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STRUCTURE-ACTIVITY-RELATIONSHIP OF THE POLYPHENOLS INHIBITION OF α-AMYLASE AND α-GLUCOSIDASE

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Abstract

Background: Diabetes mellitus (DM) is a serious public health challenge, projected by WHO to be one of the 7 leading cause of death by 2030. Medicinal plants have been demonstrated to be useful in DM local management because of polyphenols present in these plants. For an alternative treatment approach especially with polyphenols-rich herbs, knowledge of comparative efficacy of the polyphenols will lead to enhanced therapy especially in postprandial hyperglyceamic control.
Materials and Methods: Vegetative parts of *Anacardium occidentale, Abelmoschus ecsulentus and Ceiba pentandra*, prominent in the local management of DM were identified, collected and subjected to alcoholic extraction. From the crude extracts were isolated agathisflavone, quercetin 3-*O*-glucoside, quercetin 3-*O*-diglycoside, mangiferin, isomangiferin and pentagalloyl glucose, belonging to flavonoid, xanthones and tannins structural classes. These polyphenols were evaluated for their potentials to inhibit both α-glucosidase and α-amylase. Physicochemical parameters of the polyphenols were evaluated and molecular docking experiments were carried out to gain insight into the observed inhibitory activity.
Results: quercetin 3-*O*-glucosidewas the most potent of the polyphenols against the two enzymes. Increase in the number of phenolic hydroxyl group did not increase the inhibitory activity and neither computation of the binding energies with the enzymes nor physicochemical parameters of the polyphenols could explain the observed inhibitory activity against the enzymes, across the structural classes. Thus, only the bioassay against the enzymes α-glucosidase and α-amylase correlated well with the use of the plants in treating diabetic mellitus
Conclusion: Medicinal plants rich in quercetin 3-*O*-glycoside may have better treatment outcomes in postprandial hyperglycaemia control.

Key words:- polyphenols, diabetes mellitus, α-glucosidase, α-amylase, molecular docking, Postprandial hypergleamic control.

List of Abbreviation: DM: Diabetes mellitus, WHO: World Health Organisation, NIDDM: Non-Insulin Dependent Diabetes Mellitus, SDGT-1:Sodium-dependent glucose transporter 1, HRMS: High Resolution Mass Spectrometry, LCMS: Liquid Chromatography Mass Spectrometry, NMR: Nuclear Magnetic Resonance, PGG: Pentagalloyl Glucose

Introduction

The concept of drug-repurposing for orthodox drug could be extended to herbal medicine, especially in relation to some vegetables that are being used for culinary purposes (Taiwo *et al*., 2016). Studies had demonstrated that these vegetables are rich in polyphenols and that the health benefits derivable from the vegetables most times are as a result of the presence of polyphenols in these vegetables (Pandey *et al.*, 2009; Manach *et al* 2004). Prominent classes of polyphenols include flavonoids (flavonols, flavones, flavanones, anthocyanins, proanthocyanidins and catechins), phenolic acids (such as caffeic acid and gallic acid), stilbenoids (eg resveratrol), and selariscinins (Pandey *et a*l., 2009; Scalbert *et al.*, 2005 ; Manach *et al*., 2004). One of the diseases that polyphenols had been demonstrated to modulate is

non-insulin dependent diabetes mellitus (NIDDM). Diabetes mellitus, a significant public health concern, has been projected by WHO to be the seventh leading cause of mortality in 2030s (Danaei, 2011; WHO, 2011). Approaches to contain diabetes include life style adjustment that involves keeping a normal body mass ratio, regular physical exercise, refraining from smoking and a healthy diet. Both α-amylase and α-glucosidase as well as glucose absorption in the intestine by sodium-dependent glucose transporter 1 (SGLT1) may all be inhibited by polyphenols. Polyphenols may also enhance insulin-dependent glucose uptake, stir up 5′ adenosine monophosphate-activated protein kinase, affect the microbiota genome, initiate insulin secretion, decrease liver glucose output and have anti-inflammatory effects (Kim *et al.,* 2016; Xiao, 2015; Stefek, 2011 and Dembinska-Kiec, 2008).

Controlling postprandial blood glucose levels has been found to be highly important, as postprandial hyperglycemia is more critical than fasting blood glucose (Ye *et al*, 2010). Two enzymes had been found to be critical in influencing postprandial hyperglyceamia namely α-1,4-Glucan-4-glucanohydrolases, found in saliva and pancreatic juice and is known as α-amylase while the other group of enzymes is the α-glucosidases. They catalyze the breakdown of food carbohydrates and starches to produce glucose for intestinal absorption (Ye *et al*, 2010). This subsequently leads to increase in blood glucose levels in humans. Any approach therefore that leads to inhibiting the function of these enzymes in patients with NIDDM may reduce hyperglycaemia. Though polyphenols had been found to inhibit α-glucosidase and α-amylase, Cao *et al* (2018) concluded that the report on the structure-activity relationship of polyphenols as anti-diabetic agents are few. We therefore considered that the understanding of the structure activity relationship across various classes of polyphenols, may help in the choice of which particular vegetables or fruits (rich in a particular polyphenol) will interest diabetic people and be selected as sources of their dietary polyphenols.

Materials and Methods

The three medicinal plants used in this study have history in local management of diabetes, which informed their choice. All the plant vegetative parts were collected, identified by Mr A.A. Ogunlowo, Curator of the Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University and air dried. Voucher specimens with number FPI 2221*(A. esculentus*), FPI 2107 (*A.occidentale*) and FPI 2435 (*C. pentandra*) were deposited in the Faculty Herbarium. The vegetative part was air-dried at room temperature and milled. The TLC of the crude extracts (CE) obtained from the plants were monitored with EtOAc:MeOH:H_2O AcOH (10:2:1:0.2). Adsorption chromatography (open column) was carried out on Kieselgel 60 (ASTM 230–400 mesh, Merck) while gel permeation was achieved using Sephadex LH-20 (Pharmacia) which had been pre-swollen in specified solvent before loading onto the column. All thin layer chromatography analyses were carried out at room temperature using silica gel 60 GF_{254} pre-coated aluminum backed plates (Merck, 0.25 mm thick). The spots on TLC plates were viewed under UV light (254 and 366 nm) and also with the use of 1% w/v vanillin/H_2SO_4. ^1H and ^{13}C NMR spectra (for both 1D and 2 D experiments) were obtained on the Bruker AV400 (IconNMR) Spectrometer at 400 and 100 MHz respectively while the HRMS analyses were carried out on an Agilent Quadrupole Time-of-Flight LCMS machine at the School of Chemistry and Physics of the Kwazulu-Natal University in Pietermaritzburg, South Africa.

