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Alpha-amylase Inhibitory Compounds from *Musa cavendishii*

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Authors' contributions

All authors contributed to the design of the work, analysis, interpretation of data, drafting the work, agreement of the work and resolved final approval of the version to be published.

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ABSTRACT

Aims: To investigate the ethyl acetate fraction from the methanol extract of the leaves of *Musa cavendishii* and to evaluate the alpha-amylase inhibitory activity of its isolated phytochemicals and their value as potential anti-obesity products.
Study Design: Isolation and identification of phytochemicals from the ethyl acetate fraction and investigation of their alpha-amylase inhibitory activity through *In vitro* screening and docking study.
Place and Duration of Study: Faculty of Pharmacy, Mansoura University, between June 2013 and July 2016.
Methodology: Different phytochemicals were isolated and purified using different chromatographic techniques from the ethyl acetate fraction of the extract of dried powdered leaves of *Musa cavendishii*. Elucidation of the structures of the isolated compounds was performed using different spectroscopic methods (^{1}H NMR, ^{13}C NMR, NOESY, HSQC and HMBC). Alpha-amylase inhibition assay was used to evaluate the inhibitory activity of the isolated compounds. Furthermore, docking study was conducted to get an insight about the binding mode of the active compounds within the active site of the target enzyme.
Results: (6*S*,9*S*)-roseoside **1-A** was reported form the titled plant for the first time along with six reported compounds viz., (6*S*,9*R*)-roseoside **1-B**, kaempferol-3-*O*-rutinoside **6**, *p*-hydroxybenzoic acid **2**, rutoside **7**, catechuic acid **5**, quercetin **4** and kaempferol **3**. The diglucosides, kaempferol-3-

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O-rutinoside and rutoside exhibited better alpha-amylase inhibitory activity (80.45 and 98.0% inhibition, respectively) than the positive standard alph-amylase inhibitor (acarbose) used (57.0%) at a concentration of 50 µg/mL. In an agreement with the results of alpha-amylase inhibition assay, the docking results indicated that these two diglucosides **6** and **7**, showed the highest binding scores (-8.16 and -7.68 kcal/mol) compared to positive standard acarbose (-5.40 kcal/mol).
Conclusion: This study showed that flavonoid diglucosides e.g. kaempferol-3-*O*-rutinoside and quercetin-3-*O*-rutinoside (rutoside) could be potential inhibitors for alpha-amylase and could be used as anti-obesity phytochemicals.

Keywords: Musa cavendishii; alpha-amylase; anti-obesity; flavonoids; roseoside.

1. INTRODUCTION

Inhibition of the carbohydrate hydrolyzing enzymes such as alpha-glucosidase and alpha-amylase is an important strategy for treatment of obesity and other related health problems such as cardiovascular disorders (mainly heart disease and stroke), type 2 diabetes, musculoskeletal diseases (especially osteoarthritis) and certain types of cancer (endometrial, breast, and colon) [1,2].

An impressive research arena for the development of new natural slimming drugs is the search of alpha-amylase inhibitors from plant origin. Several natural plants extracts were reported to suppress alpha-amylase activity such as *Phyllanthus amarus* and *Triticum aestivum* [3]. Natural products generally have the advantage of being milder in their activity than totally synthetic drugs, of having reduced toxic and systemic undesired effects [2]. Plant constituents with alpha-glucosidase and alpha-amylase inhibitory activity include polyphenolic compounds and glycoproteins [4]. For example, anthocyanins and ellagitannins present in several plants such as raspberries and strawberries have been reported to have alpha-glucosidase and alpha-amylase inhibitory activity, respectively [5]. Furthermore, theaflavins and catechins present in green and black teas have been reported to inhibit alpha-amylase and alpha-glucosidase activity as well as decrease starch digestion [6]. Several compounds had been isolated from Musa sp. with different biological activities. Catecholamines such as norepinephrine, Leucocyanidin, quercetin and its 3-*O*-galactoside, 3-*O*-glucoside, and 3-*O*-rhamnosyl glucoside, acylsteryl glycosides such as sitoindoside-I, sitoindoside-II, sitoindoside-III, sitoindoside-IV and sterylglycosides as sitosterol gentiobioside, sitosterol myo-inosityl-*β*-D-glucoside have been isolated from fruits of *M. paradisiaca*. Several triterpenes such as cyclomusalenol, cyclomusalenone, 24-methylene cycloartanol, stigmast-7-methylene cycloartanol, stigmast-7-en-3-ol, lanosterol and β-amyrin were previously reported [7-9].

So far, the phytochemical compositions of the leaves of *Musa cavendishii* have not been fully investigated. In this report, seven compounds viz., (6*S*,9*S*)-roseoside, (6*S*,9*R*)-roseoside, *p*-hydroxybenzoic acid, kaempferol, quercetin, catechuic acid, kaempferol-3-*O*-rutinoside and quercetin3-*O*-rutinoside (rutoside) were isolated. The alpha-amylase inhibitory activity of the isolated compounds were evaluated and compared with virtual docking experiment. Most of the isolated compounds showed remarkable alpha-amylase inhibitory activity in *In vitro* assay relative to the positive standard acarbose.

2. MATERIALS AND METHODS

2.1 General Methods

The 1D- and 2D-NMR spectra were performed on Jeol 500 MHz ^{TM} spectrometer. Silica gel G60-230 (Merck, Germany), Sephadex LH-20 (SIGMA- ALDRICH) and RPC_{18} (40 µm BAKERBOND) were used for column chromatography. Thin layer chromatography (TLC) was performed on silica gel F_{254} plates (Merck or Machery-Nagel, Germany) using vanillin-sulfuric acid, 5% $AlCl_3$ and$FeCl_3$ spray reagents. The solvents used were of reagent grade (El-Nasr Co., Egypt).

