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Article

Inhibition of pancreatic α-amylase activity by a weight-loss herbal formula RCM-107

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Abstract

Reducing carbohydrates digestion by having low glycaemic index (GI) foods has been linked to weight loss. Inhibiting related enzymes is an alternative way to decrease carbohydrate digestion. RCM-107, an eight-herb formula that is modified from the RCM-104, has indicated significant weight-loss action in clinical trials. However, no research has been conducted to study its effect on the activity of porcine pancreatic alpha-amylase (PPA), which is involved in carbohydrate absorption. In this paper, we used fluorescence PPA inhibition assay to investigate the inhibitory effects of RCM-107 and the individual herbs present in this herbal mixture on amylase activity. Subsequently, molecular docking predicted the key active compounds that may be responsible for the enzyme inhibition. According to our results, both the RCM-107 formula and several individual herbs displayed α-amylase inhibitory effects. Also, marginal synergistic effects of RCM-107 were also detected. In addition, alisol B, (-)-epigallocatechin-3-gallate (EGCG) and plantagoside have been predicted as the key active compounds that may be responsible for the α-amylase inhibition effect of RCM-107 according to inter-residue contact analysis. Finally, Glu233, Gln63, His305, Asp300 and Tyr151 are predicted to be markers of important areas with which potential amylase inhibitors would interact.

Keywords: obesity, herbal medicine, molecular docking, α-amylase

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Introduction

Obesity has been defined as a chronic disease by the Obesity Society (TOS) in 2018 due to its emerging epidemiological trend [1]. It increases the risk of developing other metabolic disorders such as hypertension, type 2 diabetes, cardiovascular diseases and myocardial infarction [2, 3]. At least 2.8 million adults die due to being overweight or obese each year [4]. Globally, over 1.9 billion adults were overweight while more than 650 million adults were obese in 2016 [4].

Currently there is a variety of therapeutic targets available for weight reduction, such as pancreatic lipase, alpha-amylase, glucagon like peptide-1 (GLP-1) receptor and serotonin 2C receptor [5-7]. Along with the general reduction in use of appetite suppressants which act on the central nervous system (CNS), *eg*: fenfluramine, d-fenfluramine and rimonabant [5], drugs that act on the periphery have gained wider use [5]. Some periphery-acting drugs have proven successful in weight management with mild intolerances, especially those which reduce the digestion and absorption of nutrients [6].

In humans, various forms of carbohydrates account for between 40% to 80% of total caloric intake [5, 6]. Low GI foods (GI value < 55), polymeric form of carbohydrates that are absorbed slowly, have been linked to glycemic control and weight loss [8]. An alternative to low GI foods are products that can decrease carbohydrate absorption via suppressing related enzymes such as pancreatic α-amylase [6, 8]. Alpha-amylase is responsible for catalysing the hydrolysis of α-(1, 4)-glycosidic linkages of starch components and glycogen. Therefore, suppressing this enzyme could result in a general decrease of the main dietary carbohydrates absorption [5]. The known alpha-amylase inhibitors such as acarbose have been used as an off-label agent to assist weight loss [9]. In addition, the supplement Phase2® *Phaseolus vulgaris* white bean extract demonstrated weight-loss effects in human clinical trials via its amylase inhibitory activity [10].

Chinese herbal formulas are therapeutic herbs traditionally used in combination rather than individually. Many *in vitro* and *in vivo* studies [11-16] have demonstrated the synergistic actions of herbal formulas, indicating that herbal formulas show significantly better pharmacological effects than single herbs for different conditions, including obesity [17]. Park et al. [16] reported a stronger weight-reducing effect of two herbs (*Panax ginseng* and *Veratrum nigrum)* used in combination rather than separately on high-fat diet induced mice. Active ingredients such as phenolic and flavonoid contents from a given herbal medicine can be responsible for their synergy effects [17]. However, it is challenging to evaluate these synergistic effects accurately as the exact chemical and pharmacological properties of Chinese herbs are not clearly defined [18]. An integrated systematic analysis approach, including literature, experimental and computational studies have been developed in order to assist analysing complex multi-ligands-targets synergistic actions [17, 18]. Additionally, molecular docking has been commonly used in drug design, aiming to predict binding sites of a ligand with a target protein. The effect of a ligand on the target protein could be predicted by comparing its binding site with an established drug (e.g. inhibitor), which has known action on that protein. Also, molecular docking has been applied to identify key active compounds that can bind to the corresponding targets at the known active site [18].

In this paper, we have investigated the RCM-107 formula, modified from our previous studied RCM-104 formula, which demonstrated significant effects on weight loss in clinical trials [19]. RCM-107 contains eight Chinese herbs, including *Camellia sinensis* (green tea), *Poria*, *Nelumbinis folium* (lotus leaf), *Alismatis rhizoma*, *Plantaginis semen*, *Cassiae semen*, *Sophorae flos* and *Gardeniae fructus*. To date, no scientific evidence has been found to indicate the mechanisms of action for the use of the RCM-107 in weight management, specfically with regards to individual herbs and chemical compounds within this formula for their possible roles in alpha-amylase inhibition.

Therefore, this present work aims to firstly determine the effects of RCM-107 and individual herbs in this formula on the inhibition of porcine pancreatic alpha-amylase (PPA) using experimental amylase inhibition assays. The porcine form of the enzyme is an appropriate choice for this work due to the high sequence similarity between human, porcine and mouse pancreatic

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alpha-amylase [20]. The results from the present study can therefore readily be extrapolated to understand the mechanisms of action of the formula on a range of mammals, including humans.

