HOSTED BY

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

LC-MS metabolomics and molecular docking approaches to identify antihyperglycemic and antioxidant compounds from *Melastoma malabathricum* L. Leaf

Oke Anandika Lestari [a,b], Nurheni Sri Palupi [a,c], Agus Setiyono [d], Feri Kusnandar [a,c], Nancy Dewi Yuliana [a,c,e,*]

[a] *Department of Food Science and Technology, IPB University, Bogor 16680, Indonesia*
[b] *Department of Food Technology, Faculty of Agriculture, Tanjungpura University, Pontianak 78124, Indonesia*
[c] *Southeast Asian Food and Agricultural Science and Technology (SEAFAST) Center, IPB University, Bogor 16680, Indonesia*
[d] *Department of Clinic, Reproduction and Pathology, IPB University, Bogor, Indonesia*
[e] *Tropical Biopharmaca Research Center, IPB University, Bogor 16680, Indonesia*

ARTICLE INFO

Keywords:
Metabolomics
Karamunting
Senduduk
Glucosidase inhibitor
Antidiabetic herbal

ABSTRACT

The dried leaves of Melastoma malabathricum L., locally named Karamunting or Senduduk, is traditionally consumed in many regions in Indonesia as herbal tea to cure different illnesses, including diabetes. To date, information on the compounds responsible for their antidiabetic activity is still very rare. The study aimed to identify bioactive compounds of M. malabathricum L. leaves using LC-MS based metabolomics and molecular docking approaches. The leaves brewed with different methods were subjected to LC−MS measurements and several bioactivity tests (*in vivo* and *in vitro* antihyperglycemic, and *in vitro* antioxidant). LC−MS data were linked to the activity data using multivariate data analysis. Molecular docking using alpha-glucosidase, alpha-amylase, and insulin receptor as protein targets was used to verify the results and study the interaction between the identified compound and protein targets. As results, isoquercetin and myricitrin were identified as compounds strongly associated with alpha-amylase inhibitors, while rutin and epicatechin were identified as alpha-glucosidase inhibitors. Quercitrin, citric acid, quercetin, epicatechin, isoquercitrin, and 7-hydroxycoumarine were strongly correlated with both antihyperglycemic and antioxidant activities. The results of metabolomics were confirmed with molecular docking studies, which showed that some of these compounds acted as competitive inhibitors, while others acted as non-competitive ones. Possible synergism between epicatechin and citric acid in their interaction with IR was detected. Metabolomics combined with molecular docking efficiently identified and confirmed several antihyperglycemic and antioxidant compounds from M. malabathricum L., leaf. This study provides scientific evidence for the traditional use of M. malabathricum L. as an antidiabetic herbal.

1. Introduction

Melastoma malabathricum L. (MM) is a botanical that has traditionally been used to cure a variety of diseases in Asia (particularly in Indonesia, Malaysia, India, and China) (Joffry et al., 2012). The MM locally named Karamunting (Kartina et al., 2019; Triawanti et al., 2017), Cengkodok (Sanjaya et al., 2021) or Senduduk (Aslam et al., 2017). The fruit is one of the 36 types of edible fruits in Benguel, Philippines (Chua-Barcelo, 2014). As recently reviewed, a number of scientific studies reported MM leaf to have a variety of health-related bioactivities (Zheng et al., 2021). Some of these studies focused on the antidiabetic and antioxidant properties of MM. A methanolic extract of MM, which contained flavonoid as a major constituent, demonstrated liver protective and antioxidant effects in experimental rats (Mamat et al., 2013), whereas the ethanolic extract was reported to lower blood glucose level and improve the lipid profile of alloxan-induced diabetic rats (Balamurugan et al., 2014). MM leaf water extract was also reported to have *in vitro* antioxidant activity (DPPH) (Lestari et al., 2022). MM leaves are generally consumed by brewing or boiling them in two cups of water and concentrating them into half the volume (Lestari et al., 2022).

* Corresponding author.
E-mail addresses: oke.anadika.l@faperta.untan.ac.id (O. Anandika Lestari), hnpalupi@apps.ipb.ac.id (N. Sri Palupi), agusse@apps.ipb.ac.id (A. Setiyono), fkusnandar@apps.ipb.ac.id (F. Kusnandar), nancy_dewi@apps.ipb.ac.id (N. Dewi Yuliana).

https://doi.org/10.1016/j.sjbs.2024.104047
Received 1 March 2024; Received in revised form 4 June 2024; Accepted 7 June 2024
Available online 14 June 2024

Type 2 diabetes is closely associated with insulin resistance (Tahrani, 2017). Correspondingly, type 1 diabetes can cause insulin resistance (Vladu et al., 2020). Postprandial hyperglycaemia is exacerbated by increased oligosaccharide conversion into monosaccharides by carbohydrate digesting enzymes such as alpha-amylase and alpha-glucosidase (Poovitha and Parani, 2016). Therefore, suppressing the activity of these enzymes may prevent postprandial hyperglycaemia, allowing for insulin resistance and hyperglycaemic conditions to be controlled. Many studies have discovered a link between oxidative stress and diabetes, as well as complications involving the heart, liver, kidneys, and eyes (Asmat et al., 2016). In a recent population-based cohort study involving 5769 participants, it was reported that the total dietary antioxidant capacity was associated to a lower risk of type 2 diabetes and insulin resistance (van der Schaft et al., 2019).

Water extract of MM leaf in our previous research was found to contain 4-O-caffeoylquinic acid, quercimeritin, digipro lactone, 3-O-*trans*-coumaroylquinic acid, norbergenin, and arteamisinine I (Lestari et al., 2022). In another study, MM leaf was found to contain Kaempferol-3-O-(2″,6″-di-O-p-*trans*-coumaroyl)-glucoside, quercetin and quercitrin, which showed strong antioxidant activity using the Ferric Thiocyanate Method. Quercetin was the most active antioxidant (DPPH method), whereas α-amyrin and kaemferol-3-O-glucoside had strong anti-inflammatory activity (Susanti et al., 2008). Despite numerous studies on MM leaves with antihyperglycemic and antioxidant properties, studies identifying the responsible compounds in MM are still rare. Most of the studies used crude or half-fractionated extracts. Identification of the active components in traditional medicine such as plant extracts is crucial since many factors may affect the phytochemical composition, which eventually determine the efficacy (Yuliana et al., 2013). However, it is not an easy task due to the complexity of plant extracts, which contain various overlapping compounds in a single extract. Their pharmacological activities may have synergistic or antagonistic effects (Williamson, 2001), which are not defined when experimenting with a pharmacological activity using a single component or a certain fraction.

The metabolomics approach can be used to quickly identify antidiabetic phytochemicals in crude or half-fractionated plant extracts by linking the plant metabolome with the results of antidiabetic tests *in vitro* or *in vivo* (Yuliana et al., 2011). Compounds identified from metabolomics-based studies can be studied further with molecular *in silico* docking techniques to obtain a visualization of the ligand−protein complex interaction (Prieto-Martínez et al., 2018). The antihyperglycemic and antioxidant activities of MM leaves extracted with organic solvents have been previously reported (Idris et al., 2022; Mayasari et al., 2021). However, no reports on the identification of antihyperglycemic and antioxidant compounds from MM leaf extract and their interaction with target enzymes/receptors have been published. Thus, the objective of this study was to determine the compounds responsible for the antihyperglycemic (glucose AUC OGTT *in vivo*, alpha-glucosidase inhibitor and alpha-amylase inhibitor *in vitro*) and antioxidant (DPPH and FRAP *in vitro*) activities of MM leaf brew using LC/MS-based metabolomics. Twelve different brewing conditions were used to create chemical variations as required for the metabolomics study. The compounds identified as alpha-amylase inhibitors and alpha-glucosidase inhibitors were subjected to molecular docking analysis to determine possible interaction between the enzymes and their ligands.

2. Materials and methods

2.1. Plant materials

The plant was identified by the Herbarium Bogoriense, Botanical Research Center for Biology, Indonesian Institute of Sciences (LIPI). Dried herbarium voucher specimens (roots, stems and leaves) were deposited there with voucher number 841/IPH.101/if.07/VIII/2020-#5847. MM leaves were collected from a garden in West Kalimantan, Indonesia. The leaves were harvested in March 2021 between 06.00–08.00 am. The plant's name was verified with the MPNS database (https://mpns.kew.org; accessed on September 20, 2021). The accepted scientific name is Melastoma malabathricum L. (*Melastomataceae*). The MM leaves used were the top six leaves after the shoot. The leaves were soaked in warm water (40 °C) for 2 min, quickly immersed in cold water (15 °C) for 2 min, and then dried without sunlight at a temperature of 32.9 ± 1.3 °C and a relative humidity of 66.1 ± 6.6 % until the moisture content was < 12 % (±72 h), as measured by the gravimetric method (Lestari et al., 2022). The dried leaves were powdered using a dry blender and then sieved through an 18-mesh size. Prior to use, the powder was stored in a sealed plastic bag in the refrigerator.

2.2. Brewing conditions

Brewing was conducted using hot water (90 ± 2 °C). Twelve different brewing conditions were used to prepare the extract (Table 1). The aim of using different brewing conditions were to provide chemical variations among the extracts. This is necessary step when one intends to identify the compounds associated with bioactivity using multivariate data analysis. The brewing process was initiated by weighing the leaf powder and placing it in a 150 mL beaker glass, adding 100 mL of hot water (90 ± 2 °C), stirring until well mixed and allowing to settle according to the treatment time. Stirring was repeated every 5 min. Brewing without ultrasonication was carried out at room temperature according to the brewing time. The brewing with ultrasonication was carried out by placing the sample in the ultrasonicator (digital ultrasonic cleaner with a frequency of 42 kHz, Krisbow, China, containing 600 mL of room temperature water) according to the treatment time. The two brewing methods were done in triplicate. Thus, there were 36 samples in total. The brew from each treatment was filtered with filter paper (Mokhastock filter paper 01) and diluted with distilled water up to 100 mL.

2.3. LC−MS analysis of MM brews

The 36 brews of MM leaves were diluted with methanol (1:1) and filtered with 0.22 μm PTFE syringe filters, 5 μl was then taken and injected to the LC-MS/MS. The LC-MS/MS chemical profile measurements were carried out according to previous study (Faraone et al., 2019) (UHPLC Vanquish with mass spectrometry Q Exactive Plus Orbitrap HRMS, Thermo Fisher Scientific, Munich, Germany). The

Table 1
Brewing conditions of M.M dried powdered leaves.

