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Antioxidant and antidiabetic compounds identification in several Indonesian underutilized Zingiberaceae spices using SPME-GC/MS-based volatilomics and *in silico* methods

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ABSTRACT

This study aimed to identify compounds in 12 minor Zingiberaceae spices grown in Indonesia linked with in vitro α-glucosidase inhibitor and antioxidant (DPPH, FRAP, CUPRAC) activities using SPME-GC/MS volatilomics. The results illustrated that *Zingiber aromaticum* Val., *Alpinia malaccensis* (Burm.f.) Roscoe, *Amomum compactum* Sol. ex Maton, and *Zingiber purpureum* Roscoe had the highest α-glucosidase inhibitor and DPPH, FRAP, CUPRAC antioxidant activities, respectively. Also, the total phenolic content positively influenced DPPH, FRAP, and CUPRAC antioxidant activities. The strongest positive correlation with α-glucosidase inhibitor and DPPH antioxidant activities was found in eucalyptol; whereas o-cymene and terpinen-4-ol had the strongest correlations with FRAP and CUPRAC antioxidants, respectively. Furthermore, the molecular docking analysis revealed that all compounds with a strong correlation with α-glucosidase inhibitor activity (based on their OPLS VIP score) had binding energies (−5.06 – −6.26 kcal/mol) close to Acarbose (−10.11 kcal/mol). Thus, this study provided vital information on the volatile compounds in underutilized spices associated with their health beneficial properties.

Introduction

Spices are important food ingredients that play an essential part in our daily diet. Humans widely use spices, either as condiments and spices or directly eaten. Spices refer to vegetable products or a mixture thereof without any extraneous materials and are used for flavoring, seasoning, and providing aroma to foods (de Guzman & Siemonsma, 1999). However, spices may not only function as flavor enhancers and food preservatives. Many studies reported the bioactive properties of different spices, which indicate they may have a role in preventing non-communicable chronic diseases (Opara, 2019). These studies were conducted in vitro, in vivo, and to some extent, also include human studies. Various spices commonly used in the daily diet were recently reported to possess antioxidant and immunomodulatory properties in vitro, such as aromatic ginger, torch ginger, and mango ginger at a reasonable concentration (Safriani, Rungkat, Yuliana, & Prangdimurti, 2021). The authors reported that the total phenolics of those spices had a significant correlation with their antioxidant activity but not with immunomodulatory activity. The potential of common spices such as turmeric, black cumin, ginger, garlic, saffron, black pepper, and chili pepper in preventing the different types of cancers was comprehensively reviewed (J. Zheng et al., 2016). Several phytochemicals which might associate with spices' anti-cancer activity were described, which include 6-gingerol, 6-shogaol, and 6-paradol in ginger; organosulphur in garlic;

Abbreviations: GC/MS, Gas chromatography/mass spectrometry; SPME, Solid phase micro extraction; PCA, Principal component analysis; OPLS, Orthogonal projection to the least square; AGI, α-glucosidase inhibitor; TPC, Total phenolic content; TFC, Total flavonoid content; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, Ferric reducing antioxidant property; CUPRAC, Cupric ion reducing antioxidant capacity; CA, *Curcuma aeruginosa* Roxb; CZ, *Curcuma zedoria* Roscoe; ZC, *Zingiber purpureum* Roscoe; ZO, *Zingiber ottensii* Val.; BR, *Boesenbergia rotunda* L. Mansf.; CH, *Curcuma heyneana* Val. & Zijp; CP, *Curcuma purpurascens* Blume; CT, *Curcuma petiolata* Roxb; ZA, *Zingiber aromaticum* Val.; ZZ, *Zingiber zerumpet* L. Roscoe ex Sm.; AC, *Amomum compactum* Sol. ex Maton; AM, *Alpinia malaccensis* (Burm.f.) Roscoe.

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and piperine in black pepper. Studies in humans also showed that various spices exhibited bioactive properties related to anti-inflammatory, antioxidant, diabetes, and obesity at the reasonable concentrations for daily consumption (Opara, 2019).