Isolation of compounds

Isolation of xanthones from *Ceiba pentandra* inflorescence

The powdered plant part (2.4 Kg) was extracted with 100% Methanol (2.5 L x 3) and the crude extract (CE) was concentrated *in vacuo* to give 384.7 g. 20 g of the CE was dissolved in 100 ml of water and defatted with n-hexane. 2 grams of the aqueous fraction was subjected to solid phase extraction with mobile phase in successive order of reducing polarity with MeOH:H2O (20:80, CP1; 50:50, CP2, 80:20, CP 3 and 100:0). Pooling after the TLC analysis of the eluate gave fractions CP1-CP3. Fraction CP 3 was subjected to gel permeation on Sephadex LH-20 using 10-30% methanol in ethyl acetate as the mobile phase. 5 mL of the eluate was collected in each test tube. Analysis of the eluate gave fractions CP3a-CP3e. Fraction CP3c 0.318 g (eluted with 20% methanol) gave a mixture of two spots which was subjected to repeated purification on silica gel to give greenish yellow powder isomangiferin (0.054 g, R_f 0.54) and dull yellow powder mangiferin (0.085 g, R_f 0.52)

Isolation of quercetin 3-*O*-glucoside and quercetin 3-*O*-diglycosides from *Abelmoschus esculentus* fruit

The powdered plant part (1.0 Kg) was extracted with 100 % Methanol (2.5 L) and the CE was concentrated in vacuo to give 164.8 g. 50.4 g of the CE was dissolved in 100 mLs of water and was defatted with *n*-hexane. The aqueous fraction was concentrated to dryness *in vacuo* and 2.1 grams of the aqueous fraction was subjected to solid phase extraction on Phenomenex cartridges using the following solvent mixtures: MeOH:H2O (20:80, AE 1 (0.942 g); 50:50, AE 2 (0.568 g); 80:20, AE 3 (0.207 g) and 100:0, AE 4 (0.184 g)). Fraction AE 3 was subjected to repeated purification on silica gel and Sephadex LH-20 column to give quercetin 3-*O*-glucoside (yellow powder 1 (0.085 g, R_f 0.52) and quercetin 3-*O*-diglycoside (brown powder, 0.034 g, R_f 0.44)

Isolation of pentagalloyl glucose and agathisflavone from *Anacardium occidentale* leaf

The powdered leaves were extracted with 96 % ethanol. The extract was filtered and concentrated in-vacuo at 400C to give 289 g (11.56 % w/w) of the crude ethanolic extract. The crude extract was dissolved in 200 mL of water and was partitioned between water and n-hexane (3 x 500 mL), water and ethyl acetate (8 x 250 mL) and water and n-butanol (4 x 200 mL) to give *n*-hexane (26.2 g), ethyl acetate (15.8 g*), n*-butanol (14.6 g) and aqueous (210.4 g) fractions. The ethyl acetate fraction (15.1 g) was dissolved in methanol and adsorbed on silica gel and allowed to dry.

The dry powder was packed into a column of silica gel (30 x 3 cm) and eluted in descending mode with solvent of increasing polarity from 100% Hexane to 100% ethyl acetate to 50% methanol. The fraction eluted with 100% ethyl acetate was subjected to repeated fractionation on silica gel and Sephadex LH-20 to give pentagalloyl glucose (0.188 g) and agathisflavone (0.026 g).

In-vitro α-amylase inhibitory assay

This assay was conducted according to the method of Bahman *et al*. (2008) with slight modification. The generation of reducing sugar was quantified by the reduction of 3, 5- dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid. Acarbose was used as a positive control. The α-amylase inhibition was calculated and expressed as percentage of inhibition as follows:

Inhibition (%) = [([(AE100)-(A0)]- [Atest -Ablank]) /{(A100)-(A0)}]*100
Where:
AE100. is absorbance of 100% enzyme activity,
A0 is 0% enzyme activity (only solvent without enzyme),
Atest, test sample (with enzyme) and
Ablank is blank (a test sample without enzyme)

In-vitro α-glucosidase inhibitory assay

The enzyme inhibition studies were carried out spectrophotometrically in a 96-well microplate reader using a procedure reported by Li *et al*. (2005). Dutrao (Model SM600, Shanghai Yongchuang Medical Instrument Co. Ltd) spectrophotometric microplate reader was used for the enzyme inhibitory activity assay.

The inhibition (%) was calculated using the formula:
[(Acontrol– Asample)/Acontrol]×100 %,
Where, Acontrol is the absorbance of the control, and Asample, the absorbance of the sample

Computation of binding affinities

The three dimensional x-ray crystallographic crystal structures of alpha glucosidase (6OBX.PDB) and alpha amylase (3TOP.PDB) complexed with acarbose respectively, were retrieved from the RCSB database. 3D models were each built for the natural products using the ChemBioOffice suite of molecular modeling software. The model generation protocol employed steepest descent energy minimization for structural optimization after which each model was saved in relevant formats. AutoDock Tool (Santos-Martins, 2014; Goodsell, 1996) was employed both for the calculation of Gasteiger partial charges for both the ligands and the two enzyme structures after that the bound inhibitors (acarbose and montrelin A analog) had been removed. In-house scripts were employed for preparing the docking files as well for performing the docking screens which employed AutoDock Vina (Trott and Olson, 2010).