Code	Brewing Conditions			TDS (ppm)
	Ultrasonication	MM leaves (g/100 ml)	Time (min)	
T2005	No	20	5	1720.0 ± 20.0
S2005	Yes	20	5	1687.7 ± 5.8
T2015	No	20	15	1643.3 ± 5.8
S2015	Yes	20	15	1543.3 ± 5.8
T0705	No	7	5	1100.0 ± 17.3
S0705	Yes	7	5	1033.3 ± 15.3
T0715	No	7	15	1080.0 ± 10.0
S0715	Yes	7	15	1043.3 ± 5.8
T0305	No	3	5	563.3 ± 32.1
S0305	Yes	3	5	576.7 ± 5.8
T0315	No	3	15	600.0 ± 17.3
S0315	Yes	3	15	616.7 ± 23.1

Note: Total dissolved solids (TDS). The first letter of the code represents the treatment without (T) or with ultrasonication (S). The first two numbers indicate the amount of the leaf (20, 7, or 3 g/100 ml). The next two numbers show the brewing time (5 or 15 min).

column was an Accucore C18+ (100x2.1 mm, 1.5 μm). The electrospray ionization (ESI) method in positive and negative ionization modes was used in the mass range of 100–1500 *m/z*. The operating temperature was 30 °C. The mobile phase was in a gradient mode that consisted of solvent A (0.10 % formic acid in water) and solvent B (0.10 % formic acid in acetonitrile). The gradient flow was as follows: 0–1 min. (5 % B), 1–25 min. (5–95 % B), 25–28 min. (95 % B), 28–30 min. (5 % B). The flow rate was 0.2 mL/min. Samples were introduced into the MS via electrospray ionization using the following conditions: envelope gas flow rate 15 (arbitrary units), aux gas flow rate 3 (arbitrary units), ESI voltage 3.8 (kV), capillary temperature 320 (°C). The collected spectrum was scanned over the *m/z* value range from 155 to 2000 atomic mass units (Xcalibur ver. 4.0). Chromatogram data was processed using MZmine to obtain a chemical profile of the MM leaf brewed water. Compounds were identified and characterized using the compound discoverer 3.2 program (Thermo Scientific Inc, Waltham, MA, USA). Compounds with similarity index greater than 60 % were selected. The fragmentation patterns (MS2) were compared with various online databases (PubChem, ChEBI, and HMDB). The final data obtained included the compound name, formula, molecular weight (MW), retention time, similarity of mzCloud spectra (%), and relative intensity (%). Some compounds were also putatively identified by comparing the spectra with previously reported data.

2.4. Antihyperglycemic activity

An *in vivo* oral glucose tolerance test (OGTT) was performed using a male Sprague−Dawley (150–200 g) animal model to determine the glucose AUC (area under the curve) (Truong, 2020). The animals were placed at an ambient temperature (25 °C), 40–60 % humidity, and a 12-hour light/dark cycle. Standard food and water were delivered ad libitum. Before the test, the animals had undergone the adaptation stage for 2 weeks and were fasted for 6 h. The animal model was divided into 14 groups. Each group consisted of 3 rats. After fasting, 12 groups were given 1.78 mL/kg HED (Shin et al., 2010) of sample. 15 min later, glucose (glucose monohydrate 1.1 g/kg HED) was provided. The other two groups were the control (distilled water and glibenclamide 0.14 mg/kg HED). Blood glucose levels were measured at 0, 30, 60, 90, 120, and 180 min. Data on glucose levels were analysed with the curve of the relationship between the time (x) and each group's average blood glucose level (y). The AUC value was calculated using the trapezoid approach. Ethical approval of the use of experimental animals was issued by the Animal Ethics Commission of the Bogor Agricultural Institute No. 212–2021/IPB, and the experiments were conducted based on the Guide to Care and Use of Laboratory Animals or the Regulation of the Minister of Agriculture of the Republic of Indonesia (No. 44/Permentan/OT.140/5/2007). Alpha-amylase and alpha-glucosidase enzyme inhibition tests were conducted as previously described (Cheng et al., 2015; Sancheti et al., 2009). All measurements were performed in triplicate.

2.5. In vitro antioxidant activity

The scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric-reducing antioxidant power (FRAP) of MM leaf extract were measured to determine the *in vitro* antioxidant activity based on previous studies (Chang et al., 2020; Vignoli et al., 2011). The DPPH antioxidant activity was calculated as an inhibition percentage using the following formula: [(DPPH absorbance – DPPH + sample absorbance)/DPPH absorbance)] × 100 %. The FRAP antioxidant activity was expressed in g Trolox per 100 mL. All measurements were performed in triplicate.

2.6. Molecular docking study

The chemical structures of 3D 4-coumaric acid (CID: 637542), isoquercetin (CID: 10813969), malic acid (CID: 525), myricetin (CID: 5281672), myricitrin (CID: 5281673), choline (CID: 305), citric acid (CID: 311), epicatechin (CID: 72276), rutin (CID: 5280805), and shikimic acid (CID: 8742) were obtained from the NCBI-PubChem database (https://www.ncbi.nlm.nih.gov/) in SDF format. Acarbose with CID: 41,774 was downloaded from the NCBI PubChem database as a standard drug for docking simulation. The RSCB PDB database (https://www.rcsb.org/) was used to obtain the 3D structures of alpha-amylase (PDB ID: 4 W93), alpha-glucosidase (PDB ID: 3 W37), and insulin (PDB ID: 2 B4S). The ligands and receptor structure preparation were established as follow: 4-coumaric acid, isoquercetin, malic acid, myricetin, myricitrin, choline, citric acid, epicatechin, rutin, shikimic acid, and acarbose were prepared by minimizing energy using PyRx software. Receptor preparation (alpha-amylase and alpha-glucosidase) was conducted using Discovery Studio software. Receptor preparation was purposed to terminate water molecules or another ligand bound to receptors. After the preparation, all ligands and receptors were saved in Protein Data Bank (.pdb) format using open babel (Tri et al., 2020). Hex 8.0 software was used to simulate compound-target protein interactions with a grid dimension of 0.6, receptor range of 180, and ligand range of 180. The parameters used in this molecular docking were the correlation type of Shape+Electro+DARS and a threshold score of 0.0.

2.7. Data analysis

The results of OGTT test of brewed MM leaf water was analysed using one-way ANOVA, followed by Duncan's test using SPSS v.26 statistical software. Results were considered statistically significant at $p < 0.01$ for *in vivo* analysis and $p < 0.05$ for *in vitro* analysis. The PCA and OPLS analysis were conducted using SIMCA ver. 16 (Sartorius Stedium Data Analytics AB, Sweden) with Pareto scaling. PCA was used as a preliminary assessment to observe the classification pattern between MM leaf extracts obtained with different brewing conditions. OPLS was used to correlate the LC−MS/MS data (X variable) with antihyperglycemic and antioxidant activities (Y variable). Compounds correlated with each activity were identified using VIP and Y-Related Profile value. The model is expressed with model accuracy (R^2Y) and prediction accuracy (Q^2Y). Model validation was carried out using the CV ANOVA test and permutation test. The docking results were saved with the PDB extension and visualized using the Discovery studio program to display 3D and 2D views and their interactions (Tri et al., 2020).

3. Results

3.1. LC−MS/MS data of MM brews

In the LC−MS/MS analysis of the 12 MM brews, 62 compounds were identified and are summarized in Table S1 (Supplementary data). These compounds were categorized into fourteen groups of compounds: benzoic acids, carboxylic acids, cinnamic acids, coumarins, flavonoids, fatty acids, formyl, organooxygen, organonitrogen, organic acids, phenol lipids, quinolines, tannins, and triterpenoids. Of these 62 compounds, 17 were previously identified in this plant.

3.2. Antihyperglycemic and antioxidant activity profile of MM brew obtained with different brewing conditions

The antihyperglycemic and antioxidant activities of MM extracts using 12 brewing conditions are shown in Fig. 1. The animal models treated with MM brew had a glucose AUC of 618 to 7260 mg/dL. The negative control animal and positive control given glibenclamide groups had glucose AUC values of 4915 ± 242 mg/dL and 670 ± 151 mg/dL, respectively. Changes in blood glucose levels from 0 to 180 min in each treatment are shown in Figure S1 (Supplementary data). The MM brews had alpha-glucosidase inhibitor activity of 46.04 % to 93.54 % (0.16×10^{-3} to 4.60×10^{-3} mg/mL acarbose equivalent), alpha-amylase inhibitor activity of 67.31 % to 85.07 % (equivalent to acarbose

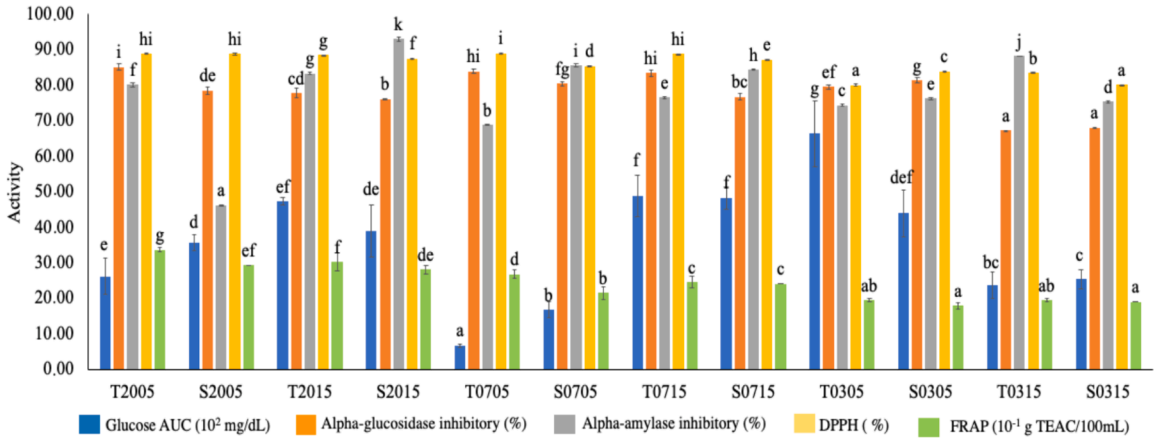

Fig. 1. Antihyperglycemic and antioxidant activity profile of MM brew obtained with different brewing conditions. Sample code explanation can be seen in Table 1. Different letters on the top of the bar chart indicate a significant difference ($P < 0.01$ for *in vivo* analysis and $P < 0.05$ for *in vitro* analysis) in each parameter. Glucose AUC of negative control and glibenclamide group of animal model was 4915 ± 242 mg/dL and 670 ± 151 mg/dL, respectively. Acarbose (10^{-3} mg/mL) was used as standard in alpha-glucosidase inhibitory and alpha-amylase inhibitory tests, with the activity of 81.67 ± 0.36 % and 72.71 ± 0.06 %, respectively. Vitamin C (0.5 mg/mL) was used as a control in DPPH radical scavenging activity, with the activity of 70.39 ± 0.24 %.

concentrations of 1.44 to 3.80 mg/mL), DPPH radical scavenging activity of 79.64 % to 89.02 % (52.75 to 59.18 mg/100 mL vitamin C equivalent), and FRAP potential of 1.71 to 3.44 g TEAC/100 mL.