Based on the literature searching, antioxidant and antidiabetes are two bioactivities that eminently reported in spices. Some studies identified the phytochemicals responsible for the activity, while others used crude extracts. For example, Curcumin contained in *Curcuma longa* L. had in vitro α-glucosidase inhibitor activity with IC_{50} value was 29.31 $nmolL^{-1}$, lower than Acarbose (22.80 $\mu molL^{-1}$) (Taslimi & Gulçin, 2017). The antioxidant activity of spices from the Zingiberaceae family is also frequently reported. For example, hot water extract of clove had the strongest DPPH antioxidant activity, while marjoram had the highest superoxide anion scavenging activity among 13 common spices tested in the study. Additionally, cloves had the highest amount of total phenolics, while marjoram had the highest total flavonoids content (Kim, Yang, Lee, & Kang, 2011).

More recent research compared the chemical composition and antioxidant activity of essential oils and crude extracts of four Zingiberaceae spice plants, namely cardamom, turmeric, ginger, and galangal (Ivanović, Makoter, & Razboršek, 2021). It was found that monoterpenes are volatiles primarily found in the essential oils of cardamom and galangal. Sesquiterpenes were the predominant volatiles identified in turmeric and ginger. α-Terpinyl acetate was found in the largest quantity in cardamom but the compound was found in trace amounts in other spices. In terms of antioxidant activity, galangal showed the highest FRAP and ABTS antioxidant value. Although they reported that galangal had the highest total phenolic content, the author did not study the correlation between the volatiles profile of these spices with their antioxidant properties. Indeed the unique spices characteristics are that they have a strong smell and taste attributed to volatile and semi-volatile compounds in spices.

Volatile compounds of several well-known Zingiberaceae spices have been widely studied. For example, zingiberene, borneol, δ-sabinene, turmerone, d-α-phellandrene, and cineole were the main volatiles in turmeric (Chane-Ming, Vera, Chalchat, & Cabassu, 2002). The fennel's volatiles and semi-volatiles composition were recently studied to indicate their quality during 5 years of storage using SPME-GC/MS. They found that monoterpenes can be used as freshness indicators because their concentration was significantly decreased after 5 years of storage (Maikhunthod & Marriott, 2013). SPME is preferred since it offers rapid, simple, inexpensive and solvent-free extraction techniques coupled with the gas chromatography technique. This method is very useful for volatiles and semi-volatile compounds screening and allows qualitative and quantitative analysis using internal or external calibration (Prosen & Zupančič-Kralj, 1999).

Although there is no fixed definition, the term underutilized spices that we used here refers to spices whose uses are limited, particularly in Indonesia. Despite huge research on beneficial health effects and chemical profiling on various spices have been done, more interest is given to the major spices, whereas other lesser-known spices is limited. Therefore, the main objectives of this study were to conduct volatile compounds profiling of 12 minor Zingiberaceae spices grown in Indonesia using the SPME-GC/MS technique and to identify the volatile compounds correlated with α-glucosidase inhibitor activity and antioxidant activity of the spices. The spices samples used in this study are not so commonly consumed as compared, for example, to ginger, turmeric, or galangal. Thus, such scientific information for these spices is not yet available. Antioxidant activity was determined using three different methods: DPPH, FRAP, and CUPRAC. Two multivariate data analysis techniques were used to study the correlation, principal component analysis (PCA) and orthogonal projection the least square (OPLS) analysis. The volatile compounds positively correlated with the activities were selected based on VIP and Y-related profiles. In silico analysis was conducted to confirm the activity, especially α-glucosidase inhibitor activity. Total phenolic and total flavonoid contents (TPC and TFC, respectively) of each spices, were additionally measured, and whether they correlated with the study bioactivities were determined. However, this is the first study that comprehensively conducted chemical profiling and bioactive compounds identification on the 12 Zingiberaceae minor spices grown in Indonesia.

Materials and methods

Samples preparation

Twelve spices used in this study which includes their local names, *Curcuma aeruginosa* Roxb (temu hitam), *Curcuma zedoria* Roscoe (temu putih), *Zingiber purpureum* Roscoe (bangle), *Zingiber ottensii* Val. (bangle hitam), *Boesenbergia rotunda* L. Mansf. (temu kunci), *Curcuma heyneana* Val. & Zijp (temu giring), *Curcuma purpurascens* Blume (temu tis), *Curcuma petiolata* Roxb (temu putri), *Zingiber aromaticum* Val. (lempuyang wangi), *Zingiber zerumpet* L. Roscoe ex Sm. (lempuyang), *Amomum compactum* Sol. ex Maton (kapulaga), and *Alpinia malaccensis* (Burm.f.) Roscoe (laja goah). Except for *Amomum compactum* Sol. ex Maton where the fruit part was used, the rhizome parts of all spices were used in this study. These spices were collected from the experimental garden of the Indonesian Spice and Medicinal Research Institute, Bogor, Indonesia. Dedi Rosadi from the Indonesian Spice and Medicinal Research Institute identified the spices. Samples were collected fresh, washed, then immediately stored in a freezer at − 20 °C. The samples were dried by the freeze dryer for the bioactivity test for 72 h. The dried samples were ground into powder and stored in the freezer until the extraction. The voucher specimen of each spice was kept in the Food Chemistry Laboratory, Department of Food Science and Technology, IPB University.