In addition, key active components that could act as potential PPA inhibitors from the individual compounds within these herbs in the RCM-107, obtained from literatures [21-34], will be prediced via molecular docking.

2. Methods

2.1 Amylase inhibition assay

2.1.1 Materials

A EnzCheck® Ultra Amylase Assay Kit (E33651) and phosphate buffer saline (PBS) tablets (18912) were obtained from Life Technologies Australia Pty Ltd (Mulgrave, AUS). Porcine pancreatic α-amylase (PPA) (50 mg/5mL, 10102814001), Dimethylsulfoxide (DMSO, 472301-500mL) and acarbose (A8980-1G) were purchased from Sigma-Aldrich (Australia). RCM-107 capsules (AUST L 285569) were provided by Tong Kang Xiao Chinese medicine clinic and herbal granules (Nong's, HK) consisting of *Poria*/Fu ling, lotus leaf/He ye, *Alismatis rhizome*/Ze xie, *Plantaginis semen*/Che qian zi, *Cassiae semen*/Jue ming zi, *Sophorae flos*/Huai Hua, *Gardeniae fructus*/Zhi zi, green tea/matcha were supplied by GL natural health care clinic.

2.1.2. Sample preparation

Each RCM-107 capsule contains 500 mg herbal powder mixed with starch. Herbal powder and granules were obtained by water extraction of the raw materials via boiling and lyophilisation. Twenty milligrams of the RCM-107 herbal powder and 8 herbal granules were weighed and dissolved in 4 mL of distilled water contained 2% DMSO. The sample was vortexed for 5 min and sonicated for 10 min then filtered through a Millex-HP 0.45μm filter (Millipore). Dilution from the stock solution (5 mg/mL) was performed to achieve a final concentration of 300 μg/mL. In addition, serial dilution of the RCM-107 formula was conducted to yield a seven calibration point ranging from 10-800μg/mL. The positive control acarbose was prepared in the same manner.

2.1.3 Fluorescence assay

Fluormetric assay of PPA activity using DQ™ Starch as a substrate was determined using a modified method previously described by [35]. Twenty-five microliters of each individual herbal extract at the final concentration of 300 μg/mL, the RCM-107 formula extraction (10-800μg/mL) and 25 μL of the PPA solution (final concentration 12mU/mL) were pre-incubated in a 96-well plate with 25 μL PBS buffer (The incubation buffer consisted of 10mM sodium phosphates, 2.68mM KCl, 140 mM NaCl and 1mM $CaCl_2$, pH 6.9), in dark at room temperature for 20 min. To initiate the enzyme reaction, 25μL of 200 μg/mL DQ™ starch solution was added. Fluorescence was measured with the *CLARIOstar*® microplate reader (BMG labtech) at an excitation and emission wavelength of 485nm and 530nm, respectively over 30 min. Acarbose (a known pancreatic alpha-amylase inhibitor) was used as a positive control. The concentration of DMSO was 0.16% and showed no effects on PPA activity. The inhibition activity (%) of the PPA was calculated by the following equation:

$$\left(1-\frac{\text{F sample}-Fsample\ background-F\ blank}{F\ control-Fcontrol\ background-F\ blank}\right)*100$$

F_{sample} and $F_{sample\ background}$ represent a fluorescence value of the sample solution with or without substrate, while $F_{control}$ and $F_{control\ background}$ are a fluorescence value of the control (contains no inhibitors) with or without substrate, respectively. F_{blank} is the fluorescence value of the selected blank, which consists of substrate and buffer.

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126 2.1.4. Statistical analysis

127 Samples and controls were run in triplicates. Results are presented as mean ± standard error
128 from 3 independent experiments. Wells that contain either samples and buffer or enzyme and buffer
129 only were used to detect background auto-fluorescence. The concentration providing 50% inhibition
130 (IC_{50}) was calculated by non-linear regression (values are mean ± SD) and statistical significance was
131 assessed with one way analysis of variance (ANOVA) followed by the Tukey multiple comparison
132 test via Graphpad Prism Software. Results with a P value < 0.05 has been considered statistically
133 significant.

134 *2.2 Molecular Docking*

135 2.2.1 Compound screening

136 A total of 149 ligands were obtained from the literature, including the major bioactive chemical
137 constituents from each herb present in RCM-107 [21-34]. Other compounds which met the common
138 requirements for potential favorable therapeutic efficacy, such as good oral bioavailability (OB) ≥
139 30%, drug likeness (DL) ≥ 0.18 and intestinal epithelial permeability (Caco-2) ≥ -0.40 were also
140 included [15, 36].

141 2.2.2 Software and Input files

142 The docking software PyRx version 0.8, together with Autodock Vina, was used for all docking
143 calculations (https://pyrx.sourceforge.io/). The structures of small molecules (ligands) and
144 macromolecules (target proteins) required to initiate structure-based virtual screening were
145 obtained and pre-processed as follows.
146 The structures of small molecule were obtained from **Pubchem or TCMSP.** The names of
147 ligands were searched from Pubchem and their 3D structures were downloaded in the form of SDF
148 files; Subsequently, the Online SMILES Translator and structure file generator
149 (https://cactus.nci.nih.gov/translate/) was then used to translate the SDF files into PDB files, which
150 are recognized by PyRx and Autodock Vina. The canonical SMILES sequences were compared
151 when more than one structure appeared for the same ligand. If the SMILES sequences were
152 different, all structures of this ligand would be collected for docking, otherwise the first structure
153 on the list would be selected.
154 The PPA structure (PDB CODE: 1OSE) was obtained from the **RCSB Protein Databank**
155 (www.rcsb.org). The 3D protein structures were modified using Visual Molecular Dynamics (VMD)
156 by removing water molecules to obtain protein-only structures and the prepared structure was
157 saved in PDB format.