The brewing condition without ultrasonication at a concentration of 7 g/100 mL brewed for 5 min (sample T0705) showed the lowest glucose AUC value, which was not significantly different ($P > 0.01$) from that of glibenclamide (positive control) (Fig. 1). Sample T0705 also had high alpha-amylase inhibitory and DPPH radical scavenging activities, which were not significantly different ($P > 0.05$) from those of the brewing conditions with the highest activity (T2005). Sample T0705 also had the highest FRAP potential ($p < 0.05$) among samples prepared with brewing conditions at the same concentration (7 g/100 mL).

3.3. Multivariate data analysis of MM LC–MS/MS data

3.3.1. Principal component analysis (PCA)

PCA was conducted as an initial preview of the chemical variations of the MM brew LC–MS data. The PCA resulted in 6 principal components (PCs), which explaining 88.1 % of the total variation. The PCA results show that the R^2Y and Q^2 values were more than 0.4, indicating an acceptable model (Worley and Powers, 2013). The PCA score plot of the second two components (PC1 = 41.8 %, PC2 = 22.6 %) shows that all samples can be classified into six groups, as shown in Fig. 2 a; (1) brewing without ultrasonication at an MM concentration of 3 g/100 mL and brew time of 5 min (sample T0305, light green), (2) brewing with ultrasonication at an MM concentration of 3 g/100 mL and brew time of 5 min (S0305, yellow), (3) brewing with and without ultrasonication at a concentration of 3 g/100 mL for 15 min (T0315, S0315, light blue), (4) brewing with a concentration of 7 g/100 mL (S0705, T0715, S0715, dark green), (5) brewing without ultrasonication for 5 min (T0705, dark blue), and (6) brewing with or without ultrasonication at a concentration of 20 g/100 mL with brew timed of 5 and 15 min (T2005, S2005, T2015, S2015, red). The overview of the identified compounds distribution over the 12 MM brews is shown in X- variant plots derived from the OPLS model (Supplementary data Figure S2). Representative phytochemicals responsible for the six clusters are depicted in the loading plot in Fig. 2b. Arteamisinine I, a gamma lactone, was found to be predominant in samples with brewing conditions at low concentration and short brewing time for Group 1, while isoleucine was the discriminating compound in Group 2. Quercitrin and choline were the predominant compounds in Group 3 and Group 4, respectively. Epicatechin and citric acid were the predominant compounds in Group 5, whereas isoquercitrin, phenylalanine, tryptophan, and epicatechin were predominant in Group 6.

Since the PCA of LC-MS data was reliable, as seen from the value of R^2X (0.881) and Q^2 (0.448), the supervised multivariate data analysis using OPLS was continued to elucidate compounds associated with the tested bioactivities.

3.3.2. Orthogonal projection to the least square analysis (OPLS)

Five OPLS models were built to identify compounds obtained from LC–MS/MS measurements that correlated with the OGTT, alpha-glucosidase inhibitory, alpha-amylase inhibitory, DPPH antioxidant, and FRAP antioxidant potentials (Fig. 3). All OPLS models were validated by employing permutation tests (100 permutations for each model). The values of R^2 and Q^2 in all permutation models were consistently lower than those in the original model, indicating adequate performance (Supplementary data Figure S3). In addition, all models had satisfying performance, as shown by R^2Y and Q^2 values, which were higher than 0.4 (Worley and Powers, 2013) (Supplementary data Table S2).

The classification of samples based on their respective bioactivities was observed using OPLS score plots (Fig. 3a–e). The chemical compounds responsible for each activity were determined based on the most positive Y-related profile value and a VIP value higher than 0.5. The standard error does not adjoin the axis regarded as significant. The Y-related profile shows the bioactivity response, and the variable influence on projection (VIP) value indicates the significance of chemical compounds on the bioactivity response. For the alpha-glucosidase inhibitory-, alpha-amylase inhibitory-, DPPH antioxidant-, and FRAP antioxidant-OPLS models, significant active compounds were selected from the most positive Y-related compounds and those with the highest VIP value, whereas for OGTT-OPLS, compounds with the most negative Y-related-profile value and the highest VIP score are considered active compounds. This is because a smaller glucose AUC mg/dL value indicates higher antihyperglycemic activity in the sample (Mechchate et al., 2021b). Table 2 summarizes the ten most significant compounds for each OPLS model, which were selected based on the VIP and Y-related profile values as described above.

The score plot of the OPLS of the LC–MS/MS and *in vivo* OGTT data (Fig. 3. a) shows an explicit separation between MM brewed with different conditions and the antihyperglycemic activity (lower OGTT). The samples were coloured according to the three activity levels, high (glucose AUC values below normal, i.e., 618 to 3330 mg/dL), low (glucose AUC values of 3331 to 5256 mg/dL), and no activity (glucose AUC of 5257 to 7260 mg/dL). Based on the VIP and Y-related values,

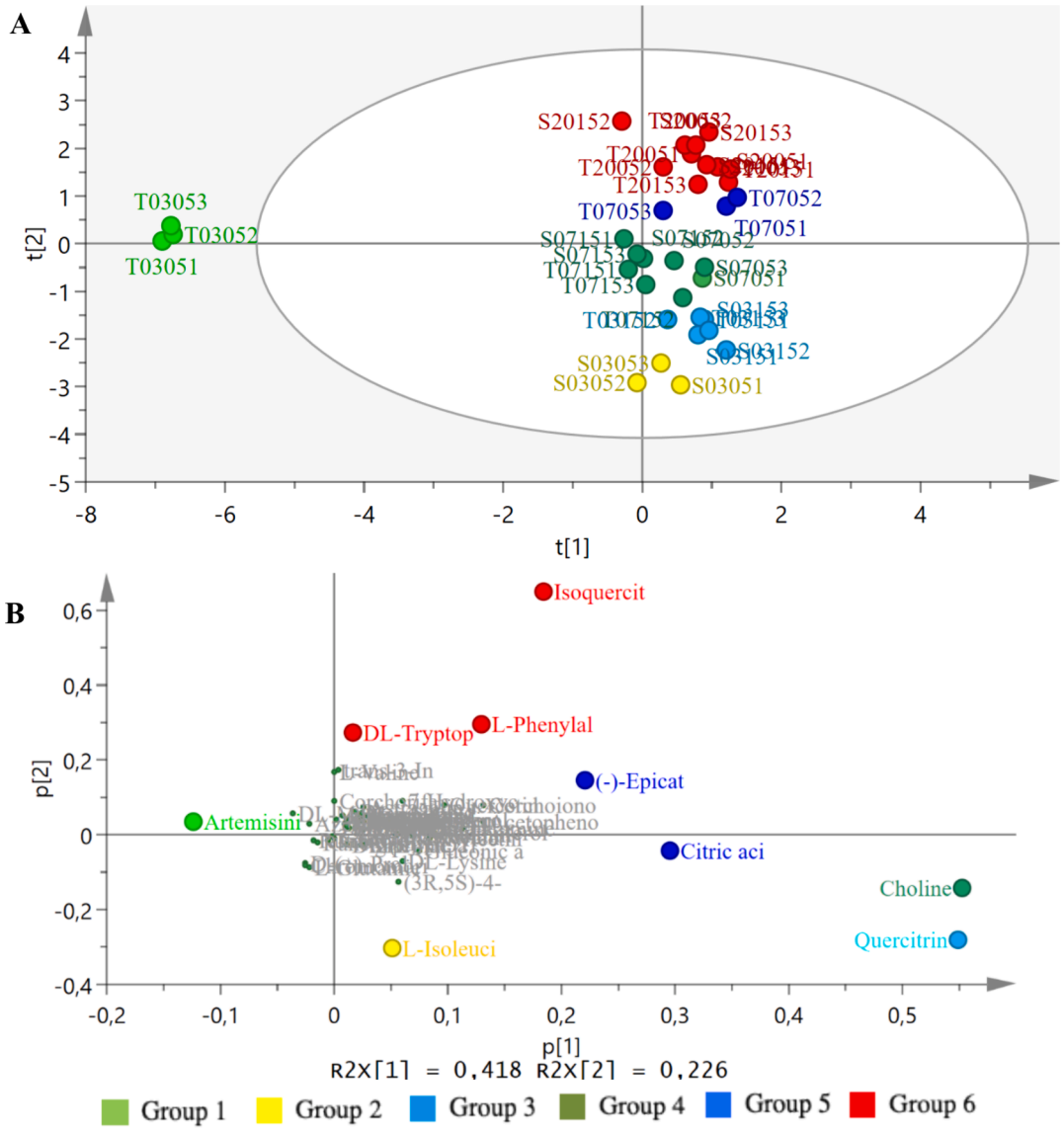

Fig. 2. PCA phytochemical profile of brewed MM leaf water based on LC−MS. (A) Score plot and (B) loading plot (R^2X 0.881 and Q^2 0.448). Sample code explanation can be seen in Table 1.

chemical compounds strongly associated with the OGTT anti-hyperglycemic activity of the brewed MM leaf water were identified as quercitrin, kaempferol-3-rhamnoside, citric acid, myricetin 3-O-beta-D-galactopyranoside, quercetin, epicatechin, gluconic acid, choline, and catechins (Table 2).