Samples extraction for bioactivity studies

Spice powder (20 g) was added with methanol 80% with a volume twice the spice powder, then ultrasonicated (Bransonic Ultrasonic Cleaner 8510E MTH, USA) for 30 min at room temperature. The mixture was filtered, and the residue retained in the filter was again ultrasonicated with the same amount of methanol 80%. Finally, the filtrate was pooled and dried using a rotary evaporator (Buchi Rotavapor R-300, Buchi Labortechnik Switzerland) at 40 °C until a dry extract was obtained.

Solid phase microextraction

The frozen spice samples were thawed, cut, sliced thinly into a uniform size, and put in a headspace vial (4 g). The internal standard (dichloromethane) was added as much as 2 μL. The vial was tightly closed with a PTFE silicon septum. SPME DVB/CAR/PDMS from the Supelco (Sigma Aldrich) was used for the extraction. The SPME fiber was conditioned at 250 °C for 2 min before use. While the sample was heated at a temperature of ± 40 °C, the SPME was then inserted into the headspace vial and was exposed to the sample without touching it for 30 min. The SPME was then released and directly injected into the GC/MS injection port with a desorption time of 2 min (Huang, Wang, Chu, & Qin, 2012).

GC–MS analysis

In this step, GC (Shimadzu Nexis GC-2030) connected to an MS detector (Shimadzu GC–MS-QP2020 NX) was used. The analysis condition was as follow: stabilized wax column (60 m, 0.25 mm ID, film thickness 0.25 m), injector temperature 80 °C, the column temperature is adjusted in gradient with the initial temperature of 40 °C maintained for 5 min, the temperature is then increased 4 °C/min until it reached a temperature of 150 °C. Finally, the temperature is increased by 30 °C/min until it reached the final temperature of 250 °C and was maintained for 5 min. Helium was used as mobile phase at 1 mL/min, split injection mode

(1:20). MS condition was operated with EI ionization mode; detector temperature was 230 °C, interface temperature was 250 °C. It ran 40 min as previously described (Huang et al., 2012) with a slight modification. Compound identification was performed by comparing the mass spectra with NIST Library (NIST14 standard version) and comparing the LRI value with external standards. The LRI value was calculated using the method as described elsewhere (Dool & Kratz, 1962). a homologous series of an *n*-alkane solution (C10-40, Polyscience, Niles, IL, USA; 5 mg/L) in dichloromethane was used to calculate the LRI of each analyte under the identical chromatographic conditions as the samples.

Determination of TPC

First, the sample concentration of 1000 μg/mL was prepared by weighing 10 mg of extract, then dissolved in 10 mL of 50% methanol. Next, a 100 μL sample or gallic acid was mixed with 200 μL Folin-Ciocalteu 10%. The mixture was then added by 800 μL Na_2CO_3 0.7 $molL^{-1}$, and incubated in a dark room at room temperature for 2 h. Next, 200 μL of the mixture was transferred to 96-well microplates. Finally, the absorbance was read at 750 nm with a microplate reader. Total phenol expressed in mg gallic acid equivalent (GAE)/g dry extract (Ainsworth & Gillespie, 2007).

Determination of TFC

Initially, the sample's extract (concentration 1000 μg/ml) of 10 μL was mixed with 60 μL of methanol, 10 μL of $AlCl_3$ (10% w/v), 10 μL of CH_3COOK (1 $molL^{-1}$) and 120 μL of distilled water. The mixture was then incubated at room temperature for 30 min. Next, the absorbance was measured at 415 nm using a microplate reader. The standard used is quercetin (concentration 20–200 μg/mL), and the total flavonoids were expressed in mg quercetin equivalent (QE) per g of sample extract (Lee, Sancheti, Bafna, Sancheti, & Seo, 2011).