Results

Spectroscopic data of isolated compounds

Quercetin 3-*O*-glycoside: ^{1}H NMR (300 MHz, CD_3OD): δ 7.73 (1H, d, J=2.1 Hz), 7.60 (1H, dd, J= 8.5, 2.1 Hz), 6.88 (1H, d, *J*=8.6 Hz), 6.41 (1H, d, *J*=2.1 Hz), 6.22 (1H, d, J=2.1 Hz), 5.25 (d, 1H, *J* = 7.6 Hz). ^{13}C NMR (75 MHz, MeOD): δ 179.5 (C-4), 166.0 (C-7), 163.0 (C-5), 159.0 (C-2), 158.5 (C-9), 149.9 (C-3′), 145.9 (C-4′), 135.6 (C-3), 123.2 (C-6′), 123.1 (C-1′), 117.6 (C-2′), 116.1 (C-5′), 105.7 (C-10), 104.3 (C-1″), 99.9 (C-6), 94.7 (C-8), 78.4 (C-5″), 78.1 (C-3″), 75.7 (C-2″),71.1 (C-4″),62.6 (C-6″). TOF ES MS m/z 463.0880 $[M-H]^-$,

Quercetin 3-*O*- diglycoside: ^{1}H NMR (300 MHz, CD_3OD): δH: 7.72 (1H, d, J=2.0), 7.68 (1H, dd, J=8.4, 2.0 Hz), 6.88 (^{1}H, d, *J*=8.2 Hz), 6.42 (1H, d, *J*=1.9 Hz), 6.22 (1H, d, *J*=1.8 Hz), 5.25 (1H, d, *J*=7.3 Hz), 4.17 (1H, d, *J*=7.6 Hz). TOF ES MS m/z 649.1378 $[M+Na]^+$.

Agathisflavone (2) was isolated as brown amorphous powder. ^{1}H NMR (400 MHz, CD_3OD) δH: 7.95 (2H, d, *J* = 8.9 Hz, H-2′,6′), 7.57 (2H, d, *J* = 8.8 Hz, H-2‴, 6‴), 6.99 (2H, d, *J* = 8.8 Hz, H-3′,5′), 6.72 (1H, s, H-6), 6.71 (1H, s, H-3), 6.63 (1H, s, H-3″), 6.57 (2H, d, *J* = 8.8 Hz, H-2‴, 6‴), 6.39 (1H, s, H-6″). ^{13}C NMR (100 MHz, CD_3OD). δC: 183.3 (Cq, C-4/C-4″), 164.2 (Cq, C-2,9/2″,9″), 162.8 (Cq, C-5/5″), 162.6 (Cq,C-7/7″), 162.2 (Cq, 4′/4‴), 129.3 (C-3′,5′/3‴,5‴), 123.6 (Cq, C-10/10″), 123.4 (Cq, C-8/1′/6″/1‴), 117.1 (CH, C-2′,6′/2‴,6‴), 103.4 (CH, C-3/3″) and 94.6 (CH, C-6/8″). LC-ESI-MS m/z 561$[M + Na]^+$, 537 $[M-1]^-$

Pentagalloyl glucose. IR (cm-1) 3324, 1698, 1608. ^{1}H NMR (400 MHz, CD_3OD). δH: 6.91 (2H, s, H-2′/6′), 6. (2H, s, H-2″/6″), 6.99 (2H, s, H-2‴/6‴), 7.06 (2H, s, H-2⁗/6⁗), 7.12 (2H, s, H-2⁗′/6⁗′). 5.08 (1H, d, *J*=8.0 Hz). ^{13}C NMR (100 MHz, CD_3OD). δc : 93.8 (CH, C-1), 72.2 (CH, C-2), 74.1 (CH, C-3), 69.8 (CH, C-4), 74.4 (CH, C-5), 63.1 (CH2, C-6). Galloyl i: 119.7 (CH, C-1′), 110.3 (CH, C-2′/6′), 146.3 (CH, C-3′/5′), 140.0 (CH, C-4′), 166.2 (CH, C-7′), Galloyl ii: 120.2 (CH, C-1″), 110.4 (CH, C-2″/6″), 146.4 (CH, C-3″/5″), 140.1 (CH, C-4″), 166.9 (CH, C-7″); Galloyl iii; 120.3 (CH, C-1‴), 110.4 (CH, C-2‴/6‴), 146.4 (CH, C-3‴/5‴), 140.3 (CH, C-4‴), 167.0 (CH, C-7‴); Galloyl iv; 120.4 (CH, C-1⁗), 110.5 (CH, C-2⁗/6⁗), 146.4 (CH, C-3⁗/5⁗), 140.3 (CH, C-4⁗), 167.3 (CH, C-7⁗). Galloyl v: 121.1 (CH, C-3⁗′/5⁗′), 110.7 (CH, C-2⁗′/6⁗′), 146.5 (CH, C-3⁗′/5⁗′), 140.8 (CH, C-4⁗′), 167.9 (CH, C-7⁗′). TOF HRMS m/z 963.1135 $[M+Na]^+$ (calculated 963.1080)

Mangiferin: ^{1}H NMR (400 MHz, DMSO). Mp, U.V, IR δH : 7.38 (1H, s, H-8), 6.88 (1H, s, H-5), 6.40 (1H, s, H-1), 4.62 (1H, d, *J*=9.8Hz, H-1′), 4.06 (1H, t, *J*=9.1, 8.9 Hz, H= 3′), 3.72 (1H, d, *J*=11.3, H6′a), 3.44 (1H, d, *J*=11.3, H6′b), 3.24-3.16 (overlapping signals, H-2, H4 and H-5). ^{13}C NMR (100 MHz, DMSO). δC: 179.6 (C-9), 164.3 (C-4), 162.3 (C-2), 154.5 (C-8b), 151.2 (C-6), 144.2 (C-7), 112.2 (C-8a), 108.1 (C-9), 103.1 (C-4b), 101.7 (C-5), 93.6 (C-1), 82.0 (C-5′), 79.4 (C-3′), 73.6 (C-1′), 71.1 (C-2′), 70.7 (C-4′) and 62.0 (C-6′). TOF HRMS m/z 445.0744 (calculated. 445.0776), $[M+Na]^+$ for $C_{19}H_{18}O_{11}$.