2.2 Plant Material

The plant material was collected in June 2013, from grown plants in Mansoura University, Dakahlia, Egypt and identity of the plant was confirmed as *Musa cavendishii*. A voucher specimen was deposited in the herbarium of the College of Pharmacy, Mansoura University (013-Mansoura-4). Leaves were shade dried and powdered.

2.3 Extraction and Isolation

Leaves (1.0 kg) were exhausted with methanol and the extract evaporated in vacuo to yield 120 g dark green semisolid residue. The extract was suspended in water then partitioned successively with *n*-hexane, CH_2Cl_2, EtOAc and BuOH. The EtOAc fraction (7 g) was chromatographed over wet packed column (60 x 2.5 cm i.d.); sephadex LH20 with medium pressure using CH_2Cl_2-MeOH mixtures of increasing polarity starting with 100% CH_2Cl_2 and the effluents (15 mL each) were collected. Similar fractions grouped together and subjected for further purification. Detailed isolation procedure is illustrated in Fig. 1.

2.4 Spectral Analysis

NMR spectra (^{1}H and ^{13}C) were recorded on a Jeol 500 MHz for 1H measurements and 125 MHz for ^{13}C measurements, respectively. methanol-d_6 was used as a solvent. Chemical shits are given in ppm with TMS as internal standard. APT, 2D NMR, H-H NOESY, HSQC and HMBC experiments were applied to gain reliable assignments. Experimental data were processed using Jeol Delta software v5.0.4.4.

(6*S*,9*R*)-roseoside **1-A**: ^{1}H NMR (methanol-d_6, 500 MHz): δ= 2.14 (1H, d, *J*= 16.5, H-2_{ax}), 2.48 (1H, d, *J*= 16.5, H-2_{eq}), 5.85 (1H, s, H-4), 5.83 (1H, brs, H-7), 5.85 (1H, brs, H-8), 4.38 (1H, m, H-9), 1.27 (1H, d, *J*= 6.5, Me-10), 1.00 (1H, s, Me-11), 1.00 (1H, s, Me-12), 1.89 (1H, s, Me-13), 4.30 (1H, d, *J*=7.5, H-1`), 3.14 (1H, m, H-2`), 3.18 (1H,m, H-3`), 3.28 (1H, m, H-4`), 3.21 (1H, m, H-5`), 3.58 (1H, m, H-6`a). **^{13}C** NMR (methanol-d_6, 500 MHz): δ= 41.1 (C-1), 49.3 (C-2), 199.7 (C-3), 125.8 (C-4), 166.0 (C-5), 78.7 (C-6), 130.2 (C-7), 133.9 (C-8), 76.0 (C-9), 19.9 (C-10), 22.1 (C-11), 23.4 (C-12), 18.2 (C-13), 101.4 (C-1`), 73.9 (C-2`), 76.7 (C-3`), 70.3 (C-4`), 76.7 (C-5)`, 61.4 (C-6`).

(6*S*,9*S*)-roseoside **1-B**: ^{1}H NMR (methanol-d_6, 500 MHz): δ= 2.11 (d, *J*= 17.0, H-2_{ax}), 2.57 (d, *J*= 17.0, H-2_{eq}), 5.86 (1H, s, H-4), 5.93 (1H, d, *J*= 15.5, H-7), 5.68 (1H, dd, *J*= 15.5, 7.5, H-8), 4.48, (1H, qui, *J*= 7.0, H-9), 1.25 (1H, d, *J*= 6.0, Me-10), 1.00 (1H, s, Me-11), 1.00 (1H,s, Me-12), 1.91 (1H, s, Me-13), 4.23 (1H, d, *J*= 8.0, H-1`), 3.14 (1H, m, H-2`), 3.18 (1H, m, H-3`), 3.28 (1H, m, H-4`), 3.21 (1H, m, H-5`) , 3.47 (1H, dd, *J*= 11.5, 6.0, H-6`a), 3.54 (1H, dd, *J*= 11.5, 6.0, H-6`b). **$^{13}$C** NMR (methanol-$d_6$, 500 MHz): δ = 41.0 (C-1), 49.4 (C-2), 200.0 (C-3), 125.8 (C-4), 166.0 (C-5), 78.7 (C-6), 132.4 (C-7), 132.3 (C-8), 73.6 (C-9), 20.9 (C-10), 22.1 (C-11), 23.4 (C-12), 18.2 (C-13), 99.9 (C-1`), 73.3 (C-2`), 76.9 (C-3`), 70.3 (C-4`), 77.0 (C-5`), 63.0 (C-6`).