The OPLS score plot in Fig. 3b classifies the samples based on their alpha-amylase inhibitor activity into 3 groups, namely, high (85.67 % to 80.25 %), medium (75.87 % to 80.24 %), and low (75.86 % to 67.28 %) inhibition of alpha-amylase groups. With a similar approach to that used in the OGTT-OPLS analysis, the alpha-amylase inhibitors from MM leaf brew were identified, which included isoquercitrin, malic acid, phenylalanine, tyrosine, 4-coumaric acid, digiprolactone, myricetin, myricitrin, kaempferol, and 7-hydroxycoumarine (Table 2).

The score plot of the alpha-glucosidase inhibitor − OPLS model is presented in Fig. 3 b. It shows a clear separation between samples with different brewing conditions according to their alpha-glucosidase inhibitor activity. Chemical compounds that strongly inhibit alpha-glucosidase in MM leaf brew included choline, rutin, shikimic acid, lysine, epicatechin, desmanthin-I, tryptophan, quercetin, quercitrin, and citric acid (Table 2).

Explicit separation of the samples based on their DPPH radical scavenging and FRAP potential activity was also found in the DPPH-OPLS and FRAP-OPLS score plots (Fig. 3 d and 3 e, respectively). The most potent chemical compounds in the DPPH radical scavenging activity of MM brew included isoquercitrin, choline, phenylalanine, tyrosine, epicatechin, citric acid, quercitrin, acetophenone, corchoionol C 9-glucoside, and quercetin (Table 2).

3.3.3. *Molecular docking study*

To confirm the results of the OPLS analysis, molecular docking studies were conducted for five major compounds identified as potential antihyperglycemic and antioxidant compounds in the respective OPLS models (excluding amino acids) by using alpha-glucosidase and alpha-amylase as the protein targets (Figure S4). Additionally, molecular docking for epicatechin and citric acid was also conducted using insulin receptor (IR) (Fig. 4). These two compounds were predominant in sample T0705, and strongly associated with the OGTT anti-hyperglycemic activity. The interactions between the ligan and the receptor were indicated by the binding energy, the amino acid residue binding site, and the types of chemical bonds involved in the binding (Table 3).

The molecular docking study results show that acarbose bound to

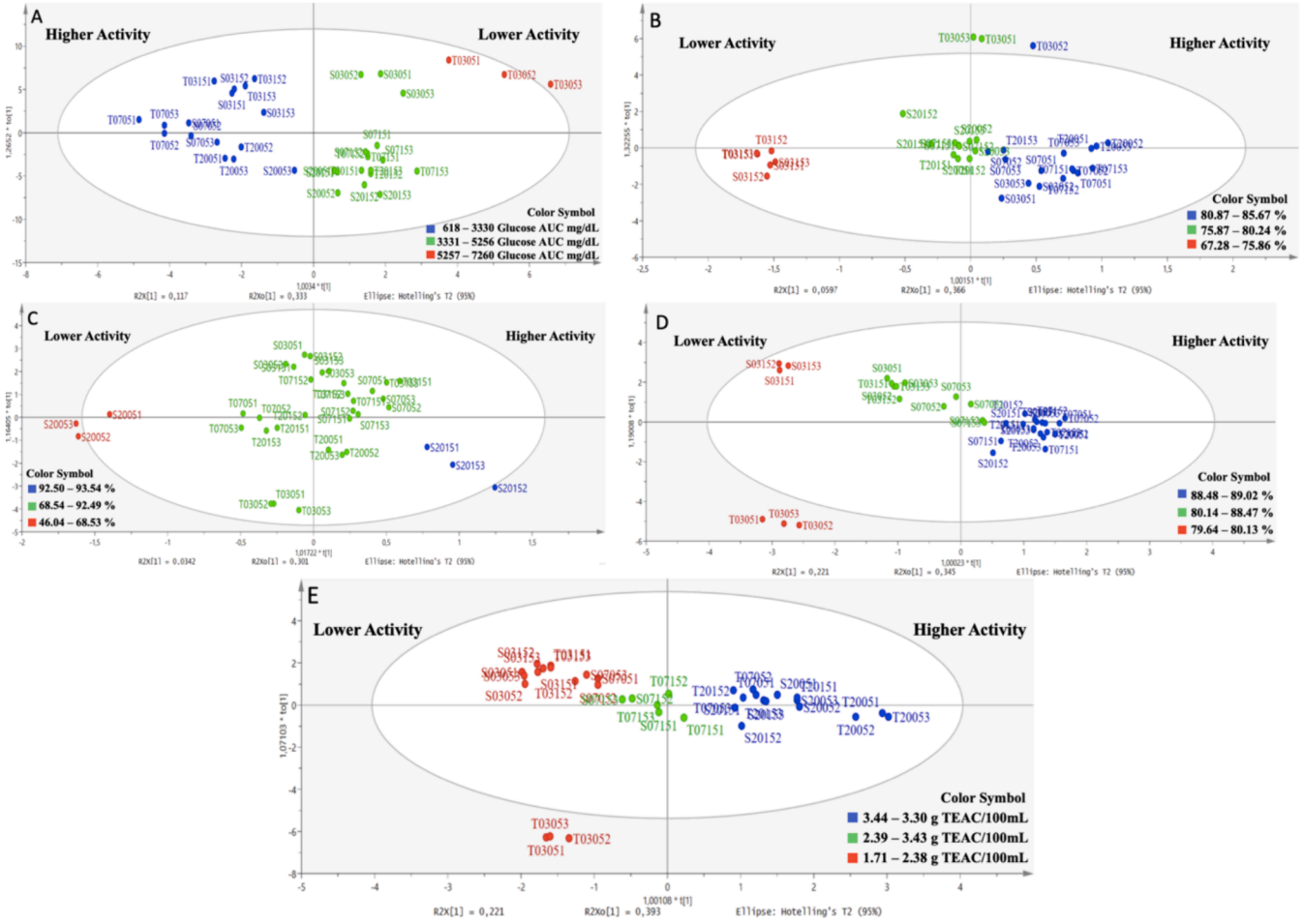

Fig. 3. OPLS score plot showing the bioactivity classification of samples based on the antihyperglycemic activity: (a) OGTT, (b) alpha-amylase inhibitory and (c) alpha-glucosidase inhibitory activity, and based on the antioxidant activity: (d) DPPH and (e) FRAP potential. Sample code explanation can be seen in Table 1.

alpha-amylase with a binding energy of −350.5 kcal/mol. The interaction occurred at ASP197 and TRP59 residues through hydrogen bonding and at ILE235, ASP197, and TRP59 through hydrophobic bonding. The compounds identified as potential alpha-amylase inhibitors in the OPLS analysis had slightly higher energy levels than acarbose (−298.5 to −183.1 kcal/mol, Table 3), for example, myricitrin (−298.5 kcal/mol) and isoquercetrin (−296.3 kcal/mol). The binding between myricitrin and alpha-amylase involved THR163, HIS305, TYR62, ASP197, ASP300, TRP59, TRP58, and LEU165 amino acid residues and was stabilized by hydrogen bonding and electrostatic and hydrophobic interactions. Isoquercetin interacted with GLN63, TYR62, and HIS305, which involved hydrogen and hydrophobic bonds. The compound 4-coumaric acid with the highest binding energy (−145.7 kcal/mol) interacted with only two amino acid residues, ASP197 and ALA198, stabilized by electrostatic and hydrophobic interactions.

Acarbose binding with alpha-glucosidase involved GLN219, GLY690, SER692, SER693, TYR740, LEU707, and GLN708 amino acid residues. The binding is stabilized with hydrophobic bonding with a binding energy of –333.9 kcal/mol. The identified alpha-glucosidase inhibitor from the OPLS analysis had a higher binding energy (−287.8 to −158.1 kcal/mol) than acarbose (Table 3), with rutin (−287.8 kcal/mol) and epicatechin (–223.9 kcal/mol) being the strongest. Four amino acid residues, LYS309, ASP305, ARG670, and LEU669, were involved in the binding of rutin to alpha-glucosidase. The binding was stabilized with hydrogen bond, electrostatic, and hydrophobic interactions. Choline had the lowest binding energy (−158.1 kcal/mol) at GLU301, ASP666, GLU792 and ASP666, which involved electrostatic and hydrogen bonds.

4. Discussion

A total of 62 chemical compounds were identified using LC-MS from 12 brewing conditions of MM leaf. Eight compounds (gallic acid, 4-O-Caffeoylquinic acid, arteamisinine I, quercetin, 3-O-*trans*-coumaroylquinic acid, digiprolactone, norbergenin, and quercimeritrin) were previously identified in our previous study with water extract of M.M leaves (Lestari et al., 2022). Kaempferol, epicatechin, rutin, glutamic acid, tyrosine, quercitrin, lysine, proline, valine, tyramine, and tryptophan were previously reported in M.M leaves by other researchers (Joffry et al., 2012; Susanti et al., 2008; Zheng et al., 2021). Isoquercitrin, citric acid, catechin, and shikimic acid have never been reported in the leaves of MM, but the other compounds were previously identified in other studies (Joffry et al., 2012; Susanti et al., 2008; Zheng et al., 2021). Many of these compounds have been found in other plants and are reported to have antidiabetic and antioxidant activity. Kaempferol isolated from *Cucumis sativus* was the compound responsible for the blood-sugar lowering and antioxidant activities of C. sativus extract in an alloxan-induced diabetic rat model. The same study reported that the compound also exhibited *in vitro* alpha-glucosidase and alpha-amylase inhibitor activity with IC_{50} values of 51.24 μg/mL and 29.37 μg/mL, respectively (Ibitoye et al., 2018). A mixture of rutin, catechin and epicatechin was shown to have antidiabetic activity in aloxan-induced diabetic rats, which was measured by the OGTT method (Mechchate et al., 2021a).

The brewing conditions (T0705) with the best OGTT result did not have the highest alpha-glucosidase inhibitory activity. A recent study reported that alpha-amylase inhibitors incorporated into yoghurt

Table 2
Chemical compounds that have significant antihyperglycemic or antioxidant bioactivity in steeped MM leaf water.