158 2.2.3. Autodock Vina

159 Both selected protein (1OSE) and ligand files were loaded to PyRx as macromolecules and
160 ligand, respectively. Proteins are fixed, while ligands were set to have rotatable torsions. Protein
161 and ligand hydrogens were automatically added using the PyRx hydrogen (H) repair functionality.
162 A box of size X: 8.3130; Y: 73.5614; Z: 146.1870 were defined around the centre of the protein, with
163 the exhaustiveness parameter set to 64 for all dockings. The two-dimensional (2D) ligand and target
164 interaction can be obtained from Discovery Studio Visualizer (DSV) Version 4.5, which can be found
165 from BIOVIA (https://www.3dsbiovia.com/products/collaborative-science/biovia-discovery-studio/). The
166 three-dimensional (3D) image and binding sites were observed and displayed in VMD.

167 **3. Results and discussion**

168 *3.1. Amylase inhibition assay*

The RCM-107 formula and 6 individual herbs present in this formula displayed significant PPA inhibitory activities after screening at 300μg/mL (Figure 1). The RCM-107 formula exhibited the highest inhibition rate followed by *Gardeniae fructus* (64%), lotus leaf and green tea (60%, 54% and 51%, respectively). However, our data has shown that differences between those three herbs and the RCM-107 formula were not statistically significant ($P > 0.05$). *Sophorae flos* did present significant PPA inhibition ($P < 0.0001$) but at a much lower inhibition rate (29%) compared to the above four samples. In addition, *Cassiae semen* and *Plantaginis semen* displayed an inhibition rate of 17% and 16%, respectively and were statistically significant ($P < 0.05$).

Only two single herbs *Poria* and *Alismatis rhizome* were statistically *non*-significant ($P > 0.05$) on PPA. *Alismatis rhizome* slightly increased the enzyme activity rather than suppressing it. Even though the inhibitory potency of the RCM-107 formula, *Gardeniae fructus,* lotus leaf and green tea are less active than acarbose, the data suggests they may act as a milder inhibitor of PPA. On the other hand, *Poria* and *Alismatis rhizome* had little or no effects on suppressing this enzyme.

Both the RCM-107 formula and the known amylase inhibitor, acarbose, demonstrated their effects by suppressing PPA in a dose-dependent manner (Figure 2). The enzyme activity was reduced dramatically from 85% to 3% when the concentration of the RCM-107 formula was raised from 10-800 μg/mL. The inhibitory potency of acarbose was presented by achieving the maximum inhibition (98%) at the concentration of 500 μg/mL. Acarbose showed a lower IC_{50} (16.31± 1.74 μg/mL) compared to the RCM-107 formula (119.5 ± 17.14 μg/mL). This data indicates that the RCM-107 formula can inhibit pancreatic α-amylase but not as potently as the positive control.

It is worthy to note that RCM-107 is an 8-herb combination at 2:1(w/w) ratio, in which the proportion of green tea is 2-fold higher than the other 7 herbs in this formula, due to its role as a key herb ("Jun") [18]. However, in the present amylase inhibition assay, equal concentrations of the herbal formula and individual herbs were used. This difference in the relative proportion of green tea in the actual formula compared to that in the present assay study may partly contribute to the present lack of observable synergistic effects of RCM-107 in inhibiting PPA enzymatic activity. Nonetheless, it may be proposed that *Gardeniae fructus,* lotus leaf and green tea are the leading herbs in RCM-107 that contribute to the action of α-amylase suppression as they present statistically significant inhibitory effects. In addition, it is proposed that increasing the proportion of individual herbs, such as *Gardeniae fructus* or lotus leaf, in the multiple-herbal combination may serve to improve the significance of synergistic effects of RCM-107 [18].

3.2 Molecular docking studies of the active site of porcine pancreatic amylase and herbal ligand-target interactions

In order to predict the likely main active compounds within the RCM-107 formula which serve to most effectively inhibit alpha-amylase, molecular docking was used to predict the predominant binding mode of potential active components with the selected protein target alpha-amylase [18]. Furthermore, the predicted interactions between bioactive herbal ligands and the targeted protein were compared with a positive control (a known inhibitor, acarbose) as well as an endogenous substrate (starch), in order to propose the key residues that may be required for a potential inhibitor to interact with, and which may therefore be responsible for the suppression of the activity of the protein target [18]. Thus, we take two approaches to propose potentially effective PPA inhibitors from RCM-107: first, by determining ligands which show the highest predicted binding affinity values; and second, by determining those ligands which share the most common interactions with PPA as the positive control, acarbose.

Acarbose was docked to the crystal structure alpha-amylase as a positive control and three binding poses were predicted to lie at the known active site, which consists of a V-shaped depression located at the carboxyl end of the 'domain A' β-barrel [20, 37]. There are four main classes of interactions present in this known α-amylase antagonist. Firstly, H-bonds are formed with acidic residues such as Glu233 and Asp300. Secondly, H-bonding with basic residues Lys200 and His305 are also present. Thirdly, interaction with the backbone of Gly residues, such as Gly306. Finally,

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aromatic ring-ring interactions with Trp58 and Trp59 provides further support to firmly anchor the inhibitor into the active site.

