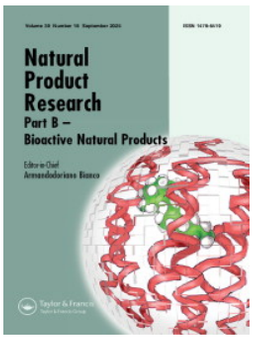

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Identification of putative α-glucosidase inhibitors and antioxidants in *Zingiber officinale* rhizome using LCMS-based metabolomics and *in silico* molecular docking

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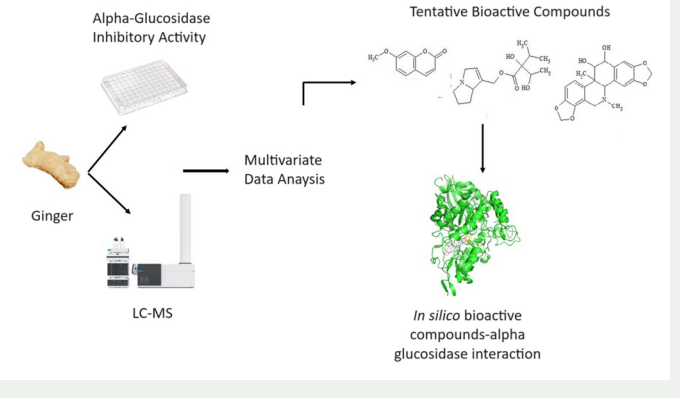
ABSTRACT

Metabolite profiling is required to reveal bioactive chemicals in ginger rhizome for supporting its traditional claim as anti-diabetic agent. This study aimed to evaluate α-glucosidase inhibitory (AGI) and antioxidant activities of the rhizome, to identify its putative α-glucosidase inhibitors, and to analyse the protein-ligand interaction of the inhibitors. The ginger extracts were tested to *in vitro* AGI assay and analysed using LCMS-based metabolomics to pinpoint the putative α-glucosidase inhibitors. The methanol extract exhibited the highest AGI activity (IC_{50} = 185.2 µg/mL) compared to the other extracts. This extract showed antioxidant activities with DPPH-IC_{50} and FRAP value of 125.0 µg/mL and 16.95 mmol TE/mgDW, respectively. The LCMS-based metabolomics revealed α-glucosidase inhibitors in the extract, namely 7-methoxycoumarin, supinine and 12-hydroxycorynoline. The presence of these compounds in ginger is being reported for the first time in this study. The activity of these compounds was supported by computational study using *in silico* molecular docking.



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1. Introduction

Diabetes is a global metabolic epidemic and increasing in epidemic proportions throughout the world. There are 425 million (age 20 - 79 years) people with diabetes worldwide in 2019. These figures were expected to increase to 700 million by 2045 (Zabidi et al. 2021). Thus, the finding of an effective medication is paramount important.

One of promising anti-diabetic natural product is *Zingiber officinale* Roscoe (ginger) rhizome (family: *Zingiberaceae*). It is used traditionally to treat a range of illnesses (Grzanna et al. 2005) including diabetes. Moreover, its anti-hyperglycaemic activity has been proven (Wei et al. 2017). Ginger contains various compounds including gingerols, shogaols, paradols, zingerone, quercetin, catechin and rutin (Ghasemzadeh et al. 2012; Ahmad et al. 2015)

Despite the current available scientific reports, research on metabolites profile related to anti-diabetic properties has not been adequately addressed in the literature. Metabolomics is one effective approach that has lately been widely used to profile the metabolites in medicinal plants (Saleh et al. 2021). One of the widely used sensitive analytical instruments is LCMS. While the interaction of these chemicals to the targeted can be evaluated utilising docking techniques *in silico*, thus verifying the results of metabolomics (Maheshwari et al. 2021).

Thereby, the aims of the present study are to evaluate the AGI and antioxidant activities of the ginger rhizome extract, to identify the putative α-glucosidase inhibitors from the extract using LC-MS-based metabolomics, and to analyse the molecular interaction of these inhibitors to the enzyme *via in silico* molecular docking analysis.