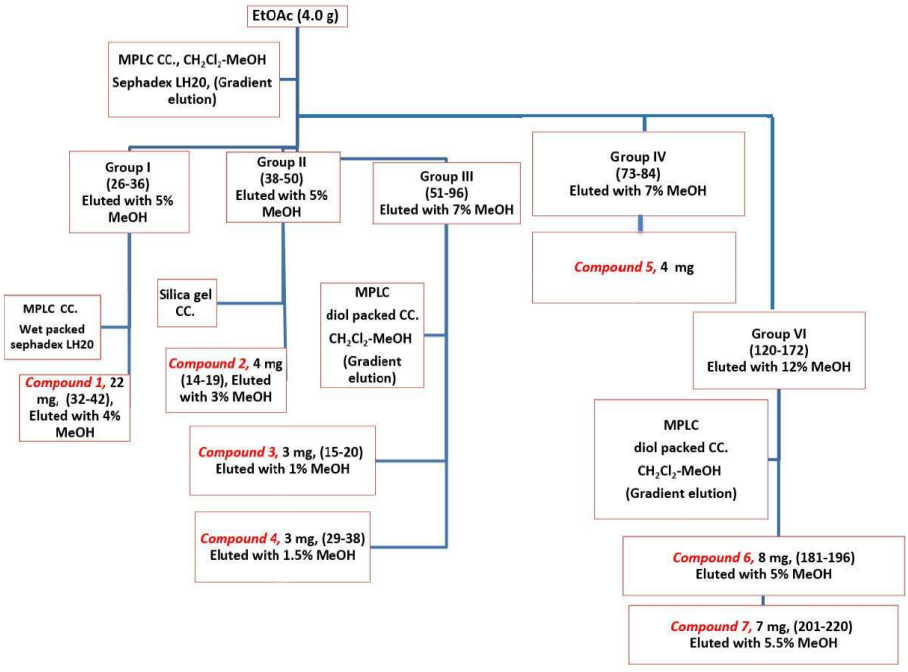

Fig. 1. Isolation procedure of compounds 1-7 from the ethyl acetate fraction of the methanol extract of the leaves of *Musa cavendishii* using different chromatographic systems

P-Hydroxybenzoic acid **2** (4 mg) was obtained as off-white solid. It is soluble in methanol, sparingly soluble in ether, petroleum ether and ethyl acetate; insoluble in water, dilute mineral acids, but soluble in alkalis. ^{1}H NMR (methanol-d_6, 500 MHz): δ= 7.84 (2H, d, 8.5, H-2/6), 6.78 (2H, d, 8.5, H-3/5), APT (methanol-d_6, 125 MHz): δ = 123.6 (C-1), 131.5 (C-2/6), 114.5 (C-3/5), 161.3 (C-4), 170.4 (C-7).

Kaempferol **3** (3 mg) was obtained as yellow amorphous powder. It is soluble in methanol. ^{1}H NMR (methanol-d_6, 500 MHz): δ= 6.18 (1H, d, *J*= 1.5, H-6), 6.39 (1H, d, *J*= 1.5, H-8), 8.07 (2H, d, *J*= 9.0, H-2`/6`), 6.89 (2H, d, *J*= 9.0, H-3`/5`). APT (methanol-d_6, 125 MHz): δ= 148.9 (C-2), 138.0 (C-3), 178.2 (C-4), 166.4 (C-7), 95.3 (C-8), 161.4 (C-9), 105.3 (C-10), 124.5 (C-1`), 131.5 (C-2`/6`), 117.1 (C-3`/5`), 159.1 (C-4`).

Quercetin **4** (3 mg) was obtained as yellow amorphous powder. It is soluble in methanol. ^{1}H NMR (methanol-d_6, 500 MHz): δ= 6.17 (1H, brs, H-6), 6.39 (1H, brs, H-8), 7.73 (1H, brs, H-2`), 6.87 (1H, d, *J*= 8.5, H-5`), 7.62 (1H, d, *J*= 8.5, H-6`). $^{13}$C NMR (methanol-$d_6$, 125 MHz): δ = 147.5 (C-2), 135.9 (C-3), 176.0 (C-4), 161.2 (C-5), 97.8 (C-6), 164.1 (C-7), 93.1 (C-8), 156.9 (C-9), 103.4 (C-10), 122.8 (C-1`), 114.6 (C-2`), 144.9 (C-3`), 146.7 (C-4`), 114.9 (C-5`), 120.3 (C-6`),

Catechuic acid **5** (4 mg) was obtained as off-white solid. It is soluble in methanol, sparingly soluble in ether, petroleum ether and ethyl acetate; insoluble in water, dilute mineral acids, but soluble in alkalis. ^{1}H NMR (methanol-d_6, 500 MHz): δ= 7.32 (1H, d, 2.0, H-2), 7.30 (1H, dd, 8.0, 2.0, H-6), and 6.68 (1H, d, 2.0, H-5). APT (methanol-d_6, 125 MHz): δ = 121.7 (C-1), 116.4 (C-2), 144.7 (C-3), 150.2 (C-4), 114.4 (C-5), 122.5 (C-6) and 168.9 (C-7).

Kaempferol-3-*O*-rutinoside **6** (8 mg) was obtained as dark yellow powder. It is soluble in methanol, sparingly soluble in ether, petroleum ether and ethyl acetate. ^{1}H NMR (methanol-d_6, 500 MHz): δ= 6.11 (1H, d, *J*=1.8, H-6), 6.31 (1H, d, *J*=1.8, H-8), 7.97 (2H, d, *J*=8.8, H-2`/6`), 6.80 (2H, d, *J*=8.8, H-3`/5)`, 5.03 (1H, d, *J*=7.5, H-1``), 4.43 (1H, S, H-1```). APT (methanol-d_6, 125 MHz): δ= 158.6 (C-2), 134.1 (C-3), 177.9 (C-4), 165.7 (C-7), 93.8 (C-8), 160.2 (C-9), 104.1 (C-10), 121.4 (C-1`), 131.0 (C-2`/6`), 114.8 (C-3`/5`), 103.3 (C-1``), 74.4 (C-2``), 76.8 (C-3``), 70.9 (C-4``), 75.8 (C-5``), 68.4 (C-6``), 101.1 (C-1```), 70.7 (C-2```), 70.9 (C-3```), 72.5 (C-4```), 70.1 (C-5```), 16.6 (C-6```).