In addition, substrate (starch) was docked into the corresponding protein to analyse its ligand-target interactions and how they differ from the known inhibitor. According to the results, all four classes of interactions mentioned earlier present (Figure 3). However, only one residue formed a H-bond for each of these classes (only Glu233 can be found under the category of H-bond with acidic residues) whereas there are at least 2 residues involved in each interaction for acarbose (*eg.* Glu233 and Asp300). Amongst these residues, three carboxyl groups Glu233, Asp300 and Asp197 are crucial for enzymatic activity, and constitute the 3rd subsite, in which Glu233 plays a crucial role in the first stage of the catalytic process as the general acid and a proton donor [20]. Therefore, acarbose serves as an effective inhibitor by preserving the key types of H-bonding and ring-ring interactions required for effective binding of ligands to the active site, and by direct binding to key residues such as Glu233, which normally functions as a nucleophilic attack moiety [20]. Additionally, it also forms additional supporting H-bonding interactions which more firmly anchors the inhibitor within the active site to prevent entry of the endogenous substrate.

Subsequently, 149 main compounds from the eight herbs present in RCM-107 were docked with the target protein (PDB ID: 1OSE). Details for the top 3 highest binding affinity ligands for each herb (**1**. Stigmasterol; **2**. Campesterol; **3**. Cassiaside; **4**. Theaflavin; **5**. Thearubigin; **6**. EGCG; **7**. Cycloartenol; **8**. Quercetin; **9**. Isorhamnetin; **10**. Beta-sitosterol; **11**. N-[6-(acridin-9-ylamino) hexyl] benzamide; **12**. Sophoradiol; **13**. Sudan III; **14.** Alisol B; **15.** Alisol A; **16.** Alisol C; **17.** Stellasterol; **18.** Eburicoic acid; **19.** Dehydroeburicoic acid; **20.** Plantagoside; **21.** Daucosterol_qt, in which compound **1-3** are extracted from *Cassia semen*; **4-6** are from green tea; **7-9** are from lotus leaf; **10-12** are from *Sophorae flos*; **1, 8, 13** are from *Gardeniae fructus*; **14-16** are from *Alismatis rhizome*; **17-19** are from *Poria*; **8, 20-21** are from *Plantaginis semen*), including their chemical structures, active site residue interactions and predicted binding energy values, are provided in Table 1, Figures 4 and 5. According to our results, docking energies of 57% of the compounds were superior to the known amylase inhibitor acarbose (-8.4 kcal/mol). In particular, compounds **14** (alisol B, -10.7 kcal/mol) and **20** (plantagoside, -10.5 kcal/mol) exhibit the highest binding affinity, with both having substantially greater affinity values than acarbose, and are proposed to be effective inhibitors of PPA.

In addition to the predicted binding affinity values, qualitative inspection of the docked site can improve the accuracy and success rate of predicting inhibitor efficacy as Autodock was reported to have a typical error of ±2 kcal/mol [38]. In particular, we propose that ligands which share similar patterns of inter-residue interactions as those of the positive control inhibitor, acarbose, may also serve as potential effective inhibitors of PPA. The predicted docking interactions between the selected highest binding affinity ligands (**1**-**21** as well as acarbose) with alpha-amylase are presented in Figure 5.

According to the docking results, compounds **5**, **6** (extracted from green tea) and **20** (obtained from *Plantaginis semen*) had more common residues with acarbose than other included ligands (in contrast, despite exhibiting the highest predicted binding affinity, compound **14** exhibited few common interactions with acarbose). Both compounds **6** and **20** displayed additional H-bonds for two main classes of interactions. Firstly, H-bonds with acidic residues Asp300 and Glu233 (compound **6** and **20**). Secondly, H-bonds with basic residues His305 and His201 (compound **6**) while compound **20** formed H-bonds with basic residues His299 and His201. Compound **5** did present H-bonds with a pair of acidic residues Glu233, Asp300 but only with one basic residue His305. In addition, there was only one aromatic ring-ring interaction with Trp59 for the compound **5**. All three compounds had no H-bonds formed with the backbone of Gly residues, which however presented in the positive control acarbose**.** This may explain the role of RCM-107 and the relevant individual herbs in inhibiting amylase activities and why they are not as potent as acarbose as observed in this study. On the other hand, both *Poria* and *Alismatis rhizome* are unlikely to be effective amylase inhibitors as their top ranked compounds formed no or minimal number of common H-bonding residues with acarbose, which is consistent with the present inhibition results. Therefore, the corresponding residues (Glu233, Gln63, His305, Asp300 and Tyr151) can be

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considered as markers of important areas in the ligand binding site. In addition, compounds **6** and **20** are more likely to act as the key compounds from RCM-107 for amylase inhibition (Figure 6). In summary, the highest ranking compound is **alisol B** while the inter-residue contact analysis showed **EGCG** and **plantagoside** shared the most common residues with the known inhibitor and presented similar interactions, suggesting they may be responsible for the amylase inhibitory action of the RCM-107 (Table 1).

Conclusion

The present work examined the alpha-amylase inhibition of the herbal formula RCM-107 and its constituent herbs, and showed that the RCM-107 can be applied as an effective α-amylase inhibitor, which act in a dose-dependent manner. Marginal (though not statistically significant) synergistic effects were observed for the RCM-107 formula when compared to the 8 single herbs. However, the lack of observable significant synergistic effects may be related to the differences between the ratio of some individual herbs in RCM-107 and the use of equal concentrations of each herb in the present inhibition assay studies. We propose that RCM-107 can be optimized via increasing the proportion of *Gardeniae fructus* and lotus leaf in the herbal mixture as those two individual herbs displayed higher amylase inhibition activities than the others. Also, alisol B, EGCG and plantagoside are proposed as the key active compounds that may play important roles in the α-amylase inhibition action of the RCM-107. Further bioassays are required to confirm this hypothesis.