No.	Compounds	VIP	Y-Related Profile
Antihyperglycaemic activity (OGTT)			
1	Quercitrin	1.52	−0.62
2	Kaempferol-3-rhamnoside	1.47	−0.59
3	Citric acid	1.45	−0.58
4	Myricetin 3-O-beta-D-galactopyranoside	1.45	−0.58
5	Quercetin	1.41	−0.58
6	(−)-Epicatechin	1.37	−0.57
7	Gluconic acid	1.43	−0.57
8	Choline	1.43	−0.56
9	Catechin	1.31	−0.56
10	Corchoionol C 9-glucoside	1.37	−0.55
Antihyperglycaemic activity (Alfa-Amylase inhibitory)			
1	Isoquercitrin	3.60	0.43
2	DL-Malic acid	2.22	0.29
3	L-Phenylalanine	1.63	0.17
4	L-Tyrosine	1.18	0.08
5	4-Coumaric acid	0.54	0.08
6	Digiprolactone	0.58	0.07
7	Myricetin	0.52	0.07
8	Myricitrin	0.52	0.07
9	Kaempferol	0.52	0.07
10	7-Hydroxycoumarine	0.60	0.06
Antihyperglycaemic activity (Alfa-Glucosidase inhibitory)			
1	Choline	2.87	0.16
2	Rutin	1.06	0.15
3	Shikimic acid	1.09	0.12
4	DL-Lysine	1.02	0.11
5	(−)-Epicatechin	1.57	0.11
6	Desmanthin-1	1.06	0.09
7	DL-Tryptophan	1.32	0.07
8	Quercetin	0.64	0.03
9	Quercitrin	2.49	0.02
10	Citric acid	1.26	0.02
Antioxidant activity (DPPH)			
1	Isoquercitrin	3.74	0.83
2	Choline	3.17	0.58
3	L-Phenylalanine	2.02	0.46
4	L-Tyrosine	1.95	0.45
5	(−)-Epicatechin	1.75	0.36
6	Citric acid	1.72	0.33
7	Quercitrin	2.69	0.25
8	Acetophenone	1.04	0.24
9	Corchoionol C 9-glucoside	0.94	0.20
10	Quercetin	0.85	0.18
Antioxidant activity (Frap potential)			
1	Isoquercitrin	4.49	1.51
2	L-Phenylalanine	2.07	0.71
3	L-Tyrosine	1.69	0.54
4	(−)-Epicatechin	1.57	0.47
5	DL-Tryptophan	1.65	0.39
6	Choline	2.51	0.29
7	Acetophenone	0.99	0.28
8	Corchoionol C 9-glucoside	0.92	0.27
9	7-Hydroxycoumarine	0.70	0.23
10	Citric acid	1.39	0.21

products had a positive correlation with the prevention of postprandial hyperglycaemia activity, which was measured with OGTT in high-fat diet mice (Wang et al., 2021). Our study also showed a positive correlation ($P < 0.01$) between alpha-amylase inhibitors and DPPH radical scavenging (r 0.553) and the FRAP potential (r 0.450), as well as DPPH radical scavenging and the FRAP potential (r 0.809). The results confirm a previous study on a methanol extract *of Phyllantus virgatus,* which indicated a positive correlation between alpha-amylase inhibitors and DPPH radical scavenging (r 0.8858), and both were correlated with the ability of the extract to stimulate glucose absorption into 3 T3-L1 cells (Hashim et al., 2013a). Based on the activity profile (Fig. 1), the brewing method without sonication at a dose of 7 g/100 mL and a 5 min brewing time (sample T0705) was a preferable method to obtain MM brew with the optimum antihyperglycemic and antioxidant activities.

The PCA results show that the different brewing conditions used in this study affected the chemical composition of brewed MM leaf water. The ultrasonication effect in this study was more visible at lower concentrations of MM (3 g/100 mL) and a shorter brew time (5 min). A previous study on black tea indicated that the total amount of phenol increased with decreasing concentrations of black tea (Wang et al., 2022). A similar result was reported with carotenoid extraction from *Chlorella vulgaris*. The carotenoid yield increased with sonication at a lower concentration of *C. vulgaris* (Dianursanti et al., 2020).

Based on *in vivo* OGTT results from this OPLS study and other studies, antihyperglycemic bioactive compounds have several mechanisms to lower blood glucose levels. It was previously reported that quercitrin, quercetin, and choline showed antidiabetic activity by increasing insulin secretion, while epicatechin and catechin acted as insulin-mimetic agents (Bharti et al., 2018). A study with diabetic animal models showed that quercetin lowered blood glucose levels and increased insulin plasma along with the doses given (Srinivasan et al., 2018). A molecular docking study of various compounds found in green tea showed that epicatechin had the lowest binding energy level to the insulin receptor (Ganugapati et al., 2011). Another molecular docking analysis confirmed the antidiabetic activity of kaempferol, identified in this study, in which kaempferol-3-rhamnoside showed a high affinity (low binding energy) to glycogen phosphorylase (Rajeswari et al., 2020). The inhibition of glycogen phosphoryl is linked to the inhibition of glycogen-glucose conversion. Citric acid intervention in a diabetic animal model for 4 weeks resulted in a lower blood glucose level than that of the positive control, and it also reduced body weight and triglyceride blood levels (Treki et al., 2018). Another antihyperglycemic mechanism was found in quercetin, namely non-competitive inhibition in binding to GLUT2 (fructose and glucose transporters), so that giving 1 g to experimental animals can inhibit the absorption of 50–100 g of glucose, but it can also inhibit SGLT1 (sodium glucose transporter) (Kwon et al., 2007). Stronger inhibition of GLUT2 and SGLT1 was also shown in green tea when compared to black tea, this was allegedly due to the higher total catechin content of green tea, in addition to the absence of an OH group on the C ring on C3″ which also determined the inhibitory effect of catechin and epicatechin (Ni et al., 2020). The inhibition occurs in the absorption system by reducing the amount of the glucose absorbed (Cermak et al., 2004).

Alpha-amylase inhibitors and alpha-glucosidase inhibitor bioactive compounds were identified in the OPLS analysis. Several of these compounds were previously reported to act as alpha-amylase inhibitors, for example, isoquercetin isolated from *Calendula officinalis* L. leaf ethanol extract (Olennikov and Kashchenko, 2014) and myricetin isolated from guava leaf ethanol extract (Wang et al., 2010). Several of these compounds have been reported to act as alpha-glucosidase inhibitors, such as rutin (Limanto et al., 2019), shikimic acid isolated from *Terminalia macroptera* leaf methanol extract (Pham et al., 2014), and desmanthin-I from *Syzygium polyanthum* leaf acetone extract.

Compounds associated with the FRAP antioxidant potential are similar to those associated with the DPPH; only the VIP and coefficient values were different. Most of the identified compounds were previously reported to have *in vitro* or *in vivo* antioxidant activity. Isoquercetin contained in *Aronia melanocarpa* extract leaves (70 %) had a positive correlation concentration with the DPPH radical scavenging activity, with $r > 0.7$ ($p < 0.05$) (Zdunić et al., 2020), whereas acetophenon from *Polygonum barbatum* methanol extract had a DPPH radical scavenging activity of 1.8x10-1 mg/mL (Mazid et al., 2011). Isoquercetin from *Tetrastigma hemsley*anum showed a stronger ability to reduce $F3^{+}$ than crude, petroleum ether and n-butanol extracts (Hashim et al., 2013b). Epicatechin from cocoa bean ethanol extract (r 0.84) and water extract

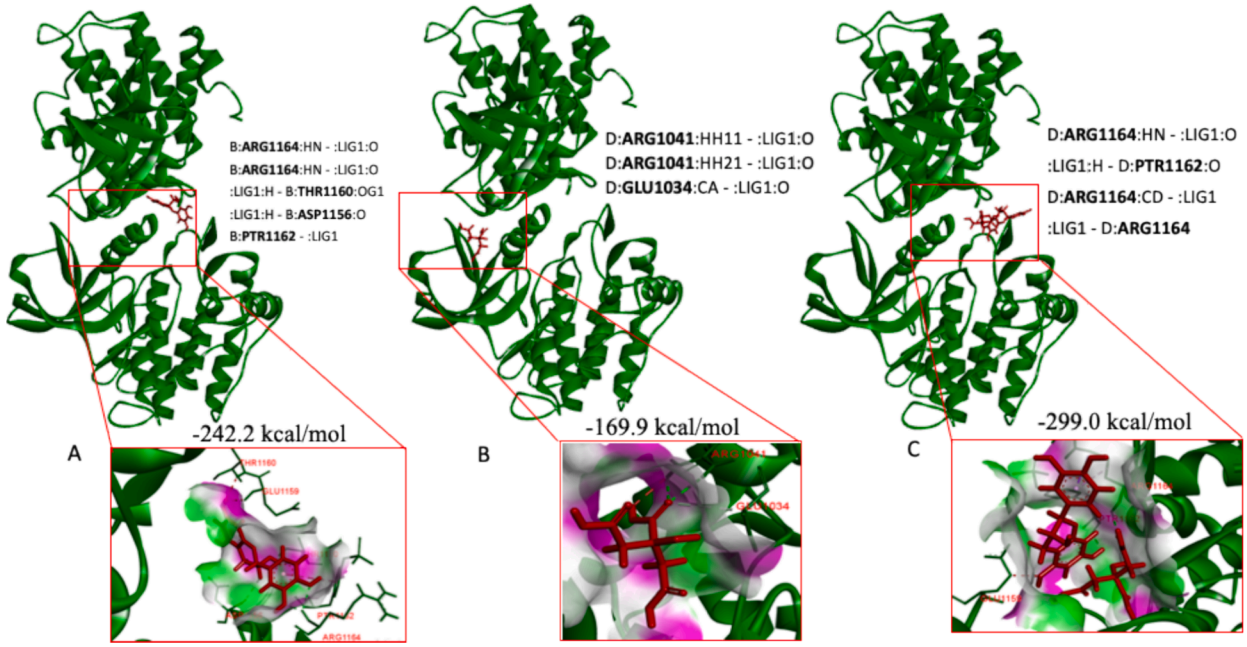

Fig. 4. Interaction between epicatechin-IR (A), citric acid-IR (B), and epicatechin-IR-citric.

(0.79) exhibited positive FRAP potential (Othman et al., 2010).