Isomangiferin: ^{1}H NMR (400 MHz, DMSO). δH : 7.25 (1H, s, H-8), 6.63 (1H, s, H-5), 6.32 (1H, s, H-1), 4.62 (1H, d, *J*=9.6Hz, H-1′), 4.07 (1H, t, H= 3′), 3.68 (1H, d, *J*=11.3, H6′a), 3.52 (1H, d, *J*=11.3, H6′b), 3.22 (1H, m, H-2'), 3.18 (1H, m, H-4') 3.20 (1H, m, H-5'). ^{13}C NMR (100 MHz, DMSO). δC: 178.8 (C-9), 164.2 (C-3), 162.2 (C-1), 156.5 (C-4a), 156.5 (C-6), 152.6 (C-10a), 145.6 (C-10), 109.5 (C-8a), 107.8 (C-2), 106.1 (C-8), 101.8 (C-9a), 93.7 (C-4), 82.0 (C-5′), 79.5 (C-3′), 73.7 (C-1′), 71.1 (C-2'), 70.8 (C-4′) and 62.0 (C-6′). TOF HRMS m/z 421.0759 (calculated 421.0771, $[M-H]^-$) for $C_{19}H_{18}O_{11}$.

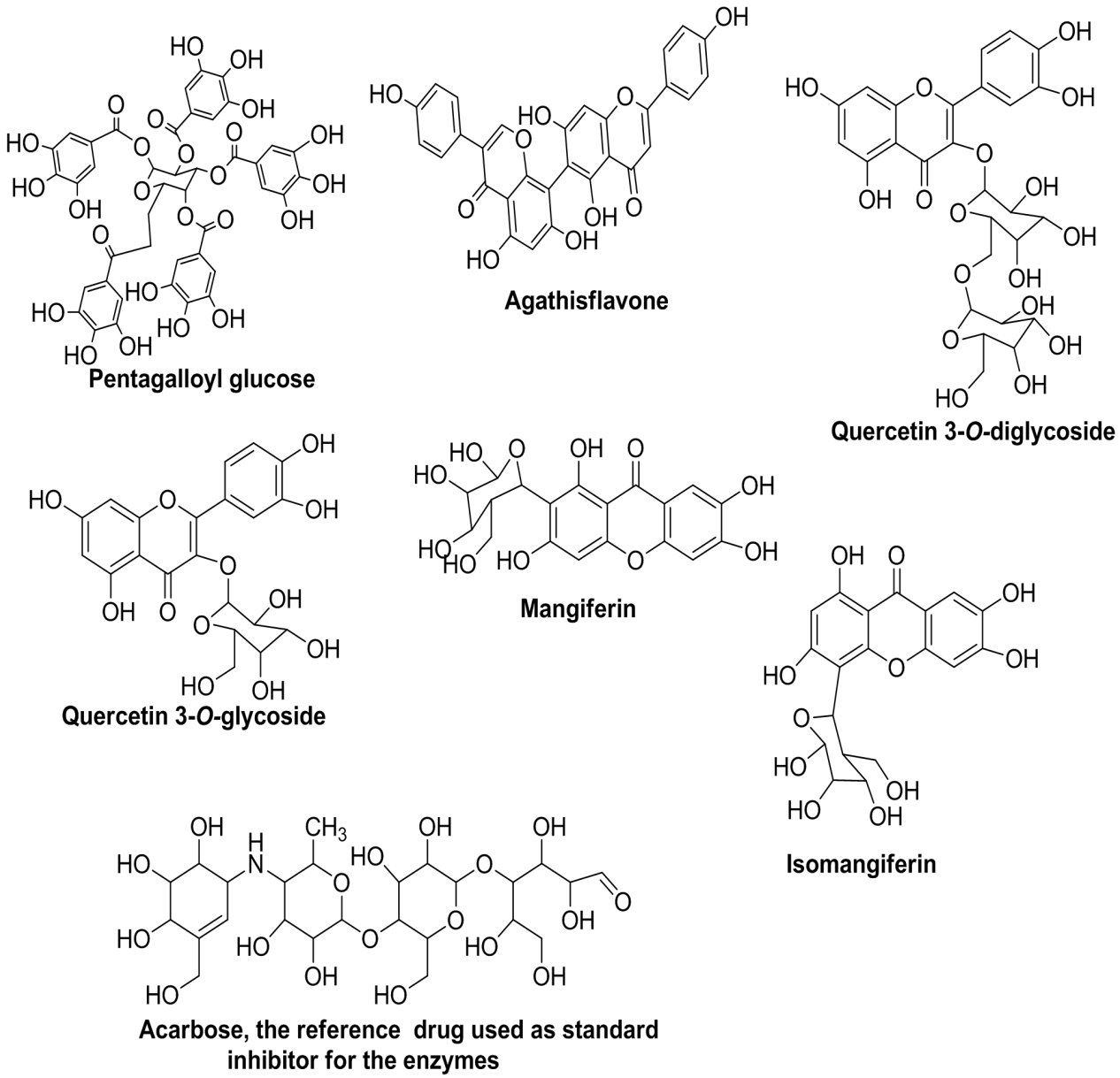

Figure 1: showing the structures of the isolated compound and that of the standard drug, acarbose

Table 1: Showing the result of the inhibition of both α-glucosidase and α-amylase

SN	Compound	IC50 (mM)	
		α-glucosidase	α-amylase
1	Acarbose	0.31 ± 0.018	1.3 ± 0.200
2	quercetin 3-*O*-glycoside	0.13 ± 0.012	0.15 ± 0.003
3	Quercetin 3-*O*-diglycoside	0.34 ± 0.068	1.63 ± 0.012
4	Agathisflavone	0.56 ± 0.037	1.45 ± 0.009
5	Pentagalloyl glucose	1.42 ± 0.003	2.34 ± 0.453
6	Isomangiferin	3.33 ± 0.017	2.68 ± 0.131
7	Mangiferin	0.84 ± 0.047	2.45 ± 0.060

Table 2: showing the binding energy of the polyphenols.