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298 **Table 1:** Top 3 ligands predicted from each herb with the strongest binding affinity and their bonds with IOSE.

Herbal names	Ligands	PubChem ID	Molecular formula	Molecular Weight (g/mol)	Hydrogen bond (amino acids)	Bond length	Unfavourable bond (amino acids)	Bond length	Lowest binding affinity (kal/mol)
Cassia semen	Stigmasterol (compound **1**)	5280794	$C_{29}H_{48}O$	412.702	GLU233	4.17	-	-	-9.8
Cassia semen	Campesterol (compound **2**)	173183	$C_{28}H_{48}O$	400.691	-	-	-	-	-9.5
Cassia semen	Cassiaside (compound **3**)	164146	$C_{20}H_{20}O_9$	404.371	GLN63 GLY106 GLY164 LEU165(Carbon hydrogen bond) VAL163 (Carbon hydrogen bond)	5.61 3.93 3.81 5.81 6.3	GLN63	3.93	-9.2
Green tea	Theaflavin (compound **4**)	114777	$C_{29}H_{24}O_{12}$	564.499	HIS201 ASP356 HIS305 GLY306 TRP59	5.38 4.62 3.66 4.12 4.1	GLN63	3.51	-10
Green tea	Thearubigin (compound **5**)	100945367	$C_{43}H_{34}O_{22}$	902.723	GLU233 (2 BONDS) ASP300 (2 BONDS) HIS305 (2 BONDS) GLN63 TRP59 VAL354	5.47;5.04 4.99;4.15 4.88;4.16 4.69 5.27 3.36	-	-	-9.8

Green tea	EGCG (compound **6**)	65064	$C_{22}H_{18}O_{11}$	458.375	HIS305 TYR151 (2 BONDS) HIS201 GLU233 ASP197 GLN63	3.97 6.55;6.43 4.96 5.81 4.28 5.35	-	-	-9.5
Lotus leaf	Cycloartenol (compound **7**)	92110	$C_{30}H_{50}O$	426.729	-	-	-	-	-9.6
Lotus leaf	Quercetin (compound **8**)	5280343	$C_{15}H_{10}O_7$	302.238	ASP300 ASP197 TYR62 GLN63 HIS305	5.12 4.69 3.68 4.21 6.44	-	-	-9.4
Lotus leaf	Isorhamnetin (compound **9**)	5281654	$C_{16}H_{12}O_7$	316.265	GLN63 (2 BONDS) TYR62 ASP197	5.81;4.2 4.37 4.36	-	-	-9.2
Sophorae flos	Beta-sitosterol (compound **10**)	222284	$C_{29}H_{50}O$	414.718	-	-	-	-	-9.6
Sophorae flos	N-[6-(acridin-9-ylamino)hexyl]benzamide (compound **11**)	146515	$C_{26}H_{27}N_3O$	397.522	GLU233 (Carbon hydrogen bond)	6.11	-	-	-9.5
Sophorae flos	Sophoradiol (compound **12**)	9846221	$C_{30}H_{50}O_2$	442.728	-	-	-	-	-9.4
Gardeniae fructus	Sudan III (compound **13**)	62331	$C_{22}H_{16}N_4O$	352.397	HIS305	4.1	-	-	-10

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Gardeniae fructus	Stigmasterol	See above	See above	See above	See above	See above			-9.8
Gardeniae fructus	Quercetin	See above	See above	See above	See above	See above			-9.4
Alismatis rhizome	Alisol B (compound **14**)	189051	$C_{30}H_{48}O_4$	472.71	VAL163 GLN63	2.96 5.31	HIS305	3.79	-10.7
Alismatis rhizome	Alisol A (compound **15**)	15558616	$C_{30}H_{50}O_5$	490.725	-	-	VAL163	5.41	-10.1
Alismatis rhizome	Alisol C (compound **16**)	101306923	$C_{30}H_{46}O_5$	486.693	GLN63 HIS305	4.17; 5.63 4.10	VAL163	4.37; 4.30	-10
Poria	Stellasterol (compound **17**)	5283628	$C_{28}H_{46}O$	398.675	-	-	-	-	-9.7
Poria	Eburicoic acid (compound **18**)	73402	$C_{31}H_{50}O_3$	470.738	HIS299	4.97	-	-	-9.6
Poria	Dehydroeburicoic acid (compound 19)	15250826	$C_{31}H_{48}O_3$	468.722	TYR62 (Pi-Doner Hydrogen bond)	3.23	-	-	-9.6
Plantaginis semen	Plantagoside (compound **20**)	174157	$C_{21}H_{22}O_{12}$	466.395	GLN63 HIS299 GLU233 (2 BONDS) ASP300 TYR151 HIS201 HIS201(Carbon hydrogen bond) HIS305	4.24 5.51 5.28;3.32 5.8 6.35 5.72 5.58 6.3	GLN63	4.88	-10.5
Plantaginis semen	Daucosterol_qt (compound **21**)	-	-	414.79	-	-	-	-	-9.8
Plantaginis semen	Quercetin	See above	See above	See above	See above	See above	-	-	-9.4

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The known amylase inhibitor	Acarbose (**Reference**)	41774	$C_{25}H_{43}NO_{18}$	645.608	GLN63 GLY104 ASP300 GLU233 GLU233 GLY306 GLU240 LYS200 ASP300 VAL163 LYS200 TYR151 HIS305 GLY304 GLY306 TYR151 LYS200 GLU233	4.64 2.83 4.45 5.24 5.16 2.83 5.86 5.69 4.51 5.26 4.76 6.05 3.51 4.44 3.22 6.46; 6.54 5.02 4.98	GLN63 HIS305	4.85 3.59	-8.4
Substrate	Corn starch	24836024	$C_{27}H_{48}O_{20}$	692.661			-	-	-6.4