Previous research showed that the alpha-amylase enzyme has active sites in the ASP197, GLU233, and ASP300 amino acid residues (Kikiowo et al., 2020), while alpha-glucosidase has active sites in GLU174, THR175, AR178, GLU196, THR197, PRO198, ARG199, VAL200, HIS201, 2, ARG203, ALA204, PRO205, GLN352, LEU355, ASP356, VAL357, VAL358, GLY359, TYR360, ARG608, VAL718, and ALA719 (Rahman et al., 2019). Acarbose was used as a control because it acts as an inhibitor of these two enzymes. It inhibits alpha-amylase with a competitive type inhibitor (Ranjan et al., 2019) and is a noncompetitive inhibitor for alpha-glucosidase (Son and Lee, 2013). A competitive inhibitor competes with the substrate for binding to the active site of the target protein/enzyme. Substrate and enzyme binding will be inhibited when interaction between the inhibitor and the enzyme occurs (Tri et al., 2020).

These results show that the more amino acid residues interact with the ligand through hydrogen bonding, the lower the binding energy. In addition, optimum hydrogen bonding and hydrophobicity may have a better ability to stabilize lignin (Varma et al., 2010).

The results of this molecular docking analysis show that acarbose acts as a competitive inhibitor of alpha-amylase since it binds to one of its active sites (ASP197). This was also demonstrated by myricitrin, a compound from brewed MM leaf water. The other four compounds were noncompetitive inhibitors. A noncompetitive interaction was detected between alpha-glucosidase and acarbose. A similar interaction was found with five compounds identified as alpha-glucosidase inhibitors in the OPLS analysis. This information highlights the great potential of MM brew as an antihyperglycemic. It has been suggested that synergistic effects could occur when competitive and noncompetitive inhibitors are present in the same extract (Hyeong-U et al., 2019).

The docking analysis revealed that alpha-glucosidase interacted with epicatechin's A ring (C5-OH and C7-OH), whereas alpha-amylase interacted similarly with myricitrin and isoquercetin. (Figure S4). Previous studies reported that the position and number of hydroxyl groups of flavonoids determine their α-amylase and α-glucosidase inhibition activity. The main determining groups are C5-OH, C6-OH, and C7-OH at ring A (Cermak et al., 2004), C8-OH at ring A, C3′-OH and C4′-OH at ring B, C3-OH at ring C, and the double bond position of C2 = C3 (Proença et al., 2017).

The molecular docking study showed that the epicatechin-IR-citric acid interaction had the lowest energy. These interactions indicate that the insulin mimetic activity of these compounds, alone or together, occurs on the area of kinase-linked receptors (KLRs). The KLRs, which are located in the IR at residues 980–1255, are important pathways for message transduction that mediate cells to communicate with each other (Meyts et al., 2013). These results explain why brew T0705, in which epicatechin and citric acid were the two predominant compounds as depicted from the PCA results, had the highest antihyperglycemic activity, as shown by the OGTT value (not significantly different from the control glibenclamide, $P < 0.01$). These synergistic effects show the potential of MM leaf brew for the treatment of type 1 diabetic (D1) and type 2 diabetic (D2) patients with insulin resistance. To confirm this, further *in vivo* and clinical research should be conducted.

5. Conclusion

Compounds with antihyperglycemic and antioxidant activities from MM leaf brew were tentatively identified by LC−MS-based metabolomics. Twenty-six potential antihyperglycemic and antioxidant compounds were obtained, some of which may have more than one bioactivity. Further studies by testing the pure compounds using *in vivo* experiment would be of interest to confirm our results. Another potential that we have yet to find is the synergy of epicatechin and citric acid in insulin-mimetic activity. The brewing conditions with preferable antihyperglycemic and antioxidant effects were a leaf concentration of 7 g/100 mL and brewing time of 5 min without ultrasonication (T0705). Epicatechin and citric acid were the predominant compounds in this brew, and both were shown to have synergistic effects. This study provides essential data on the brewing conditions and bioactive compounds of MM leaves as antihyperglycemic and antioxidant agents. More information about brewing conditions and compounds that have biofunctions is needed, especially for quality control markers for development of functional beverages. However, *in vivo* or clinical studies are needed to confirm this research, especially the effective dose and toxicity.

6. Author credit statement

Oke Anandika Lestari: conception and design of the study, acquisition of data, analysis and interpretation of the data, drafting of the

Table 3
Molecular docking of bioactive compounds of MM with alpha-glucosidase and alpha-amylase.

Compound	Point interaction	Chemical bond	Type	Energy binding (kcal/mol)
Acarbose with alpha-glucosidase	A:SER693:HG −:**LIG1:O**	HB	CvHB	–333.9
	A:GLN708: HE21 −:**LIG1:O**	HB	CvHB	
	:LIG1:H −: **LIG1:O**	HB	CvHB	
	:LIG1:H − **A: GLY690:O**	HB	CvHB	
	:LIG1:H − A: **GLN219:O**	HB	CvHB	
	:LIG1:H − A: **GLY690:O**	HB	CrHB	
	:LIG1:H − A: **TYR740:OH**	HB	CrHB	
	:LIG1:H −: **LIG1:O**	HB	CrHB	
	:LIG1:H −: **LIG1:O**	HB	CrHB	
	:LIG1:H −: **LIG1:O**	HB	CrHB	
Rutin with alpha-glucosidase	:LIG1:H −: **LIG1:O**	HB	CrHB	−287.8
	A:LYS309:NZ −:**LIG1**	Ec	Pi-Ct	
	A:ASP305:OD1 −:**LIG1**	Ec	Pi-Ai	
	:LIG1 − **A: ARG670**	Hp	Pi-Ay	
	:LIG1 − **A: LEU669**	Hp	Pi-Ay	
	:LIG1 − **A: ARG670**	Hp	Pi-Ay	
Epicatechin with alpha-glucosidase	:LIG1:H − **A: ASN668:O**	HB	CvHB	–223.9
	A:ARG313:NH2 −:**LIG1**	HB;Ec	Pi-Ct; Pi-DHB	
	A:LYS560:NZ −:**LIG1**	Ec	Pi-Ct	
	A:ARG313:HE −:**LIG1**	HB	Pi-DHB	
	:LIG1 − **A: ARG313**	Hp	Pi-Ay	
Shikimic acid with alpha-glucosidase	A:ARG699:HN −:**LIG1:O**	HB	CvHB	−175.0
	A:ARG699: HH11 −:**LIG1: O**	HB	CvHB	
	A:GLY700:HN −:**LIG1:O**	HB	CvHB	
	:LIG1:H − **A: ASP666:OD2**	HB	CvHB	
	:LIG1:H − **A: ILE759:O**	HB	CvHB	
Citric acid with alpha-glucosidase	A:ARG676: HH21 −:**LIG1: O**	HB	CvHB	−186.2
	A:ARG699:HN −:**LIG1:O**	HB	CvHB	
	A:ARG699: HH11 −:**LIG1: O**	HB	CvHB	
	A:ARG699:N:B −:**LIG1:O**	HB	CvHB	
	A:GLU792:HN −:**LIG1:O**	HB	CvHB	
Choline with alpha-glucosidase	:LIG1:N − **A: GLU301:OE2**	Ec	AC	−158.1
	:LIG1:N − **A: ASP666:OD2**	Ec	AC	
	:LIG1:N − **A: GLU792:OE1**	Ec	AC	
	:LIG1:H − **A: ASP666:OD2**	HB	CrHB	
Acarbose with alpha-amylase	:LIG1:H − **A: ASP197:OD1**	HB	CvHB	−350.5
	:LIG1:H − **A: TRP59:O**	HB	CrHB	
	:LIG1:H −: **LIG1:O**	HB	CrHB	
	:LIG1:H − **A: TRP59:O**	HB	CrHB	
	:LIG1:H −: **LIG1:O**	HB	CrHB	
	:LIG1:H −: **LIG1:O**	HB	CrHB	
	:LIG1:H − **A: ILE235:O**	Hp	Ay	
	:LIG1:C − **A: ILE235**	Hp	Pi-Ay	
	A:HIS201 −: **LIG1:C**			
Myricitrin with alpha-amylase	A:THR163:HG1 −:**LIG1:O**	HB	CvHB	−298.5
	A:THR163:HG1 −:**LIG1:O**	HB	CvHB	
	A:HIS305:HD1 −:**LIG1:O**	HB	CvHB	
	:LIG1:H − **A: TYR62:O**	HB	CvHB	
	:LIG1:H − **A: ASP197:OD1**	HB	CvHB	
	:LIG1:H −: **LIG1:O**	HB	CrHB	
	A:ASP300:OD1 −:**LIG1**	Ec	Pi-Ai	
	A:TRP59 −: **LIG1**	Hp	Pi-PiS	
	A:TRP58 −: **LIG1**	Hp	Pi-PiT-s	
	:LIG1:C − **A: LEU165**	Hp	Ay	
Isoquercetin with alpha-amylase	A:GLN63:HE21 −:**LIG1:O**	HB	CvHB	−296.3
	:LIG1:H −: **LIG1:O**	HB	CvHB	
	A:TYR62 −: **LIG1**	Hp	Pi-PiS	
	A:HIS305 −: **LIG1**	Hp	Pi-PiS	
Myricetin with alpha-amylase	A:THR264:HG1 −:**LIG1:O**	HB	CvHB	−274.0
	A:THR264:HG1 −:**LIG1:O**	HB	CvHB	
	:LIG1:H −: **LIG1:O**	HB	CvHB	
	:LIG1:H − **A: LEU237:O**	HB	CvHB	
	:LIG1 − **A: ALA260**	Hp	Pi-Ay	
	:LIG1 − **A: LEU237**	Hp	Pi-Ay	
	:LIG1 − **A: LYS257**	Hp	Pi-Ay	
	:LIG1 − **A: ALA260**	Hp	Pi-Ay	
	:LIG1 − **A: LYS261**	Hp	Pi-Ay	
Malic acid with alpha-amylase	A:SER245:HN −:**LIG1:O**	HB	CvHB	−145.7
	A:LYS257:HZ2 −:**LIG1:O**	HB	CvHB	
	:LIG1:H − **A: ASP236:OD2**	HB	CvHB	
	A:VAL287:CA −:**LIG1:O**	HB	CrHB	

(*continued on next page*)

Table 3 (*continued*)

Compound	Point interaction	Chemical bond	Type	Energy binding (kcal/mol)
	A:PRO288:CD −:**LIG1:O**	HB	CrHB	
4-coumaric acid with alpha-amylase	A:ASP197:OD2 −:**LIG1**	Ec	Pi-Ai	−183.1
	:LIG1 − **A: ALA198**	Hp	Pi-Ay	

Note: *bold letters indicate acceptor residues. Chemical bond: HB = Hydrogen Bond; Ec = Electrostatic; Hp = Hydrophobic. Type: CvHB = Conventional Hydrogen Bond; CrHB = Carbon Hydrogen Bond; Pi-Ct = Pi-Cation; Pi-Ai = Pi-Anion; Pi-Ay = Pi-Alkyl; Pi-DHB = Pi-Donor Hydrogen Bond; AC = Attractive Charge; Ay = Alkyl; Pi-PiS = Pi-Pi Stacked; Pi-PiT-s = Pi-Pi T-shaped.

article, and final approval of the version to be published.