Rutoside **7** (7 mg) was obtained as dark yellow powder. It is soluble in methanol, sparingly soluble in ether, petroleum ether and ethyl acetate. ^{1}H NMR (methanol-d_6, 500 MHz): δ= 6.21 (1H, d, *J*=1.8, H-6), 6.40 (1H, d, *J*=1.8, H-8), 7.66 (1H,d, *J*=2.5, H-2`), 6.86 (1H, d, *J*=8.0, H-5`), 6.87 (1H, dd, *J*= 8.0, 2.5, H-6`), 5.09 (1H, S, H-1``). $^{13}$C NMR (methanol-$d_6$, 125 MHz): δ = 157.2 (C-2), 134.3 (C-3), 178.1 (C-4), 161.7 (C-5), 98.6 (C-6), 164.7 (C-7), 93.5 (C-8), 158.0 (C-9), 104.3 (C-10), 121.7 (C-1`), 116.3 (C-2`), 144.5 (C-3`), 148.5 (C-4`), 116.3 (C-5`), 121.7 (C-6`), 103.3 (C-1``), 74.4 (C-2``), 76.8 (C-3``), 70.0 (C-4``), 75.9 (C-5``), 67.2 (C-6``), 101.1 (C-1```), 70.7 (C-2```), 70.9 (C-3```), 72.6 (C-4```), 68.4 (C-5```), 16.5 (C-6```).

2.5 Alpha-amylase Assay

2.5.1 Reagents

Porcine pancreatic alpha-amylase (EC3.2.1.1, type VI, Sigma Chemicals, USA) was dissolved in ice-cold distilled water to give a concentration of 4 unit/mL solution. Potato starch (0.5% w/v) was dissolved in 20 mM phosphate buffer (pH 6.9) containing 6.7 mM sodium chloride, was used as a substrate solution. Test compounds (1 mg/mL in DMSO) were used as stock solutions. Dinitrosalicylic acid color reagent solution (DNS) was prepared by dissolving 1 gm of 3,5-dinitrosalicylic acid in 50 mL of dist. water. Thirsty gms of sodium potassium tartrate (tetrahydrate) were added slowly, then 20 mL of 2 N NaOH was added. Previous solution was diluted to a final volume 100 mL with distilled water. Final solution must be stored not longer than 2 weeks.

2.5.2 Procedure

The α-amylase inhibition assay was performed using the chromogenic method adopted from Sigma–Aldrich [2,10]. In this experiment, 200 µL of enzyme solution was pre-incubated with the 40 µL of pure compound (1 mg/mL in DMSO) for 15 min and the reaction was started by the addition of 400 µL of starch solution. The tubes were incubated at 25℃ for 3 min. Final concentrations in the incubation mixture was 50 µg/mL for compounds, 0.25% (w/v) starch and 1 unit/mL enzyme. At 3 min from addition of starch, 400 µL mixture was removed and added into a separate tube containing 200 µL DNS color reagent solution. Previous solution was diluted to a final volume 100 mL with dist. water and placed into an 85℃ water bath for 15 min. The mixture

was removed from the water bath and alpha-amylase activity was determined by measuring the absorbance of the mixture at 570 nm using a Bioteck® Elisa plate reader. Control incubations, representing 100% enzyme activity were conducted in an identical fashion replacing plant extract with DMSO (40 µL). For blank incubations (to allow for absorbance produced by the plant extract), the enzyme solution was replaced with distilled water and the same procedure was carried out as above. The absorbance (*A*) due to maltose generated was calculated as:

A_{570nm} control or plant extract = A_{570nm} Test − A_{570nm} Blank

From the net absorbance obtained, the percentage (w/v) of maltose generated was calculated from the equation obtained from the maltose standard calibration curve (0–0.1%, w/v, maltose). Percentage of inhibition was calculated as (100 – % reaction at t = 3 min) whereby % reaction = (mean maltose in sample/mean maltose in control) × 100.

2.6 Docking Methodology

2.6.1 Docking using MOE

Molecular Operating Environment (MOE), Version 2009.10, Chemical Computing Group, Inc., Montreal, Quebec, Canada, 2009, was employed in the search for favorable binding configurations between ligands and macromolecular target. Docking studies of the isolated compounds were performed by MOE using the x-ray crystallographic structure of target protein obtained from the protein data bank. The ligand co-crystallized with the selected target protein is used as a reference for assessing the affinity of the synthesized compounds. We performed 100 docking iterations for each ligand and the top scoring configuration of each of the ligand-enzyme complexes was selected on energetic ground. In Table 1, the output of docking simulation is the scoring function which reflects the binding free energy *dG* in kcal/mol (S).

2.6.2 Steps in methodology of MOE

The docking procedure using MOE involved the following steps:

a. Conformational search is done where all items were set as default with RMS gradient of 0.01 kcal/mol and RMS distance of 0.1 Å.
b. Hydrogen atoms were added to the isolated target with their standard geometry.
c. A connect and type procedure was run for automatic check of missed bonds during isolation and crystallization.
d. The target was fixed and dealt as a rigid structure. Alpha-amylase enzyme from RCSB protein data bank (1HNY) was used in this study.
e. The pocket atoms were used to define the active site. Alpha spheres within 5 Å were used to guide the placement. For alpha-amylase enzyme, as there is no reference ligand in the x-ray crystal structure of protein, the active site could not be defined. As a result, surface mapping has been done for the protein surface. This revealed the cavities on the protein surface which could serve as potential active site. The largest cavity on alpha-amylase protein was used to explore the binding of the isolated compounds to this protein.
f. During docking, the default parameters and settings using triangle match placement method, London *d*G scoring and the number of retain (30) were employed. In addition, GridMin refinement and affinity *d*G rescoring 2 were also used.