SN	Polyphenol	Alpha glucosidase kcal/mol	Alpha amylase kcal/mol
1	*Acarbose	-9.1	-9.1
2	Quercetin 3-*O*-glycoside	-8.6	-7.7
3	Quercetin 3-*O*- diglycoside	-8.6	-9.0
4	Agathisflavone	-9.8	-8.4
5	Pentagalloyl glucose	-8.3	-9.2
6	Isomangiferin	-7.7	-8.8
7	Mangiferin	-9.0	-8.1

*Reference (Not a polyphenol)

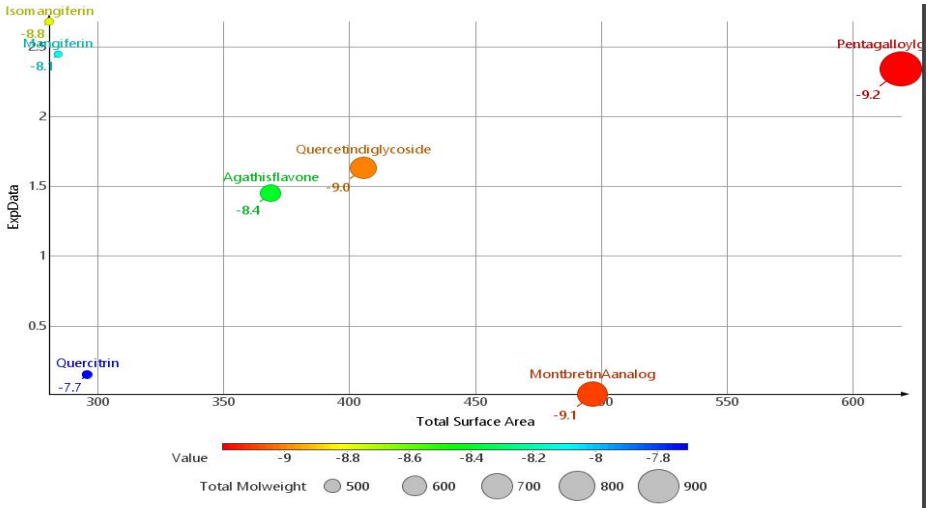

Figure 1: 4-dimensional plot of physicochemical parameters and inhibition of alpha-glucosidase

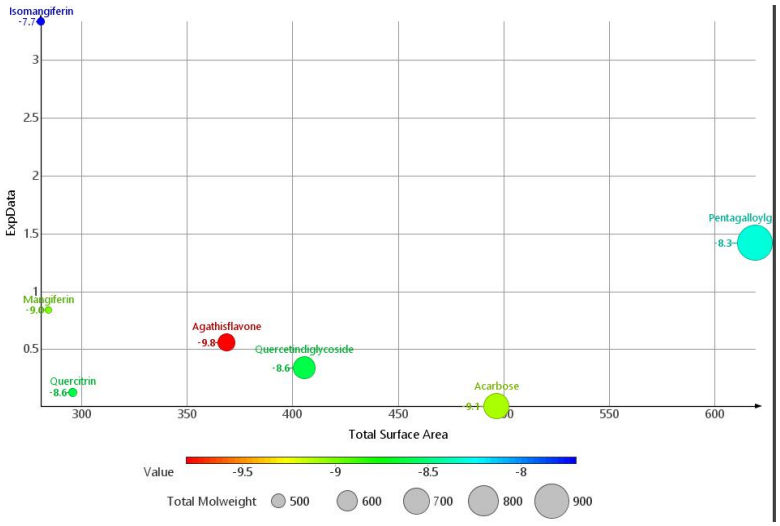

Figure 2: 4-dimensional plot of physicochemical parameters and inhibition of alpha-amylase

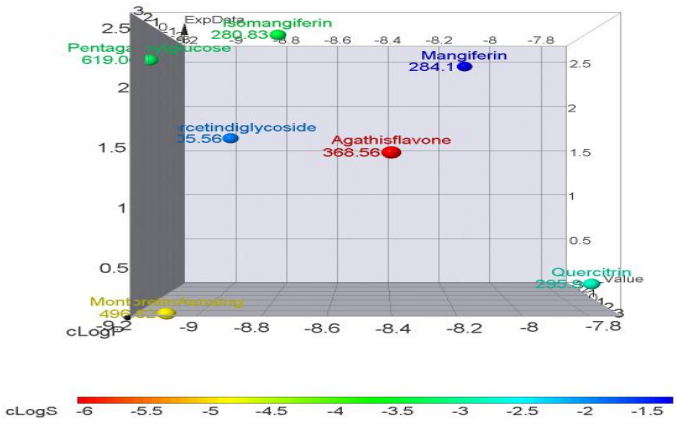

Figure 3: 4-dimensional plot of physicochemical and inhibition of alpha-glucosidase

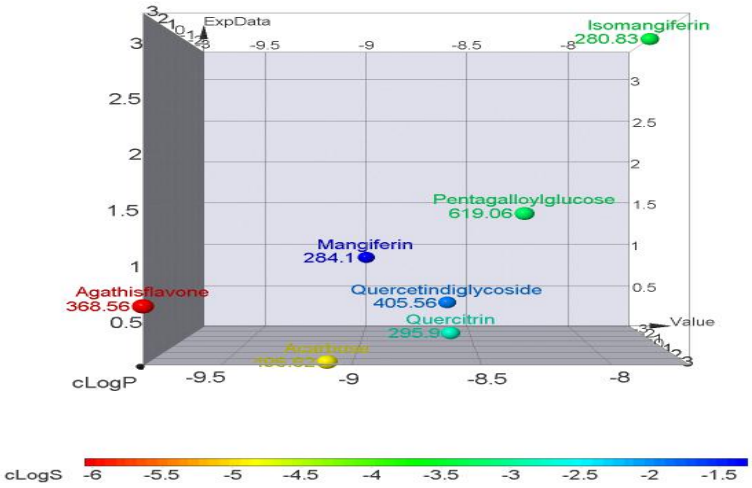

Figure 4: 4-dimensional plot of physicochemical parameters and inhibition of alpha-amylase.