2. Results and discussion

2.1. Extraction yield, α-glucosidase inhibitory and anti-oxidant activities of the ginger extract

The α-glucosidase catalyses starch and glycogen digestion in the body into glucose. The inhibition of this enzyme facilitates the maintenance of circulating glucose levels by decreasing the rate of blood sugar absorption (Irawan et al. 2022), and thus is referred to as antidiabetic properties. S2 (supplementary file) lists the percentage of AGI activity of different ginger extracts.

The percent of AGI increases from the lowest activity (20.5% inhibition) at 0% methanol concentration to the highest activity (60.7% inhibition) at 100% methanol concentration. However, the AGI-IC_{50} value can be determined only for the methanol extract, which was 185.2 µg/mL. The IC_{50} of other extracts could not be determined because they did not achieve % AGI activity greater than or equal to 50% at the plant extract concentrations higher than 2 mg/mL.

The IC_{50} value for DPPH activity inhibition of the ginger rhizome extracts ranged from 125.0 to 223.4 µg/mL. The highest DPPH inhibition activity was shown by the 75% methanol extract with the IC_{50} value of 125.0 µg/mL. Likewise, the water extract showed lower DPPH inhibitory activity (IC_{50} value = 223.4 µg/mL) compared to the methanolic extracts. The strength of the antioxidant activity is categorised to be very strong if the IC_{50} value is 50 ppm, strong at 50–100 ppm, moderate at 101–150 ppm,

and weak at >150 ppm (Irawan et al. 2022). Therefore, the extract possessing the highest DPPH inhibitory activity, the 75% methanolic extract, is categorised in the moderate activity (IC_{50} value of 125.0 µg/mL). The FRAP value ranged from 5.86 to 14.04 mmol TE/mgDW. The trend of this activity was similar to the DPPH inhibition activity. The highest FRAP value was exhibited by the 75 and 100% methanolic extracts. While the water extract exhibited the minimum FRAP value of 5.86 mmol TE/mgDW.

Several studies have explored the potential benefits of antioxidants in diabetes management. For example, a study by Maritim et al. (2003) discussed the role of oxidative stress in diabetes-related complications and emphasised the importance of antioxidants in mitigating these effects.

2.2. Multivariate data analysis

Orthogonal partial least square model (OPLS) correlated the LC-MS signals (*x*-variables) were correlated to the AGI activity (y-variable). The OPLS model was successfully generated two OPLS components, with the total variation explained (R^2Y-cum), and the total predicted variation (Q^2Y-cum) of 0.77 and 0.50, respectively. The difference between R^2Y-cum and Q^2Y-cum was 0.27 which is less than 0.3, indicating the validity of this model (Eriksson et al. 2013). The highest variation represented by OPLS component 1 (71.2%), followed by OPLS component 2 (5.6%). The validity of the calibration model was further verified using permutation, exhibiting the R^2Y and Q^2Y intercept values of less than 0.4 and 0.05, respectively, which are under acceptable range according to Erickson et al. (2013).

Supplementary file-S3 shows that the methanol 100% and 75% extracts were placed on the positive side of the OPLS component 1, whereas the less active extracts were situated on the negative side. While supplementary file-S4 displays the m/z values closed to the AGI activity dot that had a positive correlation to the bioactivity. However, only three ions (m/z 177.0545, 284.2344, and 384.4100) could be identified after comparison with the database and references.

2.3. Identification of the putative compounds related to the α-glucosidase inhibitory activity

The three putative compounds were identified as 7-methoxycoumarin, supinine and 12-hydroxycorynoline (S5-suplementary file). Supplementary file-S6 shows MS^2 fragment ions of each putative compound. The fragmentation pathway of these compounds is shown in S7 (supplementary files). Summary of MS^2 fragment ions of putative metabolites identified in the ginger rhizome extract *via* LC-MS/MS analysis with positive ionisation is shown in S8 (supplementary file).

Compound **1** belongs to the classes of coumarin with the substitution of methoxy group at C-7, also known as herniarin (Güvenalp et al. 2017). Protonation of compound **1** at carbonyl functional group formed the parent ion $[M+H]^+$ with m/z 178. Then, consequent removal of H_4O+, C_3H_3 and C_3H_2O from the parent ion generated daughter ions with m/z 156, 137, and 122, respectively.