3. RESULTS AND DISCUSSION

3.1 Identification of Compounds [1-7], Fig. 2

Careful investigation of compound **1** revealed that it is formed of a mixture of two isomers **1-A** and **1-B** with the approximate ratio of 3:1, respectively. APT spectrum showed the presence of two sets for two 6-hydroxy-ionol moieties and two sets for a glucose moiety. Most of the signals were very close or appeared as duplicates except for C-9 in both structures where it appeared at δ_C 76.0 in **1-A** and at δ_C 73.6 in **1-B** indicating that C-9 is the position of different chirality. Full ^{1}H NMR and APT data are compared with published data [11]. Thus, compound **1-A** and **1-B** were tentatively identified as (6*S*,9*R*)-dihydroxy-megastigma-4,7-dien-3-one-9-*O*-β-D-glucopyranoside (**1-A**), commonly known as *6S,9R*-roseoside and (6*S*,9*S*)-dihydroxy-megastigma-4,7-dien-3-one-9-*O*-β-D-glucopyranoside (**1-B**), known as *6S,9S*-roseoside (Fig. 1), from comparison of their spectral data with literature values [11-14]. It is

worth to mention that *6S,9R*-roseoside has been previously reported from *M. paradisiaca* [15]. However, this is the first report for the presence of both *6S,9R*- and *6S,9S*-roseosides in *M. cavendishii* and for the presence of *6S,9S*-roseoside in *Musa* spp..

The ^{1}H NMR spectrum of compound **2** showed the presence of *p*-substituted benzene ring as revealed from the two proton doublets at δ_H 7.84 (2H, *J*= 8.5 Hz) and 6.78 (2H, *J*= 8.5 Hz). The structure of *p*-hydroxybenzoic acid was confirmed for **2** from the quaternary carbon signals at δ_C 170.4 (C-7) and 161.3 (C-4).

^{1}H NMR spectrum of **3** showed a characteristic pattern for a flavonol. Two singlet at δ_H 6.18 and 6.39 were assigned to H-6 and H-8, respectively. The proton signals at δ_H 8.07 (2H, d, *J*= 9.0, H-2`/6`) and 6.89 (2H, d, *J*= 9.0, H-3`/5`) indicated a *p*-hydroxy B-ring. This was confirmed from the ^{13}C NMR spectra which showed 13 carbon signals assigned for positions 15 carbons. The most characteristic signals were the signals at δ_C 131.5 (C-2`/6`) and 117.1 (C-3`/5`) for the symmetric *p*-hydroxyphenyl group assigned for B-ring. By reviewing the published data [16], compound **3** was confirmed to be kaempferol.

^{1}H NMR spectrum of compound **4** showed five aromatic proton signals characteristic for a flavonol. Two of these signals appeared as singlet at δ_H 6.17 and 6.39 assigned to H-6 and H-8, respectively. The proton signals at δ_H 7.73 (brs, H-2`), 6.87 (d, 8.5, H-5`) and 7.62 (d, 8.5, H-6`) indicated a catechol B-ring pattern. This was confirmed from the $^{13}$C NMR spectra which showed 15 carbon signals with two characteristic carbons at $\delta_C$ 144.9 and 146.7 assigned for positions C-3`and C-4`of the *o*-dihydroxy B-ring. From the previous findings and by reviewing with the published data, compound **4** was confirmed to be quercetin [16].

The ^{1}H NMR spectrum of **5** showed the presence of three aromatic proton signals, at δ_H 7.32, 7.30 and 6.68 of an ABX system, each integrated to one proton and their "*J*" values, indicated a tri-substituted benzene ring with a 3,4-dihydroxy substitution pattern. Its ^{13}C NMR spectrum showed seven carbon signals with a characteristic quaternary carbon signal at δ_C 168.9 of an aromatic acid. Therefore, the structure of compound **5** was deduced to be 3,4-dihydroxybenzoic acid (catechuic acid).

The ^{1}H and ^{13}C NMR spectra of **6** indicated a flavonoid glucoside structure. The presence of a downfield carbon signals at 134.1 (C-3) indicates a flavonol moiety [16]. A *p*-hydroxylated B-ring was suggested from the two proton doublet at δ_H 8.03 (2H, *J*= 8.8 Hz) and 6.92 (2H, *J*= 8.8 Hz) assigned for positions 2`/6` and 3`/5`, respectively. Therefore, the aglycone moiety was suggested to be kaempferol. The presence of sugar moieties was deduced from the proton signals at the range of 3.2-4.5 ppm [17], and confirmed by the presence of 12 carbon signals which refers to presence of two sugar moieties. A characteristic methyl doublet at δ_H 1.04 refers to the presence of rhamnosyl moiety and it was confirmed by the presence of a carbon signal at δ_C at 16.5 [16]. Additionally, the presence of proton doublet at δ_H 5.02 ppm (*J*= 7.5 Hz) indicated the presence of flavonol-3-*O*-glucosyl moiety and confirmed by the downfield anomeric carbon at δ_C 103.3 and HMBC correlation between anomeric proton of glucose and C-3. The proton signal at δ_H 4.43 represented a downfield anomeric rhamnose proton due to attachment to glucose, this was confirmed through HMBC correlation between anomeric rhamnose proton H-1``` and C-6" of glucose. Thus, compound **6** was confirmed to be Kaempferol-3-*O*-rutinoside.

