: Ferric reducing potential by *Gymnema sylvestre* methanol leaf extract fractions

concentration mg/mL	ethyl acetate fraction	Aqueous fraction	ascorbic Acid
0.16	0.213±0.025	0.039±0.001	0.167±0.001
0.31	0.273±0.002	0.134±0.001	0.280±0.001
0.63	0.313±0.002	0.332±0.001	0.391±0.001
1.25	0.342±0.002	0.337±0.001	0.427±0.002
2.50	0.452±0.002	0.425±0.001	0.426±0.000

Values were expressed as mean ± SD, (n=3). Ascorbic acid was used as standard.

Table 4: LC-MS Profile of Aqueous Fraction

Peak No.	Rt	Base peak (m/z)	molecular weight g/mol	Tentative compound	Molecular formula
1	7.145	367.2	366.3	luteolin 7-sulfate. (flavone)	$C_{15}H_{10}O_9S$
2	9.548	389.3	388.3	6-[5-(2-carboxy-2-oxoethyl)-2,3-dihydroxyphenoxy]-3,4,5-trihydroxyoxane-2-carboxylic acid. (phenolic glycoside)	$C_{15}H_{16}O_{12}$
3	5.664	390.0	389.0	1,6-dimethyl 2,3,4-trihydroxy-5-(3,4,5-trihydroxybenzoyloxy)hexanedioate Dimethyl 2-galloylgalactarate	$C_{15}H_{18}O_{12}$
4	6.352	607.4	606.4	6-{[6-(3,4-dihydroxy-6-methyl-5-oxooxan-2-yl)-2-(3,4-dihydroxyphenyl)-5-hydroxy-4-oxochromen-7-yl]oxy}-3,4,5-trihydroxyoxane-2-carboxylic acid. (Flavonol glycoside)	$C_{27}H_{26}O_{16}$

Table 5: Liquid chromatography-Mass Spectroscopy (LC-MS) Profile of Ethyl Acetate

Peak No.	Rt	Base peak (m/z)	molecular weight g/mol	Tentative compound	Molecular formula
1	5.330	332.3	331.3	3,5,7-trihydroxy-2-(4-hydroxy-3,5-dimethoxyphenyl)-5H-chromen-5-yl	$C_{17}H_{15}O_7$
2	6.871	633.3	632.4	7-[4-(1,2-dihydroxyethyl)-5,11,12,13-tetrahydroxy-8-oxo-3,7 (Flavanol) dioxatricyclo[7.4.0.0^{2,6}]trideca-1(13),9,11-trien-10-yl]-3,4,8,9,10-pentahydroxy-6-oxobenzo[c]chromene-1-carboxylic acid (flavanone glycoside)	$C_{27}H_{20}O_{18}$
3	6.979	617.4	616.4	Quercetin 3-(2-galloylglucoside	$C_{28}H_{24}O_{16}$

Rt: Retention time, m/z: mass to charge ratio. Molecular weight: (Base peak-H+)

Table 6. BIOVIA docking scores (Kcal/mol) for compound identified from aqueous fraction of G.s methanol leaf extract

		Target protein			
		4w93		3wy2	
Des.	Compound	Scores	H-b	Scores	H-b
A	6-[5-(2-carboxy-2-oxoethyl)-2,3-dihydroxyphenoxy]-3,4,5-trihydroxyoxane-2-carboxylic acid. (phenolic glycoside)	-8.8	6	-8.4	6
B	1,6-dimethyl 2,3,4-trihydroxy-5-(3,4,5-trihydroxybenzoyloxy)hexanedioate Dimethyl 2-galloylgalactarate	-7.0	9	-7.7	4
C	6-{[6-(3,4-dihydroxy-6-methyl-5-oxooxan-2-yl)-2-(3,4-dihydroxyphenyl)-5-hydroxy-4-oxochromen-7-yl]oxy}-3,4,5-trihydroxyoxane-2-carboxylic acid. (Flavonol glycoside)	-7.8	3	-7.7	4
D	Acarbose (Control)	-9.9	6	-9.5	8

Scores: binding energy, Des: Designation, 4w93: human pancreatic alpha-amylase, 3wy2: Apha-glucosidase, H-b: number of hydrogen bond