Nurheni Sri Palupi, Agus Setiyono, Feri Kusnandar: the acquisition of data and drafting of the article, final approval of the version to be published.

Nancy Dewi Yuliana: conception and design of the study, analysis and interpretation of the data, drafting of the article, critical revision of the article for important intellectual content, and final approval of the version to be published.

CRediT authorship contribution statement

Oke Anandika Lestari: Data curation, Formal analysis, Investigation, Visualization, Writing – original draft, Methodology, Resources. **Nurheni Sri Palupi:** Conceptualization, Methodology, Supervision, Validation, Writing – review & editing. **Agus Setiyono:** Conceptualization, Methodology, Supervision, Validation, Writing – review & editing. **Feri Kusnandar:** Conceptualization, Methodology, Supervision, Validation, Writing – review & editing. **Nancy Dewi Yuliana:** Conceptualization, Methodology, Software, Supervision, Validation, Visualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.sjbs.2024.104047.

References

Aslam, M.S., Ahmad, M.S., Mamat, A.S., 2017. A review on phytochemical constituents and pharmacological activities of clinacanthus nutans. Int. J. Pharm. Pharm. Sci. 8, 76–91.

Asmat, U., Abad, K., Ismail, K., 2016. Diabetes mellitus and oxidative stress—a concise review. Saudi. Pharma. J. 24, 547–553. https://doi.org/10.1016/J.JSPS.2015.03.013.

Balamurugan, K., Nishanthini, A., Mohan, V.R., 2014. Antidiabetic and antihyperlipidaemic activity of ethanol extract of Melastoma malabathricum Linn. leaf in alloxan induced diabetic rats. Asian Pac. J. Trop. Biomed. 4, S442–S448. https://doi.org/10.12980/APJTB.4.2014C122.

Bharti, S.K., Krishnan, S., Kumar, A., Kumar, A., 2018. Antidiabetic phytoconstituents and their mode of action on metabolic pathways. Ther. Adv. Endocrinol. Metab. 9, 81–100. https://doi.org/10.1177/2042018818755019.

Cermak, R., Landgraf, S., Wolffram, S., 2004. Quercetin glucosides inhibit glucose uptake into brush-border-membrane vesicles of porcine jejunum. Br. J. Nutr. 91, 849–855. https://doi.org/10.1079/bjn20041128.

Chang, M.Y., Lin, Y.Y., Chang, Y.C., Huang, W.Y., Lin, W.S., Chen, C.Y., Huang, S.L., Lin, Y.S., 2020. Effects of infusion and storage on antioxidant activity and total phenolic content of black tea. Appl. Sci. (Switzerland) 10. https://doi.org/10.3390/APP10082685.

Cheng, Q., Cai, S., Ni, D., Wang, R., Zhou, F., Ji, B., Chen, Y., 2015. In vitro antioxidant and pancreatic α-amylase inhibitory activity of isolated fractions from water extract of Qingzhuan tea. J. Food Sci. Technol. 52, 928–935. https://doi.org/10.1007/s13197-013-1059-y.

Chua-Barcelo, R.T., 2014. Ethno-botanical survey of edible wild fruits in Benguet, Cordillera administrative region, the Philippines. Asian Pac. J. Trop. Biomed. 4, S525–S538. https://doi.org/10.12980/APJTB.4.201414B36.

Dianursanti, S., Maeda, A.R., Yoshino, T., Tanaka, T., 2020. The effects of solvents and solid-to-solvent ratios on ultrasound-assisted extraction of carotenoids from chlorella vulgaris. Int. J. Technol. 11, 941–950. https://doi.org/10.14716/ijtech.v11i5.4331.

Faraone, I., Rai, D.K., Russo, D., Chiummiento, L., Fernandez, E., Choudhary, A., Milella, L., 2019. Antioxidant, antidiabetic, and anticholinesterase activities and phytochemical profile of Azorella glabra wedd. Plants 8, 1–14. https://doi.org/10.3390/plants8080265.

Ganugapati, J., Mukkavalli, S., Sahithi, A., 2011. Docking studies of green tea flavonoids as insulin mimetics. Int. J. Comput. Appl. 30, 975–8887.

Hashim, A., Khan, M Salman, Khan, Mohd Sajid, Baig, M.H., Ahmad, S., 2013a. Antioxidant and α; ylase inhibitory property of phyllanthus virgatus L.: an in vitro and molecular interaction study. Biomed. Res. Int. https://doi.org/10.1155/2013/729393.

Hashim, A., Khan, M Salman, Khan, Mohd Sajid, Baig, M.H., Ahmad, S., 2013b. Antioxidant and α; ylase inhibitory property of phyllanthus virgatus L.: an in vitro and molecular interaction study. Biomed. Res. Int. https://doi.org/10.1155/2013/729393.

Hyeong-U, S., Eun-Kyeong, Y., Chi-Yeol, Y., Chul-Hong, P., Myung-Ae, B., Tae-Ho, K., Chang Hyung, L., Won Lee, K., Seo, H., Kyung-Jin, K., Sang-Han, L., 2019. Effects of synergistic inhibition on α-glucosidase by phytoalexins in soybeans. Biomolecules 9, 1–13. https://doi.org/10.3390/biom9120828.

Ibitoye, O.B., Uwazie, J.N., Ajiboye, T.O., 2018. Bioactivity-guided isolation of kaempferol as the antidiabetic principle from *Cucumis sativus* L. fruits. J. Food Biochem. 42, 1–7. https://doi.org/10.1111/jfbc.12479.

Idris, M., Purnomo, A.S., Martak, F., Fatmawati, S., 2022. Antioxidant and antidiabetic activities of melastoma malabathricum leaves extracts. J. Hunan University 49, 144–153.

Joffry, S.M., Yob, N.J., Rofiee, M.S., Affandi, M.M.R.M.M., Suhaili, Z., Othman, F., Akim, A.M., Desa, M.N.M., Zakaria, Z.A., 2012. Melastoma malabathricum (L.) smith ethnomedicinal uses, chemical constituents, and pharmacological properties: a review. Evidence-Based Complem. Alter. Med. https://doi.org/10.1155/2012/258434.

Kartina, A.M.A., Agang, M.W., 2019. Characterisation of Phytochemical Content of Karamunting (Melastoma malabatchricum L.) Using Mass Spectrometry (GC-MS). Biota : J. Ilmiah Ilmu-Ilmu Hayati 4, 16–23. https://doi.org/10.24002/biota.v4i1.2363.

Kikiowo, B., Ogunleye, J.A., Iwaloye, O., Ijatuyi, T.T., 2020. Therapeutic potential of chromolaena odorata phyto-constituents against human pancreatic α-amylase. J. Biomol. Struct. Dyn. https://doi.org/10.1080/07391102.2020.1833758.

Kwon, O., Eck, P., Chen, S., Corpe, C.P., Lee, J., Kruhlak, M., Levine, M., 2007. Inhibition of the intestinal glucose transporter GLUT2 by flavonoids. FASEB J. 21, 366–377. https://doi.org/10.1096/fj.06-6620com.

Lestari, O.A., Palupi, N.S., Setiyono, A., Kusnandar, F., Yuliana, N.D., 2022. In vitro antioxidant potential and phytochemical profiling of Melastoma malabathricum leaf water extract. Food Sci. Technol. 42, 1–9. https://doi.org/10.1590/fst.92021.

Limanto, A., Simamora, A., Santoso, A.W., Timotius, K.H., 2019. Antioxidant, α-glucosidase inhibitory activity and molecular docking study of gallic acid, quercetin and rutin: a comparative study. Mol. Cellular Biomed. Sci. 3, 67. https://doi.org/10.21705/mcbs.v3i2.60.

Mamat, S.S., Fauzi, M., Kamarolzaman, F., Yahya, F., Mahmood, N.D., Shahril, M.S., Jakius, K.F., Mohtarrudin, N., Ching, S.M., Susanti, D., Taher, M., Zakaria, Z.A., 2013. Methanol extract of Melastoma malabathricum leaves exerted antioxidant and liver protective activity in rats. BMC Complement. Altern. Med. 13, 12.

Mayasari, D., Murti, Y.B., Sudarsono, S., Pratiwi, S.U.T., 2021. Phytochemical, antioxidant and antibacterial evaluation of Melastoma malabathricum L.: an indonesian traditional medicinal plant. Trop. J. Nat. Prod. Res. 5, 819–824. https://doi.org/10.26538/tjnpr/v5i5.5.

Mazid, M.A., Datta, B.K., Nahar, L., Bashar, S.A.M.K., Bachar, S.C., Sarker, S.D., 2011. Phytochemical studies on Polygonum barbatum (L.) Hara var. barbata (Polygonaceae). Rec. Nat. Prod. 5, 143–146.

Mechchate, H., Es-safi, I., Haddad, H., Bekkari, H., Grafov, A., Bousta, D., 2021a. Combination of catechin, epicatechin, and rutin: optimization of a novel complete antidiabetic formulation using a mixture design approach. J. Nutr. Biochem. 88, 108520 https://doi.org/10.1016/j.jnutbio.2020.108520.

Mechchate, H., Ouedrhiri, W., Es-safi, I., Amaghnouje, A., Jawhari zahra, F., Bousta, D., 2021b. Optimization of a New Antihyperglycemic Formulation Using a Mixture of Linum usitatissimum L., Coriandrum sativum L., and Olea europaea var. sylvestris flavonoids: a mixture design approach. Biologics 1, 154–163. https://doi.org/10.3390/biologics1020009.