The ^{1}H and ^{13}C NMR spectra of **7** were very close to that of **6** and indicated a flavonoid glucoside structure. However, the hydroxylation pattern of B-ring refers to a 3`,4`-dihydroxy substitution [17], as revealed from the proton signals at δ_H 7.66 (1H, d, *J*= 2.5 Hz, H-2`), 6.86 (1H, d, *J*= 8.0 Hz, H-5`) and 6.87 (1H, dd, *J*= 8.0, 2.5 Hz, H-6`). This was confirmed from the downfield carbon signals at δ_C 144.5 and 148.5 of the hydroxylated positions, C-3' and C-4', respectively. Therefore, the aglycone is confirmed to be quercetin [16-18]. Based on these findings the structure of compound **7** was confirmed to be quercetin-3-*O*-rutinoside (rutoside).

3.2 Alpha-amylase Inhibitory Assay

The identified compounds were tested for their alpha-amylase inhibition using chromogenic method using dinitrosalysylic acid (DNS) as substrate. Compared to acarbose as positive standard, most of the test compounds showed inhibitory activity [Table 1]. It was observed that the flavonoid diglucoside derivatives, compounds **6** & **7** showed potent inhibition (80.45 and 98.0%, respectively). However, their aglycone derivatives **3** & **4** showed lower inhibition (52.88 and 63.14%,

respectively). Also, *p*-Hydroxybenzoic acid also exhibited remarkable activity (61.54%). Other compounds including roseoside **1**, catechuic acid **5** showed weak inhibition.

Table 1. Alpha-amylase inhibitory activity of compounds isolated from *Musa cavendishii* using DNS chromogenic method

Compounds	% inhibition*
Compound **1**	32.05
Compound **2**	61.54
Compound **3**	52.88
Compound **4**	63.14
Compound **5**	4.81
Compound **6**	80.45
Compound **7**	98.0
Acarbose	57.0
100% Enzyme	0

**100 – % reaction at t = 3 min, whereby % reaction= (mean maltose in sample/mean maltose in control) × 100*

3.3 Docking

To get an insight about the mode of interaction between the binding site of alpha-amylase enzyme and the active compounds, docking study was conducted using MOE docking software [Table 2].

Quercetin-3-*O*-rutinoside (**7**) exhibits the highest alpha-amylase inhibitory activity (98.0%) at 50µg; this may be due to the formation of highly stable complex with the enzyme. This was confirmed by running the docking utility, where the binding energy is the lowest among all compounds (-8.16 Kcal/mol). Careful investigation of the docked structure **7** [Fig. 3(i)] shows 6 points of attachments with the enzyme active site through hydrogen bonding with polar amino acids such as, Glu233, Asp197, Asp300 and Tyr151 also the characteristic side chain hydrogen bond with the greasy Ile235.On the other hand, quercetin only [Fig. 3(ii)] shows moderate inhibitory activity either *In vitro* (80.45%) or *In silico* (-6.28 kcal/mol). It may be due to weaker interaction with the active site than the rutinoside derivative. Attachment is occurring through C-ring to polar acidic Asp300 and Glu233 by side chain hydrogen bond and through A-ring with polar Tyr62 by backbone hydrogen bonds.

Table 2. Free energy of binding *d*G in kcal/mol (S) calculated by MOE for the tested compounds

Compounds	Free energy of binding (kcal/mol)
Compound **7**	-8.16
Compound **6**	-7.68
Compound **3**	-6.47
Compound **4**	-6.28
Acarbose	-5.40
Compound **1-B**	-5.13
Compound **1-A**	-4.77
Compound **2**	-4.31
Compound **5**	-3.62

Kaemferol rutinoside, has high inhibition activity at the *In vitro* scale compared to the acarbose, this could be illustrated from the docked complex [Fig. 4(i)] which has the lowest energy so, more stable complex. From Fig. 4(i), it is worth to say that the interaction is well characterized with the glycone moiety through backbone hydrogen bonding with TRP59 and side chain hydrogen bonding with GLN63, ASP197 and GLU233. However the glycone moiety is exposed out of the active site. Regarding the kaemferol only

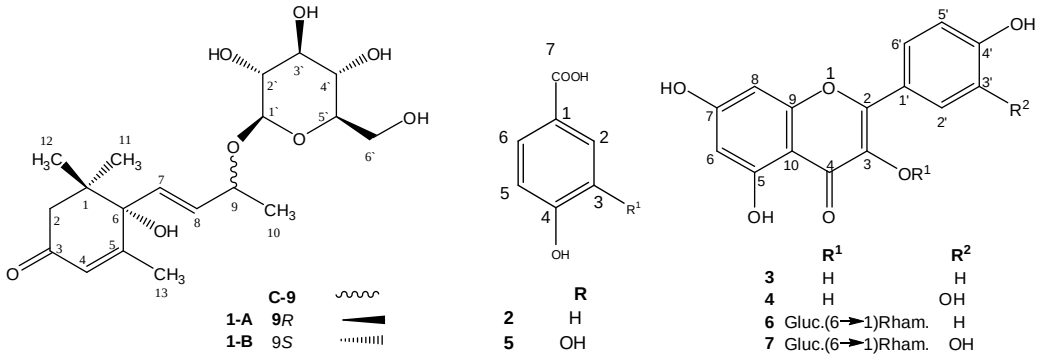

Fig. 2. Structures of the isolated compounds from the ethyl acetate fraction of *Musa cavendishii*