Discussion

Phytochemical investigation of each of the selected plants led to the isolation of polyphenolic compounds from each of the plants and the structures of the compounds were determined. Interestingly, the three plants afforded polyphenols which belong to three different classes of polyphenols ie flavonoids,tannins, and xanthones. Pentagalloyl Glucose (PGG) was isolated for the first time in *Anarcadium occidentale* as a brown amorphous powder. The compound gave strong blue black colour on thin layer chromatography when sprayed with ferric chloride. IR spectrum displayed absorption at 3324, 1698, 1608, 1536 cm-1. Positive TOF HRMS gave a signal at m/z 963.1135 $[M+Na]^+$ (calculated 963.1080) for a molecular mass $C_{41}H_{32}O_{26}$. The proton NMR spectrum displayed five singlets at δH 7.12, 7.06, 6.99, 6.96 and 6.91 characteristic of multiply substituted gallotannins. The appearance of a doublet at 5.08 ppm (*J*= 8.0 Hz) indicate the presence of a β-anomeric proton. NMR spectroscopic data are as shown under spectroscopic data above. PGG was identified as β-penta-O-galloyl glucose (PGG) by comparison of the spectroscopic data with the literature values (Torres-Leon et al., 2017). Agathisflavone was isolated as a brown amorphous powder. ESI-MS spectrum gave a peak at m/z 561 $(M+Na)^{+\cdot}$ On comparison of the NMR data of the compound with literature (Svenningsen *et al*., 2006, *Ajileye et al*., 2015, Taiwo *et al.*, 2017) it was elucidated as agathisflavone. Quercetin 3-*O*-glycoside was isolated a yellow powder. The UV spectrum at 257.78 and 356.35 nm was characteristic of quercetin flavonoid. HRTOFMS in the negative mode displayed a signal at m/z 463.0880 $[M-H^-$ (cal. 463.0877) for a molecular formula $C_{21}H_{19}O_{12}$. The ^{1}H NMR spectrum displayed signals at δH: 7.73 (1H, d, *J*=2.1 Hz), 7.60 (1H, dd, *J*= 8.5, 2.1 Hz), 6.88 (1H, d, *J*=8.6 Hz), 6.41 (1H, d, *J*=2.1 Hz), 6.22 (1H, d, *J*=2.1 Hz). An anomeric proton signal was observed at δH 5.22 (1H, d, *J*=7.4 Hz), the large coupling constant indicating a β-glycoside. Other signals were observed between δH 3.25 and 3.65. On comparison with literature data, (Lin *et al*., 2014) the compound is identified as quercetin 3-*O*-glycoside. Quercetin 3-*O*-diglycoside was isolated as a brown powder. Its UV spectrum displayed strong absorption at 257 and 357 nm, characteristic of quercetin flavonoid. The high resolution TOF ES MS gave a

signal at m/z 649.1378 $[M+Na]^+$ (calculated 649.1381) for a molecular formula $C_{27}H_{30}O_{13}Na$ The proton NMR spectrum displayed five aryl proton signals at δH: 7.72 (1H, d, *J*=2.0), 7.68 (1H, dd, *J*=8.4, 2.0 Hz), 6.88 (1H, d, *J*=8.2 Hz), 6.42 (1H, d, *J*=1.9 Hz), 6.22 (1H, d, *J*=1.8 Hz). Two anomeric protons signals were observed at δH 5.25 (1H, d, *J*=7.3 Hz), 4.17 (1H, d, *J*=7.6 Hz). The large coupling constant values for the anomeric protons indicates β-orientation for each of the sugar. The other sugar protons were observed at δH 3.99 (1H, d, *J*=11.9 Hz), 3.77 (1H, dd, *J*=12.1, 2.4 Hz) and series of overlapping proton signals between at δH 3.02 and 3.69. On comparison of the nmr data with the literature values, Quercetin 3-*O*-diglycoside was identified as 5,7,3′,4′-tetrahydroxy-3-O-[β-D- glucopyranosyl-1→6)]-β-D-glucopyranoside (Liao *et al*., 2012). Isomangiferin was isolated as greenish-yellow amorphous powder while mangiferin was obtained as a yellow powder. Both compounds displayed the same λ_{max} at 240, 259, 320 and 370 nm in the UV spectra, characteristic of the presence of xanthone moieties. HRTOFMS ES indicated that the two compounds were close isomer with the high resolution mass spectrometry giving a signal at m/z 421.0759 (calculated 421.0771, $[M-H]^-$) in the negative mode and 445.0744 (cald. 445.0776) for $[M+Na]^+$ in the positive mode for isomangiferin and mangiferin respectively. Comparison of the NMR data with the literature values (Wu *et al*., 2010) indicated that the isolated compounds were xanthones. The ^1H NMR spectrum of the compounds on comparison with literature led to the identification of the compounds as mangiferin and isomangiferin.

All the investigated polyphenols showed higher potency against alpha glucosidase than they did for alpha amylase. This pattern was also shown for the standard drug, acarbose used for the inhibition of the two enzymes (Table 1). In terms of structural requirement for the inhibition of the enzymes, compounds with flavonoidal skeleton were better in inhibiting the two enzymes than both the xanthones and the tannins. Generally, the order of inhibition was flavonoid >biflavonoid >xanthone >tannin for the α-glucosidase and flavonoids > tannins > xanthones for the α-amylase. For both α-glucosidase and α-amylase, quercetin 3-*O*-glycoside appeared to be more potent than even the agathisflavone, a biflavonoid. Additional glycosylation in quercetin 3-*O*-diglycoside decreased the potency of the flavonoid against both enzymes compared with quercetin 3-*O*-glycoside. For both enzymes, increase in the number of phenolic hydroxyl groups does not correlate with increase in inhibitory activity as demonstrated by quercetin 3-*O*-glycoside being more potent than agathisflavone, a biflavonoid and pentagalloyl glucose. It is interesting to note that within same structural class, it is possible to have differing disposition to inhibitory activity as illustrated by isomangiferin, a positional isomer of mangiferin. The V_{max} and Km for inhibition for α-glucosidase by quercetin 3-*O*-glycoside were determined to be: 0.6670 and 0.6962 while for α-amylase it was 4.854 and 0.5496 respectively (Figure 6a and 6b) indicating a higher affinity of quercetin 3-*O*-glycoside for α-amylase than for α-glucosidase.