Table 7. BIOVIA docking scores (kcal/mol) for compounds identified from ethyl acetate fraction of *Gymnema sylvestre* methanol leaf *extract*

Target protein					
		4w93		3wy2	
Des.	Compound	Scores	H-b	Scores	H-b
A	3,5,7-trihydroxy-2-(4-hydroxy-3,5-dimethoxyphenyl)-5H-chromen-5-yl	-7.1	6	-8.8	6
B	7-[4-(1,2-dihydroxyethyl)-5,11,12,13-tetrahydroxy-8-oxo-3,7 (Flavonol) dioxatricyclo[7.4.0.0^{2,6}]trideca-1(13),9,11-trien-10-yl]-3,4,8,9,10-pentahydroxy-6 oxobenzo[c]chromene-1-carboxylic acid (flavanone glycoside)	-9.0	9	-10.6	4
C	Quercetin 3-(2-galloylglucoside	-9.2	3	-9.1	4
D	Acarbose (Control)	-9.9	6	-9.5	8

Score: binding energy, Des: Designation, 4w93: human pancreatic alpha-amylase, 3wy2: Apha-glucosidase, number of hydrogen bond

RESULTS

The results on DPPH scavenging assay are shown in Table 2. The values obtained by DPPH scavenging activity for ethyl acetate fraction of the plant extract are statistically different when compared to the ascorbic acid standard, yet significantly lower than standard using the aqueous fraction. Besides this, the ferric reducing potential of the plant extract is shown in Table 3. The LC-MS profile of the aqueous and ethyl acetate fractions of methanol *G. sylvestre* leaf extract are presented in Table 4 and 5, respectively. The profile revealed numerous compounds, some which were identified using their molecular fragmentation pattern. Molecular docking analysis results are illustrated in figures 1 and 2, for some of the identified compound against two important enzymes that are linked to diabetic condition. And the summary of the docking scores are shown in Table 7, which shows the binding capabilities of the identified compounds that correspond to their inhibitory potentials.

DISCUSSION

The results on DPPH scavenging assay showed that all the fractions of the extract exhibited potential against free radical in a concentration-dependent manner. However ethyl acetate fraction of *G. sylvestre* revealed higher DPPH radical scavenging potential [(88.98±2.69)%] at 2.5 mg/ml than aqueous fraction and ascorbic acid (reference standard) [(83.86±0.64)% and (84.71±0.60)%, respectively] at 2.5 mg/ml. The free radical scavenging property was expressed as 50% inhibitory concentration (IC_{50}). The lowest IC_{50} (1.01±0.04 mg/ml) was observed for ethyl acetate fraction followed by standard ascorbic acid (1.03±0.01 mg/ml) and aqueous fraction (1.04±0.00 mg/ml), revealing that the ethyl acetate fraction exhibited greater potency for scavenging DPPH free radicals. In a research conducted by Srinivasa, and Jyothi (2019), some similarities were observed with respect to ethyl acetate fraction's highest antioxidant activity among other fractions.

Ethyl acetate fraction of *G. sylvestre* showed greater reducing ability compered to aqueous fraction and ascorbic acid, which is the reference standard. The highest

reduction in absorbance of ethyl acetate and aqueous fractions and ascorbic acid were 0.452±0.002, 0.425±0.001 and 0.426±0.000, respectively (Table 3). Interestingly, this result follows the trend of the DPPH scavenging property reported in previous studies that could be due to polarity difference, on which extraction of secondary metabolite depends. In several studies, substantial antioxidant and antidiabetic activities of methanol and ethyl acetate leaf extract of this plant have been observed (Ibrahim *et al*. 2017; Murtala *et al.*, 2017; Gunasekaran *et al*., 2019).