Compound **2** is a pyrrolizidine alkaloid. It was protonated at nitrogen in pyrrolizidine ring to produce the parent ion $[M+H]^+$ having an m/z 284. Addition of hydrogen in the pyrrolizidine ring cause the ring opening reaction and form straight imines functional group before removal of CH_2 from the parent ion, resulting daughter ion having an m/z 270. Then, the fragmentation of parent ion continued with the removal of C_4H_8O and CH_4O_4, formed other daughter ions with m/z 211 and 203, respectively.

Compound **3** is one of derivatives for benzophenanthridine alkaloid that is corynoline substituted by hydroxyl group at C-12. Protonation of this compound *via* addition of hydrogen at nitrogen in the quinoline ring formed parent ion $[M+H]^+$ with m/z 284. Then, a series of ring restructure reaction followed by removal of H_4O generate a daughter ion having m/z 363. Subsequent removal of other organic compounds, specifically C_2H_3, CH_4O, C_3H_3O and $C_{13}H_{14}NO_2$ from the first daughter ion produced another daughter ions with m/z 356, 351, 328, and 167, respectively.

Although the presence of these compounds has been documented in a variety of plants, it is herein reported for the first time that these compounds were found in the ginger through this investigation.

2.4. Molecular docking

To validate the docking parameters, the co-crystallised control ligand, alpha-D-glucose (ADG) and quercetin were employed as the controls. In this study, the complex with low binding energy is regarded as the strong-docked complex. The re-docked ADG was discovered to bind with 3A4A in the same way that its crystallographic configuration did. The rhizome mean square deviation (RMSD) of the re-docked ADG was determined to be 0.595 Å, suggesting that the docking parameters employed were able to regenerate the crystallised conformation. The docking parameters are considered suitable if the RMSD value of the redocked ligand with reference to the crystallised one is less than 1.5 A° (Wei et al. 2017). As shown in S9 (supplementary file), the ADG and quercetin had a binding energy of −6.0 and −8.4 kcal/mol, respectively.

The detail molecular interaction between these compounds to the enzyme is exhibited in S9 and S10 (supplementary files). ADG formed interaction through hydrogen bond in the active site of the enzyme, with bond distance less than 3.00 A°. While quercetin exhibited interaction through hydrogen bond, dipole-ions interactions, and dipole-dipole. The interactions took place in the active site of the enzyme (S11) which is in line with the report by Yamamoto et al. (2010).

12-Hydroxylcorynoline had higher binding affinity (lower binding energy, −10.5 kcal/mol) compared to the control ligand (−6.0 kcal/mol), followed by supinine and 7-methocxycoumarin. The lower value of binding energy indicates the stronger binding affinity of protein-ligands, thus more stable complex is formed (Zabidi et al. 2021).

Among three docked compounds, two of them interacted with the active site of the enzyme (S11), such as 7-methoxycoumarin and supinine. Both compounds interacted with some amino acid residue which was reported to be in the active site of the enzyme. Interaction to the active site of the enzyme reflects its competitive inhibition to the enzyme. While the other docked compounds, 12-hydrocoryline exhibited interactions with the allosteric binding site of the enzyme (S11) since no

interaction of this compound with the active site of the enzyme was observed (Patrick 2009), suggesting its non-competitive inhibition to the enzyme.

This finding shows that the identified putative compounds are responsible for the AGI activity proven by LC-MS-based metabolomics and supported by the *in silico* docking between the compounds and the enzyme. In addition, it explains the AGI activity of the crude extract of the ginger rhizome.

3. Conclusions

This study exhibited that the methanol extract of the ginger rhizome possessed AGI and antioxidant activities. LCMS-based metabolomics successfully identified the putative compounds possessing the AGI and the antioxidant activities, namely 7-methoxycoumarin, supinine, and 12-hydroxycorynoline. The current study is the first to show that these compounds exist in the ginger rhizome. All of the identified compounds were anticipated to have AGI and antioxidant activities. The AGI activity of the compounds is supported by *in silico* docking study. Methoxycoumarine and supine formed interactions with the active site of the enzyme, while 12-hydrocoryline interacts with the enzyme's allosteric binding site. This finding gives valuable scientific information for the advancement of forthcoming nutraceuticals.

Disclosure statement

There are no conflicts of interest declared by the authors.

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