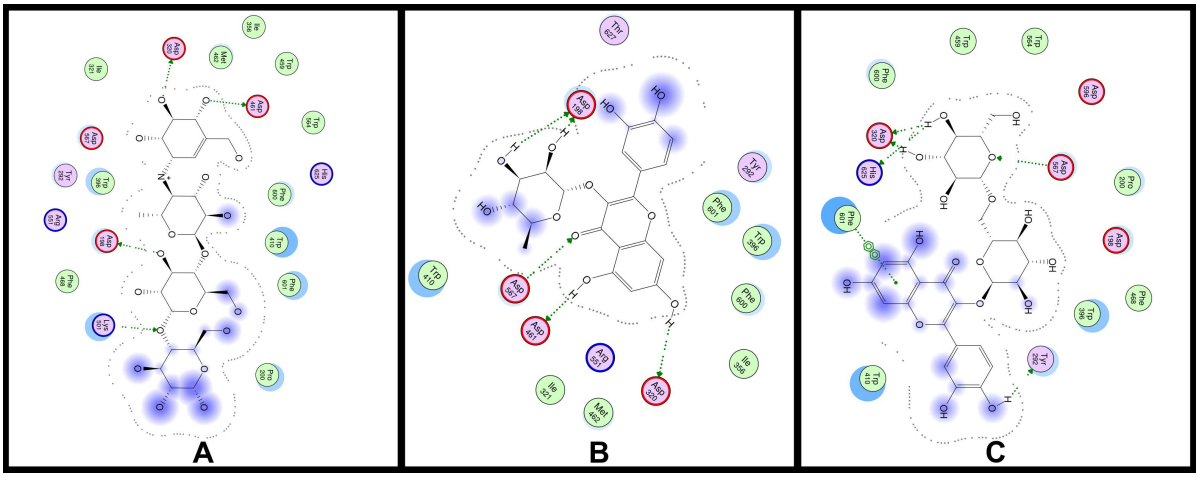

Figure 5: Binding site interaction of alpha glucosidase with A) acarbose, B), quercetin 3-*O*-glycoside and C) quercetin 3-*O*-diglycoside showing networks of hydrogen bond interactions as major driving force for inhibitor interaction.

Examination of binding site interaction by acarbose in Figure 5 reveals extensive network of hydrogen bond contacts restricted to two of three glycosidic units of the inhibitor. The third sugar unit located on the flanks in Figure 5A is solvent-exposed; together with a consideration of the molecular size of the inhibitor, it appears the third sugar group is not necessary for binding site contact which may be a direct result of the volume of the binding site that is able to accommodate three rings serially arranged as seen in acarbose, or for rings non-serially arranged in quercetin, but not five rings (quercetin 3-*O*-diglycoside) without significant solvent exposure of parts of the bound inhibitor. This partly explains why quercetin 3-*O*-glycoside is a better inhibitor of the alpha glucosidase enzyme than the diglycoside. Just like acarbose, all the natural products form crucial hydrogen bonding contacts with the binding site amino acid residues. For acarbose, hydrogen bonds with three aspartic acids (Asp198, Asp320, and Asp461 in Figure 5) are important in ligand recognition. Interestingly quercetin 3-*O*-glycoside does not just engage all three aspartic acids in hydrogen bond formation, it additionally donates the carbonyl oxygen in forming hydrogen bond with Asp567 in the binding site. The effect of a second sugar in quercetin 3-*O*-diglycoside appears to significantly affect its binding fit such that its flavonoid unit is largely solvent exposed and the molecule is only able to establish hydrogen bonding contacts with the aspartic acids. A similar hydrogen bond stabilization of inhibitor contacts was also observed in the case of alpha

amylase as presented in Figure 6. However, contrary to the pattern obtained for alpha glucosidase, quercetin 3-*O*-diglycoside was able to form more hydrogen bonding contacts with the binding site amino acids in support of the slight superiority in computed binding affinities compared with quercetin 3-*O*-glycoside

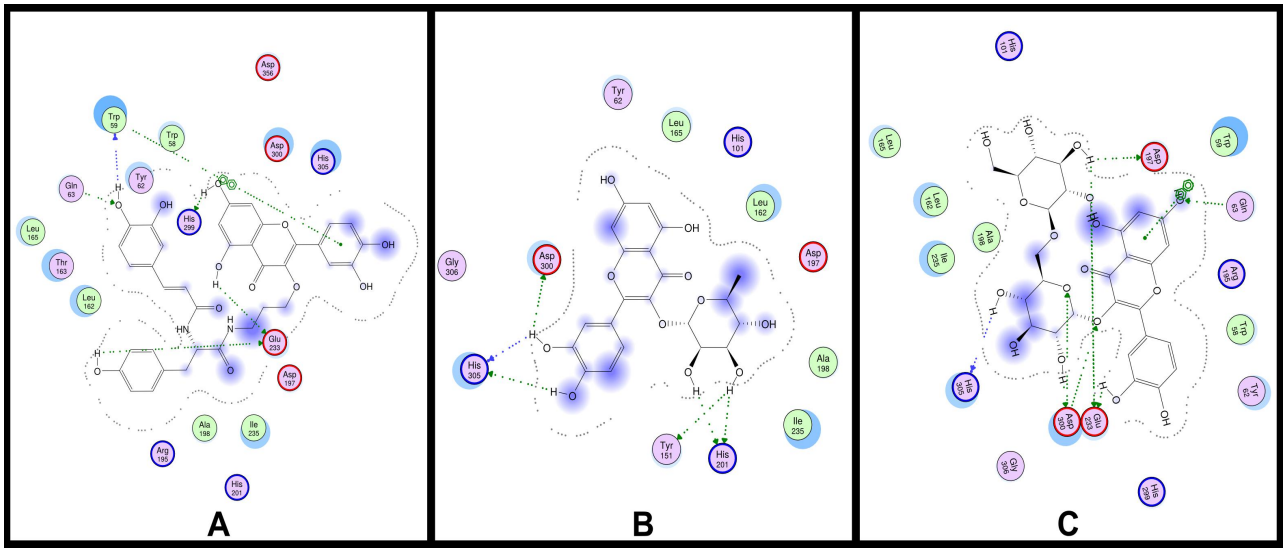

Figure 6: Binding site interaction of alpha amylase with A) acarbose, B), quercetin 3-*O*-glycoside and C) quercetin 3-*O*-diglycoside showing networks of hydrogen bond interactions as major driving force for inhibitor interaction.