The LC-MS profile of the aqueous and ethyl acetate fractions of methanol *G. sylvestre* leaf extract had different compounds. Database was used to obtain the tentative compounds, from which many compounds were identified, based on their molecular fragmentation, in both ethyl acetate and aqueous fractions of methanol *G. sylvestre* leaf. These compounds identified from the l cms profile could be the reasons for the activities observed in this study. Similarly, these compounds have been reported in many studies to possess antioxidant, anti-inflammatory and antimicrobial properties such as flavone (leutolein and leutolein glycoside) and galloy-glycoside from the aqueous fraction (Paul and Jayapriya 2009; Khan *et al.*, 2019). However, Tannin, quercetin 3-(2-galloylglucoside) and anthocyanidin were identified from the ethyl acetate fraction. Other studies on quantitative and qualitative phytochemical screening on *G. sylvestre* leaf extract have revealed the presence of flavonoids, tannins, saponins and alkaloid in this plant (Murtala *et al.*, 2017; Srinivasa, and Jyothi, 2019). Tannins are very useful secondary metabolites especially for the treatment of numerous tissue damages and show remarkable activity for cancer prevention (Aiyegoro and Okoh, 2010). Quercetin glycoside (quercetin 3-(2-galloylglucoside) has been reported to exhibit diverse biological activities, including antioxidant, anti-inflammatory, hepatoprotective, and transporter inhibiting properties (Wu *et al.,* 2012; Chen *et al*., 2019). Anthocyanidin exhibits antioxidant and anti-hypertensive activity due to its hydrogen donating ability and its ability to inhibit angiotensin-1 converting

enzyme (Cui *et al*., 2013). Significant hypoglycemic effect of ethyl acetate extract of this plant on alloxan-induced diabetic mice has been reported that explains its traditional usage as an antidiabetic herb. The hypoglycemic activities have been attributed to its phytochemical content such as flavonoid glycoside and other polyphenols capable of inhibiting alpha-amylase and alpha-glucosidase. Therefore, hyperglycemia-induced oxidative stress is prevented by glucose auto-oxidation (Ibrahim *et al* 2017; Murtala *et al*., 2017).

The molecular docking results showed higher ability of prediction for binding between ligand and protein that could be the reason for its frequent application in structure based drug design , with a substantial degree of accuracy, in which small-molecule ligands bind within the appropriate target binding site (Meng *et al*., 2011). Docking scores derived from the binding energy are used to predict the degree of affinity between protein ligand interactions (Ononamadu and Ibrahim, 2019). The lower the binding energy, the more stable the ligand target complex (i.e., large negative value of score indicate greater affinity between ligand target interactions). In the present study, potential bioactive compound identified from the aqueous and ethyl acetate fractions of *G.sylvestre* was docked against a target protein human pancreatic alpha-amylase and alpha-glucosidase. The compound designated C (quercetin 3-(2-galloylglucoside) isolated from the ethyl acetate fraction exhibited a binding energy of -9.1 Kcal/mol to the apha-amylase active site and -9.2 Kcal/mol to the alpha-glucosidase active site, close to that of compound D (arcabose) that was used as control. The 2D structure revealed conventional hydrogen bond interaction between the compound and Arg252, Ser289, Pro332 residue in the alpha-amylase active site. In alpha-glucosidase, the hydrogen bond is between His515, Asn443, Asp441, Gln439 and Arg450. This degree of binding energy and hydrogen bond interaction exhibited by this compound could make it a potential antagonist of pancreatic alpha-amylase and alpha-glucosidase, therefore contributing to the hypoglycemic activity of *G. sylvestre* leaf extract reported by

Ibrahim *et al*. (2017). And by extension, the hypoglycemic effect could also play a role in managing hyperglycemia-induced oxidative stress.

CONCLUSION

The present study revealed that fractions of methanol (ethyl acetate and aqueous) extract of *G. sylvestre* leaf exhibited noticeable antioxidant potential against DPPH free radical and ferric ion, with a comparably higher activity for ethyl acetate fraction. The LC-MS profile shows that the fractions contain flavonoids (quercetin glycoside, anthocyanin) and tannin. Molecular docking of quercetin 3 - (2-galloylglucoside) one of identified compound from the ethyl acetate fraction against some key enzymes of diabetic conditions (alpha-amylase and alpha-glucosidase) suggests anti-diabetic potential of this plant. The observed antioxidant activity could be related to the hydrogen donating ability of these compounds. Therefore, *G. sylvestre* leaf extract could be recommended as a potential natural antioxidant source to prevent oxidative stress-associated diseases such as cancer and also in drug development.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

FUNDING

None

CONFLICT OF INTERESTS

None.

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