In vitro antidiabetic and antioxidant test

α-glucosidase inhibition activity

First, 10 μL of the extract (concentration of 1000 μg/mL) was mixed with 50 μL of 0.1 $molL^{-1}$ phosphate buffer (pH 7.0), 25 μL of 10 $mmolL^{-1}$ 4-nitrophenyl-β-D-glucopyranoside solution (dissolved in 0,1 $molL^{-1}$ phosphate buffer solution pH 7.0) and 25 μL α-glucosidase isolated from *Bacillus stearothermophilus* (0.06 U/mL in 0.1 $molL^{-1}$ buffer solution pH 7.0). The reaction was incubated for 30 min at 37 °C. The reaction was stopped by 100 μL of 0.2 $molL^{-1}$ sodium carbonate solution. The absorbance reading was done in a microplate reader at a wavelength of 410 nm (Sancheti, Sancheti, & Yum-sung, 2009). The assay was repeated three times. The inhibition of α-glucosidase was expressed as percent inhibition and was calculated by Equation (1).

$$Inhibition(\%) = \left[\left(Abs_{Control} - Abs_{Sample}\right) / Abs_{Control} \right] \times 100 \quad (1)$$

DPPH radical scavenging activity

100 μL of sample extract solution (concentration 1000 μg/ml) was added to 100 μL of 125 $\mu molL^{-1}$ DPPH solution in ethanol, then homogenized and left at darkroom temperature for 30 min. The absorbance value was measured using a microplate reader at a wavelength of 517 nm. This test was repeated three times. The positive control used was Trolox with concentrations of 1, 5, 25, 50, 75, and 100 $\mu molL^{-1}$. The antioxidant capacity is expressed in Trolox Equivalent (TE) Antioxidant Capacity (μmol TE/g dry extract) (Salazar-Aranda Ricardo & Pérez-López, 2011).

FRAP assay

The FRAP reagent was prepared by mixing 300 $mmolL^{-1}$ acetate buffer (pH 3.6), 10 $mmolL^{-1}$ TPTZ solution and 20 $mmolL^{-1}$ $FeCl_3$ solution with a ratio (10:1:1 v/v/v). Standard solution using Trolox with a concentration of 80–800 $\mu molL^{-1}$ dissolved in ethanol. The TPTZ solution was prepared on the same day as the test. A total of 20 μL of sample and 180 μL of FRAP reagent were added to a 96-well microplate. The mixture was incubated at 37 °C for 15 min; then, the absorbance was measured at 595 nm using a microplate reader. The FRAP value was expressed as μmol TE/g dry extract (Sekhon-Loodu, Warnakulasuriya, Rupasinghe, & Shahidi, 2013).

CUPRAC assay

A total of 40 μL of sample extract (concentration 1000 μg/mL), 50 μL $CuCl_2$ 10 $mmolL^{-1}$, 50 μL neocuproine 7.5 $mmolL^{-1}$, and 60 μL CH_3COONH_4 1 $molL^{-1}$ pH 7 were mixed in a 96-well microplate. The mixture was incubated for 60 min; the absorbance value was measured at 450 nm using a microplate reader. Standard solution using Trolox with concentration (20–800 $\mu molL^{-1}$). The antioxidant capacity was expressed in μmol TE/g dry extract (Apak, Güçlü, Özyürek, Bektaş oğlu, & Bener, 2008).

Data analysis

Data analysis was carried out statistically by repeating the test in triples for each sample. Data is shown in the form of mean ± standard deviation. The data was processed using SPSS® (Statistical Package for the Social Science) software version 24.0. Bioactivity data from 12 samples were analyzed by one-way Analysis of Variance (ANOVA) at a 95% confidence interval. The Analysis of Variance (ANOVA) results, which showed differences, further carried out Duncan's Multiple Range Test at a 5% level. The correlation of volatile profiles with antidiabetic and antioxidants was analyzed using multivariate data analysis (MVDA). PCA and OPLS analysis was conducted using the Pareto scaling method and was performed using SIMCA software ver 16.0 (Sartorius Stedim Biotech GmbH, Göttingen, Germany). For PCA analysis of volatiles data, the Savitzky-Golay filtering method was applied to remove all noises that might interfere with the model. The model quality was assessed using the criteria of model accuracy (R^2Y), predictive accuracy (Q^2Y), and permutation test. Y-related coefficient and VIP (Variable Influence on Projection) value were used to select volatile compounds in correlation with the antioxidant and α-glucosidase inhibitor activities (Eriksson et al., 2013).