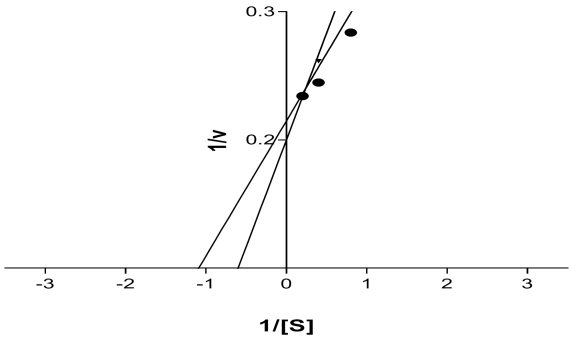

Figure 6a: Michealis-Menten plot of quercetin 3-*O*-glycoside inhibition of α-amylase

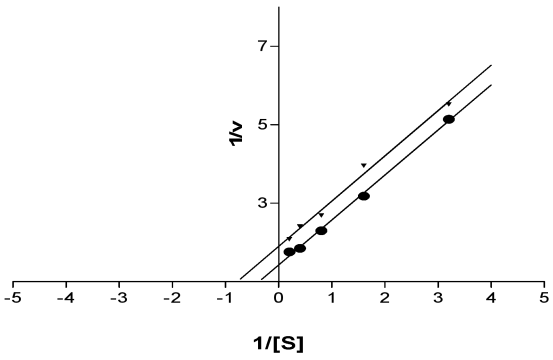

Figure 6b: Michealis-Menten plot of quercetin 3-*O*-glycoside inhibition of α-glucosidase

The Michaelis-Menten plot (Figure 6b) showed that quercetin 3-O-glycoside inhibited α-amylase competitively. This suggests that the compound competed with the substrate for binding to the active site of the enzyme thereby preventing the breaking down of oligosaccharides to saccharides. However, the non-competitive mode of inhibition obtained from the Michaelis-Menten plot (Figure 6a) for α-glucosidase indicated that quercetin 3-*O*-glycoside did not compete with the substrate for binding to the active site, in retarding the conversion of disaccharides to monosaccharides. Other factors beyond affinity for the active site on the enzyme may be responsible for the potency of quercetin 3-*O*-glycoside inhibition against α-glucosidase.

To further understand the interrelatedness of physicochemical properties (cLog P, cLog S, surface area, molecular weight) of the polyphenols and their inhibitory activities on α-glucosidase and α-amylase, both computed and measured binding profiles and some molecular descriptors were calculated and presented in Figures 1, 2, 3, and 4. All six polyphenols demonstrated good binding interaction with both enzymes. In fact agathisflavone, in binding alpha glucosidase, interacts better compared to the reference inhibitor acarbose. A similar observation was recorded for pentagalloyl glucose for alpha amylase binding where it demonstrated marginal binding superiority over acarbose, the reference inhibitor. The interaction pattern at the atomistic level obtained for all the polyphenols against the two enzymes suggests that specific physicochemical properties involvements outside of simple enzyme binding and inhibition are likely of importance in determining the patterns obtained experimentally. While saccharide units appears to be crucial for target recognition, agathisflavone an aglycone nevertheless demonstrated the strongest receptor interaction with ΔG value of -9.8 kcal/mol for alpha glucosidase. This may not be so in cell-based experiments since the presence of sugar groups in the other polyphenols will most likely help facilitate membrane transport by employing membrane-based glucose transporters that may account for higher concentrations of the glycosides at the receptor sites. No linear relationship exists between computed physicochemical properties and the binding data (Figures 1-4). This may be because the natural products belong to chemically diverse groups and a simple linear mathematical model fails to account for the relationship. However, the data effectively captures the impact of structural variations on the binding. For instance, isomangiferin with the same molecular formula as mangiferin demonstrates significantly poorer binding both experimentally and computationally. Additional glycosidic group in Quercetin 3-*O*-diglycoside did not significantly affect receptor binding positively as can be seen in the values obtained for quercetin 3-*O*-glycoside and quercetin 3-*O*- diglycoside.

Just like for alpha glucosidase, no linear relationship exists between physicochemical properties computed and the binding data. However, isomangiferin and mangiferin demonstrates similar binding suggesting that alpha amylase is less selective for the two isoforms compared with alpha glucosidase. Also, as opposed to what was obtained for alpha glucosidase, additional glycosylation in quercetin 3-*O*-diglycoside significantly affected the receptor binding, with the smaller molecular weight of quercetin 3-*O*-glycoside (it seems) being better favoured over quercetin 3-*O*- diglycoside.

Conclusion

This study demonstrated that of the three classes investigated, quercetin 3-*O*-glycoside is the most potent and that additional glycosylation reduced its inhibitory activity while the presence of polyphenolic hydroxyl groups had no influence on the inhibitory activities across the structural class. Also it was shown that the inhibitory activity of polyphenols on both α-glucosidase and α-amylase cannot be predicted by the binding energy studies of the polyphenols with the enzymes. For possible consideration of choice of medicinal plants rich in polyphenols from nature in the treatment of diabetes, a source rich in quercetin flavonoids should be prescribed when the mechanism of treatment involves control of postprandial glucose levels through inhibition of either or both α-glucosidase and α-amylase.

Conflict of Interest: The authors declare that there is no conflict of interest.

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