[Fig. 4(ii)], which has lower inhibition activity at 50 µg/ml, shows also weaker interaction with the active site through side chain hydrogen bonding with ASP197 and GLU233. Roseoside shows weak *In vitro* inhibitory activity which also correlate to its docking pattern [Fig. 5], where most of its structure is exposed out of the active site and the interaction is performed by the sugar molecules where the isomer 6*S*,9*S* [Fig. 5(i)] is attached through 3 point of interaction and isomer 6*S*,9*R* [Fig. 5(ii)] through 2 point however it is more active. Simply, it is worth to conclude that the diglycoside moiety allow more stable interaction and thus increase the activity.

p-Hydroxybenzoic acid shows a moderate inhibitory activity c.f catechuic acid that has no remarkable activity. *p*-Hydroxybenzoic acid [Fig. 6(i)] is interacted with the carboxylic group with the polar ASP300 and His 299 which allow the forming of stable complex with free energy (-4.31) more than catechuic acid (-3.62), Fig 6(ii). It is worth to say that the phenolic hydroxyl group by which catechuic acid interacted, form less stable complex than the *p*-hydroxy benzoic acid.

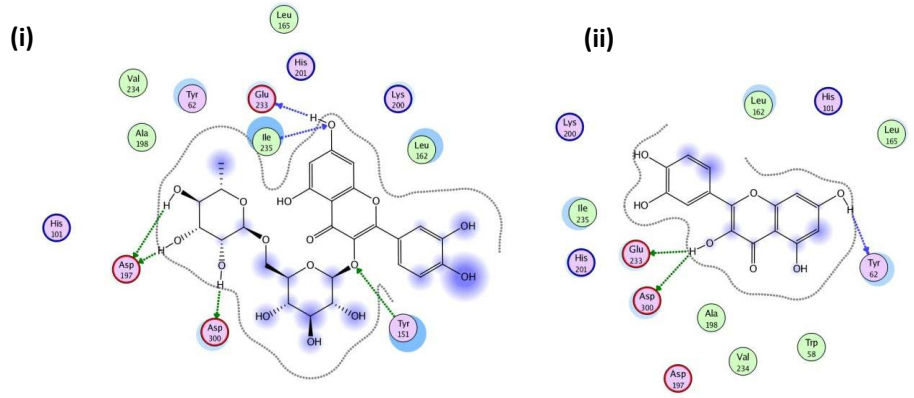

Fig. 3. Interaction diagrams of docked structures (i) compound 7 (rutin); (ii) its aglycone, 4 (quercetin) with alpha-amylase (PDB: 1HNY). Green arrow represents side chain acceptor/donor; blue arrow represents backbone acceptor/donor; blue shadow represents ligand exposure

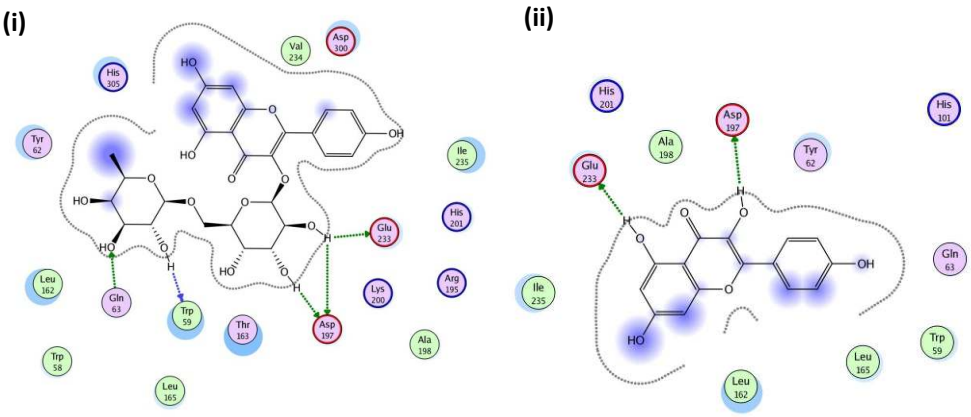

Fig. 4. Interaction diagrams of docked structures (i) compound 6 (kaempferol-3-*O*-rutinoside); (ii) its aglycone, 3 (kaempferol) with alpha-amylase (PDB: 1HNY). Green arrow represents side chain acceptor/donor; blue arrow represents backbone acceptor/donor; blue shadow represents ligand exposure

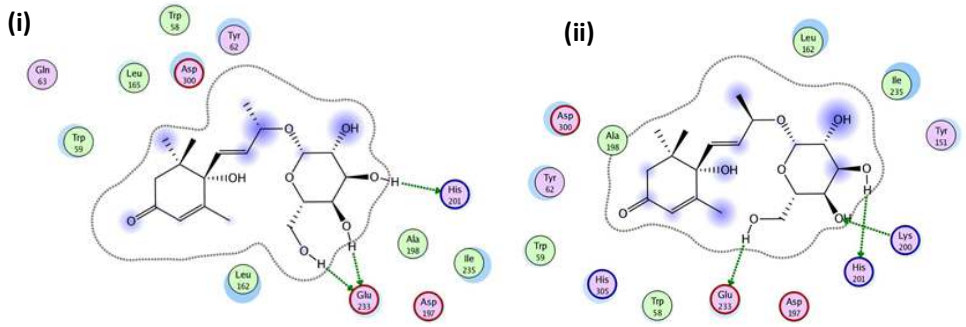

Fig. 5. Interaction diagrams of docked structures (i) compound 1-B (6*S*, 9*S*-roseoside); (ii) its isomer, 1-A (6*S*, 9*R*-roseoside) with alpha-amylase (PDB: 1HNY). Green arrow represents side chain acceptor/donor blue shadow represents ligand exposure