P.D. Meyts W. Sajid J. Palsgaard A.-M. Theede L. Gauguin H. Aladdin J. Whittaker Insulin and IGF-I Receptor Structure and Binding Mechanism 2013 National Center for Biotechnology Information.

Ni, D., Ai, Z., Munoz-Sandoval, D., Suresh, R., Ellis, P.R., Yuqiong, C., Sharp, P.A., Butterworth, P.J., Yu, Z., Corpe, C.P., 2020. Inhibition of the facilitative sugar transporters (GLUTs) by tea extracts and catechins. FASEB J. 34, 9995–10010. https://doi.org/10.1096/fj.202000057RR.

Olennikov, D.N., Kashchenko, N.I., 2014. Componential profile and amylase inhibiting activity of phenolic compounds from Calendula officinalis L. leaves. Sci. World J. https://doi.org/10.1155/2014/654193.

Othman, A., Jalil, A.M.M., Weng, K.K., Ismail, A., Ghani, N.A., Adenan, I., 2010. Epicatechin content and antioxidant capacity of cocoa beans from four different countries. Afr. J. Biotechnol. 9, 1052–1059. https://doi.org/10.5897/ajb09.1219.

Pham, A.T., Malterud, K.E., Paulsen, B.S., Diallo, D., Wangensteen, H., 2014. α-Glucosidase inhibition, 15-lipoxygenase inhibition, and brine shrimp toxicity of extracts and isolated compounds from terminalia macroptera leaves. Pharm. Biol. 52, 1166–1169. https://doi.org/10.3109/13880209.2014.880486.

Poovitha, S., Parani, M., 2016. In vitro and in vivo α-amylase and α-glucosidase inhibiting activities of the protein extracts from two varieties of bitter gourd (Momordica charantia L.). BMC Complement. Altern. Med. 16, 1–8. https://doi.org/10.1186/s12906-016-1085-1.

Prieto-Martínez, F.D., Arciniega, M., Medina-Franco, J.L., 2018. Acoplamiento Molecular: Avances Recientes y Retos. TIP Revista Especializada En Ciencias Químico-Biológicas 21. https://doi.org/10.22201/fesz.23958723e.2018.0.143.

Proença, C., Freitas, M., Ribeiro, D., Oliveira, E.F.T., Sousa, J.L.C., Tomé, S.M., Ramos, M.J., Silva, A.M.S., Fernandes, P.A., Fernandes, E., 2017. α-Glucosidase inhibition by flavonoids: an in vitro and in silico structure–activity relationship study. J. Enzyme Inhib. Med. Chem. 32, 1216–1228. https://doi.org/10.1080/14756366.2017.1368503.

Rahman, N., Muhammad, I., Nayab, G.E., Khan, H., Aschner, M., Filosa, R., Daglia, M., 2019. Molecular docking of isolated alkaloids for possible α-glucosidase inhibition. Biomolecules 9, 1–16. https://doi.org/10.3390/biom9100544.

Rajeswari, R., Sridevi, M., Vijayakumar, N., Banerjee, S., 2020. Molecular Docking interaction of Kaempferol-3-o- α -l- rhamnoside isolated from Cardiospermum halicacabum Linn with molecular targets involved in blood glucose homeostasis. J. Pharm. Sci. 12.

Ranjan, A., Singh, R.K., Khare, S., Tripathi, R., Pandey, R.K., Singh, A.K., Gautam, V., Tripathi, J.S., Singh, S.K., 2019. Characterization and evaluation of mycosterol secreted from endophytic strain of Gymnema sylvestre for inhibition of α-glucosidase activity. Sci. Rep. 9, 1–13. https://doi.org/10.1038/s41598-019-53227-w.

Sancheti, S., Sancheti, S., Yum-sung, S., 2009. Chaenomeles sinensis : a potent α -and β -glucosidase inhibitor Korean collection of herbal extract Inc, Kongju 314–701. Korea Am. J. Pharmacol. Toxicol. 4, 8–11.

Sanjaya, W., Rialita, A., Mahyarudin, M., 2021. Antifungal Activity of cengkodok leaf ethanol extract (melastoma malabathricum) against malassezia furfur growth. Jurnal Fitofarmaka Indonesia 8, 23–32. https://doi.org/10.33096/jffi.v8i1.614.

Shin, J., Seol, I., Son, C., 2010. Interpretation of animal dose and human equivalent dose for drug development. J. Korean Orient. Med. 31, 1–7.

Son, H.-U., Lee, S.-H., 2013. Comparison of α-glucosidase inhibition by Cudrania tricuspidata according to harvesting time. Biomed. Rep. 1, 624–628. https://doi.org/10.3892/br.2013.111.

Srinivasan, P., Vijayakumar, S., Kothandaraman, S., Palani, M., 2018. Anti-diabetic activity of quercetin extracted from Phyllanthus emblica L. fruit: In silico and in vivo approaches. J. Pharm. Anal. 8, 109–118. https://doi.org/10.1016/j.jpha.2017.10.005.

Susanti, D., Sirat, H., Ahmad, F., Ali, R., 2008. Bioactive constituents from the leaves of melastoma malabathricum L. Jurnal Ilmiah Farmasi UII 5, 96438.

Tahrani, A.A., 2017. Novel therapies in type 2 diabetes: insulin resistance. Practical Diabetes 34, 161–166a. https://doi.org/10.1002/pdi.2109.

Treki, M.M.A., Ashikeen, M.N., Baib, A.A.O., 2018. Effect of acid load (citric acid) on diabetes-induced rats. Int. J. Med. Toxicol. Legal Med. 21, 212. https://doi.org/10.5958/0974-4614.2018.00070.0.

Tri, A., Safitri, A., Fatchiyah, F., 2020. An in silico approach reveals the potential function of cyanidin-3-o-glucoside of red rice in inhibiting the advanced glycation end products (AGES)-receptor (RAGE) signaling pathway. Acta Informatica Medica 28, 170–179. https://doi.org/10.5455/aim.2020.28.170-179.

Triawanti, S.E., Yusriandi, R., Fauzul, Z.O., Winata, N.P., Aditya, S., Andhika Azwar, A. G., 2017. Effect of Karamunting fruit juice (Melastoma malabathricum L.) to Advanced Glycation End-Products (AGEs) and lipid profile as advanced complications of diabetes mellitus. J. Eng. Appl. Sci. https://doi.org/10.3923/jeasci.2017.171.175.

Truong, L.C., 2020. Reducing the effects of blood sugar infusion of melastoma malabathricum L.in Mus Musculus. J. Asian Multicult. Res. Med. Health Sci. Study 1, 1–10.

van der Schaft, N., Schoufour, J.D., Nano, J., Kiefte-de Jong, J.C., Muka, T., Sijbrands, E. J.G., Ikram, M.A., Franco, O.H., Voortman, T., 2019. Dietary antioxidant capacity and risk of type 2 diabetes mellitus, prediabetes and insulin resistance: the Rotterdam Study. Eur. J. Epidemiol. 34, 853–861. https://doi.org/10.1007/S10654-019-00548-9/TABLES/3.

Varma, A.K., Patil, R., Das, S., Stanley, A., Yadav, L., Sudhakar, A., 2010. Optimized hydrophobic interactions and hydrogen bonding at the target-ligand interface leads the pathways of Drug-Designing. PLoS One 5. https://doi.org/10.1371/journal.pone.0012029.

Vignoli, J.A., Bassoli, D.G., Benassi, M.T., 2011. Antioxidant activity, polyphenols, caffeine and melanoidins in soluble coffee: the influence of processing conditions and raw material. Food Chem. 124, 863–868. https://doi.org/10.1016/j.foodchem.2010.07.008.

Vladu, M., Efrem, I.C., Clenciu, D., Fortofoiu, M.-C., 2020. Research on Insulin Resistance and Chronic Kidney Disease in Patients with Type 1 Diabetes Mellitus, in: Current Topics in Medicine and Medical Research Vol. 7. doi: 10.9734/bpi/ctmamr/v7.

Wang, H., Du, Y.J., Song, H.C., 2010. α-Glucosidase and α-amylase inhibitory activities of guava leaves. Food Chem. 123, 6–13. https://doi.org/10.1016/j.foodchem.2010.03.088.

Wang, S., Guo, C., Xing, Z., Li, M., Yang, H., Zhang, Y., Ren, F., Chen, L., Mi, S., 2021. Dietary intervention with α-amylase inhibitor in white kidney beans added yogurt modulated gut microbiota to adjust blood glucose in mice. Front. Nutr. 8, 1–17. https://doi.org/10.3389/fnut.2021.664976.

Wang, S., Gan, Z., Wang, B., Zhang, N., LI, K., Yao, T., 2022. Effect of brewing conditions on polyphenols in the dark tea (Camellia sinensis L.) infusions: content, composition and antioxidant activities. Food Sci. Technol. (42. https://doi.org/10.1590/fst.36322.

Williamson, E.M., 2001. 1-s2.0-S0944711304700586-main 8, 401–409.

Worley, B., Powers, R., 2013. Multivariate Analysis in Metabolomics. Curr. Metabol. 1, 92–107. https://doi.org/10.2174/2213235x11301010092.

Yuliana, N.D., Khatib, A., Choi, Y.H., Verpoorte, R., 2011. Metabolomics for bioactivity assessment of natural products. Phytother. Res. https://doi.org/10.1002/ptr.3258.

Yuliana, N.D., Jahangir, M., Verpoorte, R., Choi, Y.H., 2013. Metabolomics for the rapid dereplication of bioactive compounds from natural sources. Phytochem. Rev. https://doi.org/10.1007/s11101-013-9297-1.

Zdunić, G., Aradski, A.A., Gođevac, D., Živković, J., Laušević, S.D., Milošević, D.K., Šavikin, K., 2020. In vitro hypoglycemic, antioxidant and antineurodegenerative activity of chokeberry (Aronia melanocarpa) leaves. Ind. Crop. Prod. 148, 1–8. https://doi.org/10.1016/j.indcrop.2020.112328.

Zheng, W.J., Ren, Y.S., Wu, M.L., Yang, Y.L., Fan, Y., Piao, X.H., Ge, Y.W., Wang, S.M., 2021. A review of the traditional uses, phytochemistry and biological activities of the Melastoma genus. J. Ethnopharmacol. 264, 113322 https://doi.org/10.1016/j.jep.2020.113322.