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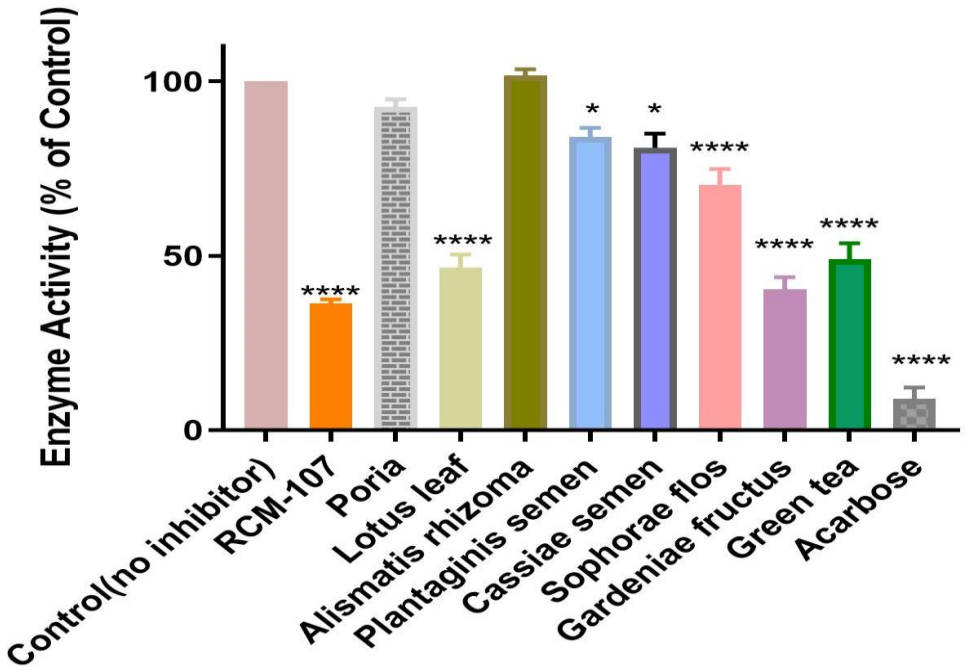

Figure 1. Suppressive effects of the RCM-107 formula, eight single herbal granules and acarbose (inhibitor) at 300 μg mL^{-1} on porcine pancreatic α-amylase activity. The enzyme activity with an absence of the samples or inhibitor was presented as 100%. Data is expressed as means ± SEM from three independent experiments, including three replicates each time. **** indicates $P< 0.0001$ as compared to the control.

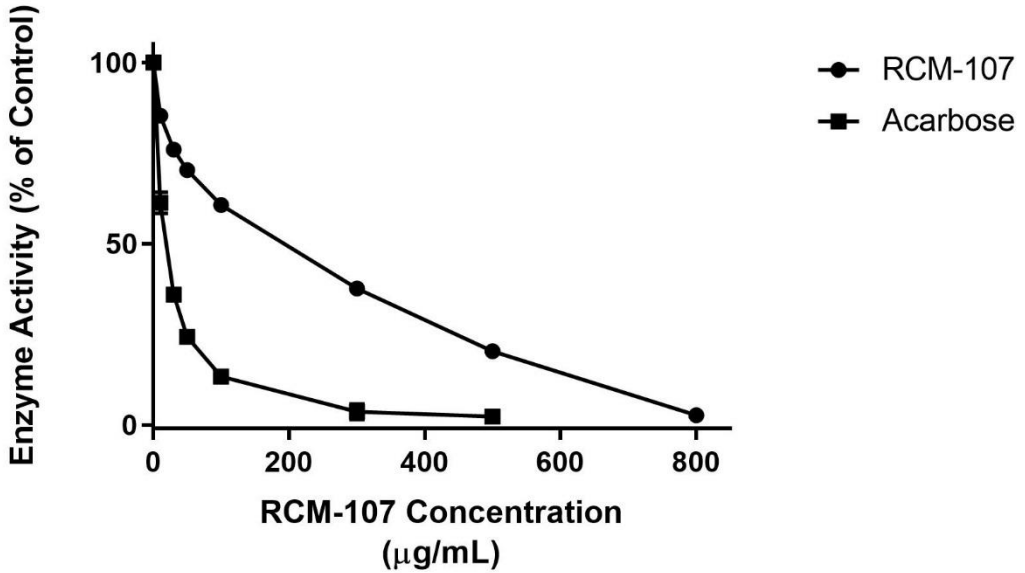

Figure 2. Dose-dependent inhibitory effects of the RCM-107 formula (1-800μg mL^{-1}) on porcine α-amylase. Data represent mean ± SEM from three independent experiments with three replicates per condition.