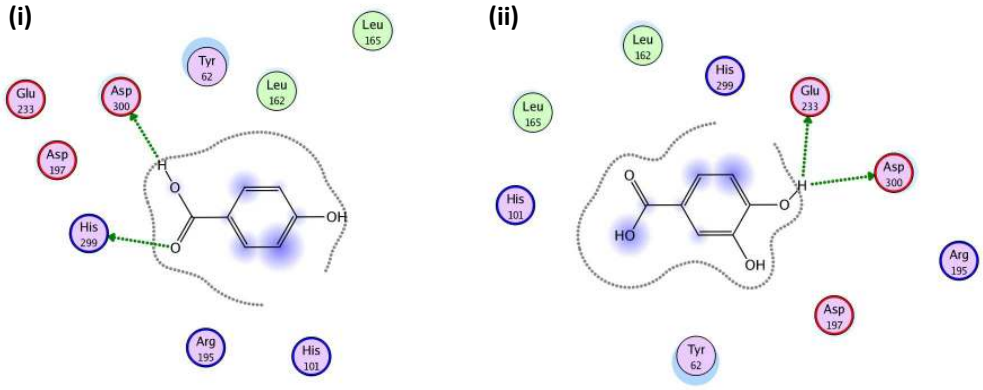

Fig. 6. Interaction diagrams of docked structures (i) compound 2 (p-hydroxybenzoic); (ii) compound 5 (catechuic) with alpha-amylase (PDB: 1HNY). Green arrow represents side chain acceptor/donor blue shadow represents ligand exposure

4. CONCLUSION

The flavonoid diglucosides; kaempferol-3-*O*-rutinoside and quercetin-3-*O*-rutinoside isolated from the leaves of *Musa cavendishii* could be potential inhibitors for alpha-amylase enzyme and could be used as safe anti-obesity phytochemicals.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

All authors stated that no conflict of interest.

REFERENCES

1. Barrett ML, Udani JK. A proprietary alpha-amylase inhibitor from white bean (*Phaseolus vulgaris*): A review of clinical studies on weight loss and glycemic control. Nutr J. 2011;10:24-24.
2. Buchholz T, Melzig MF. Medicinal plants traditionally used for treatment of obesity and diabetes mellitus – screening for pancreatic lipase and α-amylase inhibition. Phytother Res. 2016;30(2):260-266.
3. Ali H, Houghton PJ, Soumyanath A. alpha-Amylase inhibitory activity of some

Malaysian plants used to treat diabetes; with particular reference to *Phyllanthus amarus*. J Ethnopharmacol. 2006; 107(3):449-455.
4. Tundis R, Loizzo MR, Menichini F. Natural products as alpha-amylase and alpha-glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes: an update. Mini Rev Med Chem. 2010;10(4):315-331.
5. McDougall GJ, Stewart D. The inhibitory effects of berry polyphenols on digestive enzymes. Biofactors. 2005;23(4):189-195.
6. Koh LW, Wong LL, Loo YY, Kasapis S, Huang D. Evaluation of different teas against starch digestibility by mammalian glycosidases. J Agric Food Chem. 2010; 58(1):148-154.
7. Vettorazzi G. 5-hydroxytryptamine content of banans and banana products. Food Cosmet Toxicol. 1974;12(1):107-113.
8. Waalkes TP, Sjoerdsma A, Creveling CR, Weissbach H, Udenfriend S. Serotonin, Norepinephrine, and Related Compounds in Bananas. Science. 1958;127(3299): 648-650.
9. Ghosal S. Steryl glycosides and acyl steryl glycosides from *Musa paradisiaca*. Phytochem. 1985;24(8):1807-1810.
10. Bernfeld P. Amylases, α and β. Methods Enzymol. 1955;1:149-58.
11. Mamadalieva NZ, Sharopov F, Girault JP, Wink M, Lafont R. Phytochemical analysis and bioactivity of the aerial parts of *Abutilon theophrasti* (*Malvaceae*), a medicinal weed. Nat Prod Res. 2014; 28(20):1777-1779.
12. Murai Y, Kashimura S, Tamezawa S, Hashimoto T, Takaoka S, Asakawa Y, et al. Absolute Configuration of (6S,9S)-Roseoside from *Polygonum hydropiper*. Planta Med. 2001;67(05):480-481.
13. Sohn YM, Chin Y-W, Yang MH, Kim J. Terpenoid constituents from the aerial parts of *Aspleniums colopendrium*. Nat Prod Sci. 2008;14(4):265-268.
14. Wang Y-S, Liao Z, Zhu H-K, Feng X-F, Kun-Ming-Jiang, Huang R, et al. Megastigmane O-glucopyranosides from *Litsea glutinosa*. Chem. Nat. Comp. 2012; 48(2):346-349.
15. Martin TS, Ohtani K, Kasai R, Yamasaki K. A Hemiterpenoid Glucoside from *Musa paradisiaca.* J Nat Med. 2000;54(4):190-192.
16. Agrawal P, Thakur R, Bansal M. Carbon-13 NMR of Flavonoids. Elsevier Science INC; 1989.
17. Mabry TJ, Markham KR, Thomas MB. The Systemic Identification of Flavonoids. Springer-Verlag; 1970.
18. Napolitano JG, Lankin DC, Chen SN, Pauli GF. Complete 1H NMR spectral analysis of ten chemical markers of *Ginkgo biloba*. Magn Reson Chem. 2012;50(8): 569-575.

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