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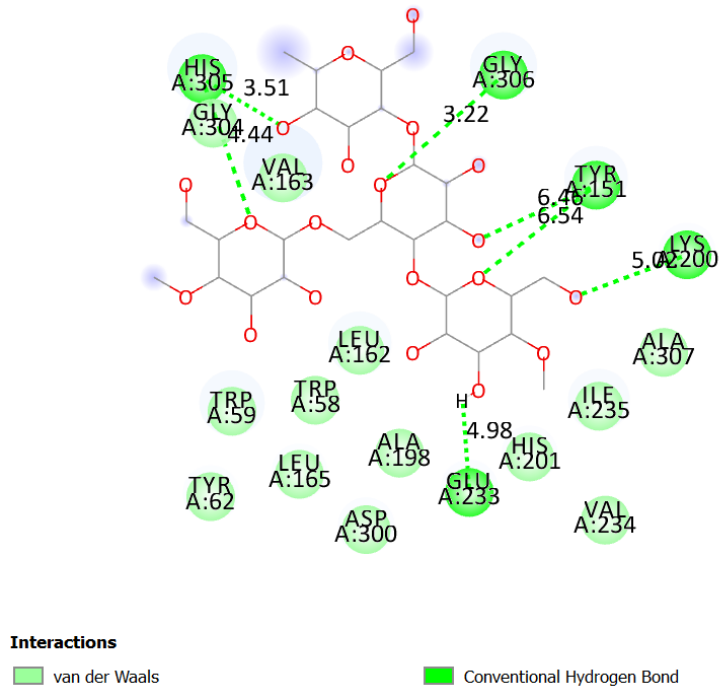

Figure 3. 2D diagram of interactions between corn starch and 1OSE.

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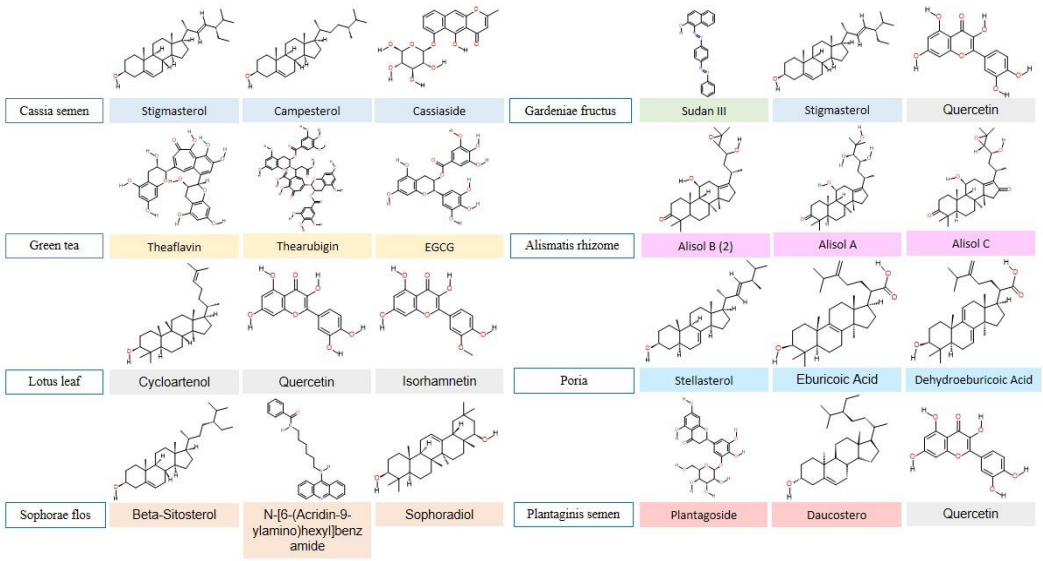

Figure 4. Chemical structures of selected compounds.

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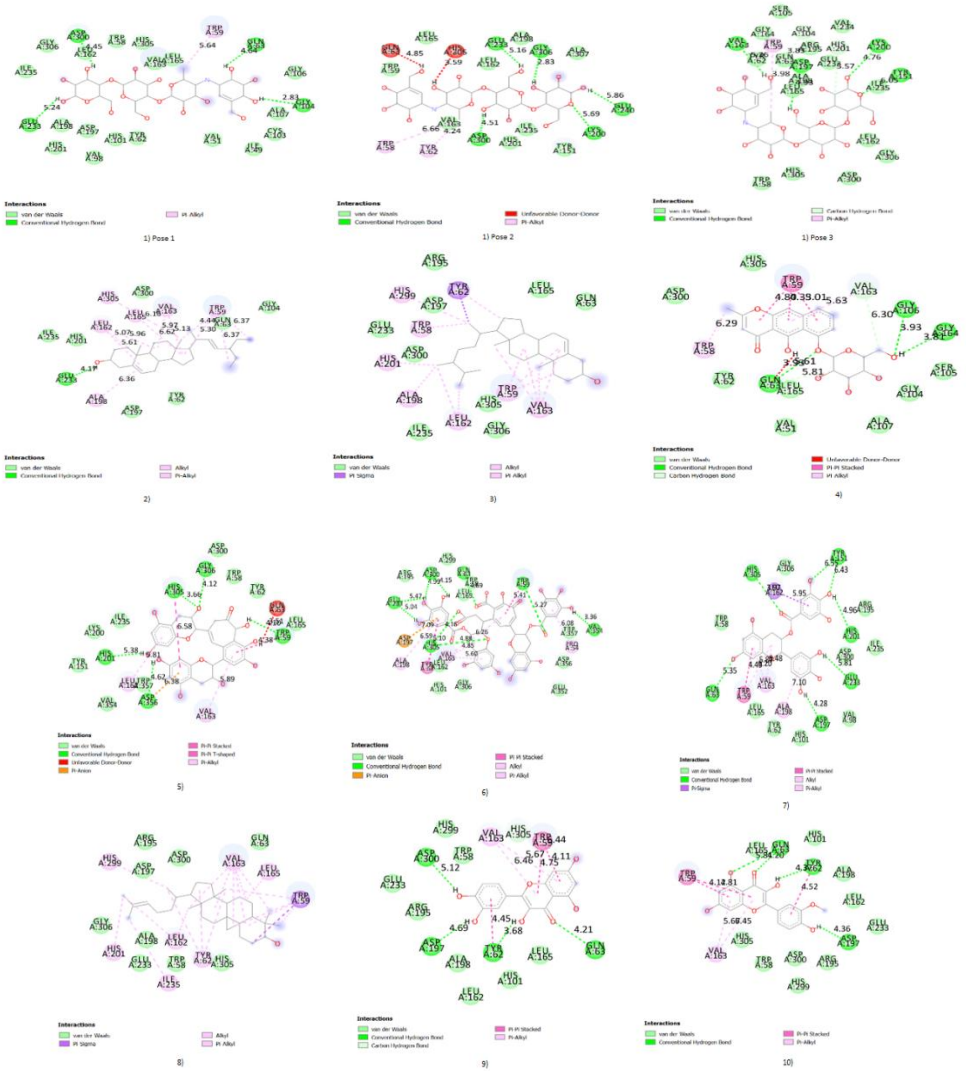

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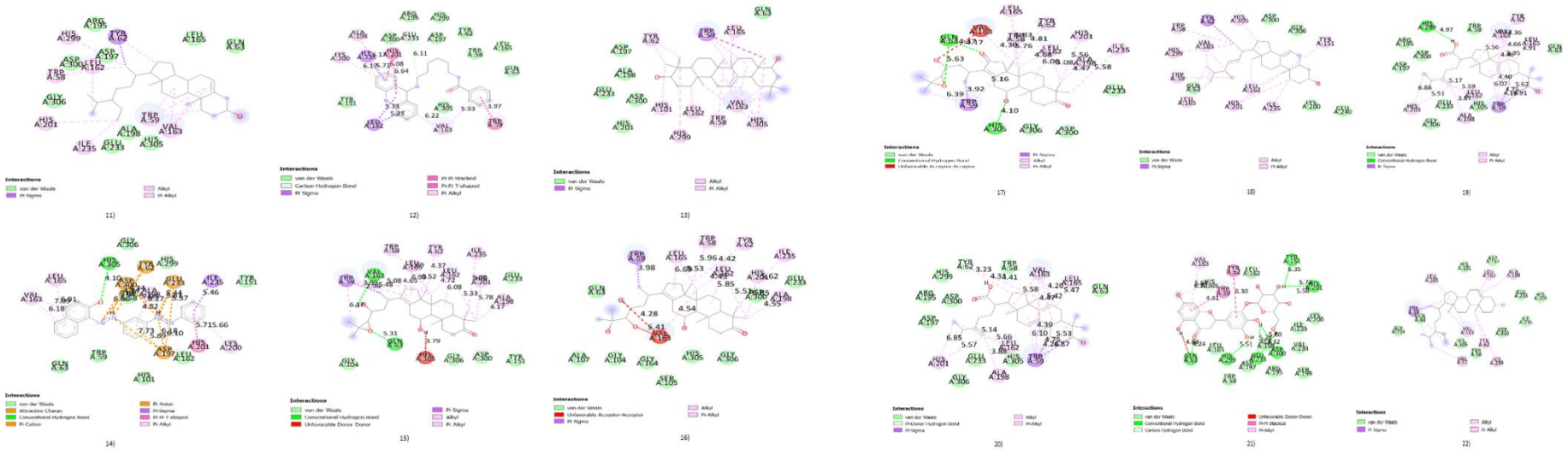

Figure 5. 2D diagram of top 3 predicted ligands from each herb with 1OSE. 1) Acarbose (3 binding poses) 2). Stigmasterol 3) Campest-5-en-3bta-ol; 4) Cassiaside; 5) Theaflavin; 6) Thearubigin; 7)EGCG; 8) Cycloartenol; 9) Quercetin; 10) Isorhamnetin; 11) Beta-sitosterol; 12) N-[6-(acridin-9-ylamino)hexyl]benzamide; 13) Sophoradiol; 14) Sudan III; 15) Alisol B; 16) Alisol A; 17) Alisol C; 18) Stellasterol; 19) Eburicoic acid; 20) Dehydroeburicoic acid; 21) Plantagoside; 22) Daucostero_qt

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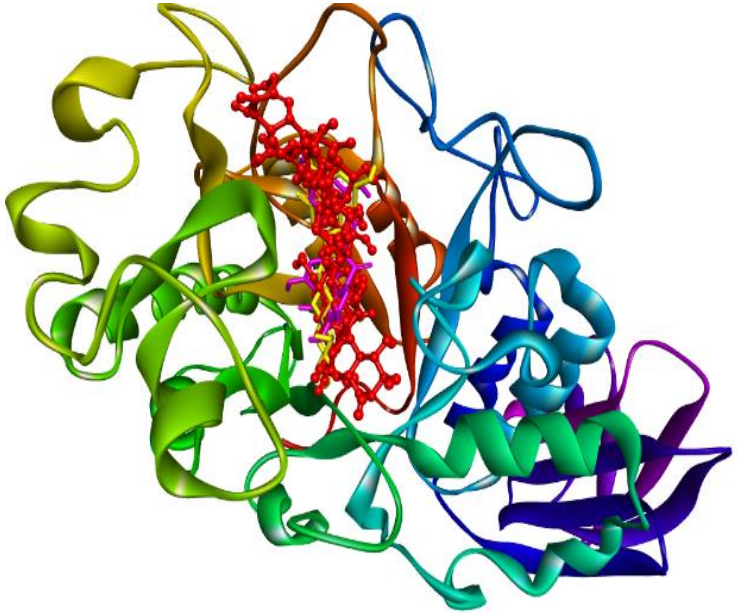

Figure 6. 3D interactions between predicted leading compounds EGCG (pink), Plantagoside (yellow), the known inhibitor acarbose (red) and 1OSE.

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