Docking analysis

Docking analysis was performed to determine the binding affinity of the putative bioactive compound to the enzyme's catalytic site. AutoDock Tools v.1.5.2 (The Scripps Research, USA) was used as a software to evaluate their interaction. The 3D structures of α-glucosidase were retrieved from the protein data bank (PDB code 3TOP). Water and native ligands were detached before docking, while polar hydrogen and computed Geister charge was added to the crystal structure. All structures of putative bioactive compounds were collected from PubChem in sdf format which then clean the 2D and 3D structure and converted to pdb format by MarvinSketch software (Advanced Chemistry Development, Inc.). The rotatable bond of this compounds were decided using AutoDock Tools v.1.5.2. The docking was carried out at cordinate 31.483 (x), 32.275 (y), and 29.233 (z) with spacing and grid box size 0.375 Å and 60 × 60 × 70, respectively (Syabana, Yuliana, Batubara, & Fardiaz, 2022). The docking output was run by employing the Lamarckian genetic algorithm. Binding energy and constant inhibition was used as parameter to evaluate the ligand-α-glucosidase interaction. Visualization of docking analysis was performed by ligplot software

(EMBL-EBI, Cambridgeshire, UK).

Results and discussion

Total phenolic and total flavonoid content, in vitro antidiabetic and antioxidant activities

TPC and TFC of the studied spices were varied (Table 1). *A. compactum* Sol. ex Maton had the highest TPC value (41.47 mg GAE/g), followed by *Z. purpureum* Roscoe (39.96 mg GAE/g). The TPC of *Z. aromaticum* Val and *C. zedoria* Roscoe were not significantly different (38.26 mg GAE/g, 38.02 mg GAE/g, respectively). The highest TFC was found in *Z. purpureum* Roscoe (144.5 mg QE/g), followed by *Z. aromaticum* Val and *C. purpurascens* Blume (80.13 mg QE/g and 77.5 mg QE/g, respectively).

Phenolic compounds, including polyphenols, are the most common antioxidants in plant food, with flavonoids, lignans, phenolic acids, and stilbenes as the four major classes of polyphenols (Shahidi & Ambigaipalan, 2015). As previously mentioned, these compounds were reported to correlate positively with bioactivity in some reports, but no correlation was observed in other studies (Ivanović et al., 2021; Safriani et al., 2021).

The result of α-glucosidase inhibitor and antioxidant activity screening of the 12 spices extracts was also quite varied, as summarized in Table 1. *Z. aromaticum* Val showed the highest percentage of α-glucosidase inhibition among other samples. Extracts of *Z. purpureum* Roscoe, *B. rotunda* L. Mansf., *A. compactum* Sol. ex Maton, *C. purpurascens* Blume, and *A. malaccensis* (Burm.f.) Roscoe also showed relatively high inhibitory activity, which was in the range of 58%–82%. Antidiabetic activity of *Z. aromaticum* Val was also previously reported but with a different mechanism from our study. It inhibited the activity of protein tyrosine phosphatase 1B (PTP1B), an enzyme found in important insulin-targeted tissues like the liver, muscle, and fat. In excess, PTP1B will impair insulin down-regulation leading to type II diabetes mellitus (Elchebly et al., 1999; Haj, Zabolotny, Kim, Kahn, & Neel, 2005). The inhibitory activity of the enzyme by *Z. aromaticum* was 84.4% at 25 μg/mL, and the identified active compounds were (5R)-2,6,9-humulatrien-5-of-8-one; kaempferol-3,40-di-O-methyl ether, and (S)-6-gingerol (Saifudin, Kadota, & Tezuka, 2013).

Antioxidants deactivate radicals in biological systems via two distinct mechanisms: single electron transfer (SET) and hydrogen atom transfer (HAT). Therefore, more than one type of assay can measure antioxidant activity in plant extracts (Dudonné, Vitrac, Coutière, Woillez, & Mérillon, 2009). Three different antioxidant measurement methods were carried out in this study. DPPH radicals can be neutralized by two modes of action, either by HAT or by SET mechanisms (Kedare & Singh, 2011). The CUPRAC assay's antioxidant activity is based on the reduction of copper (II) to copper (I), which is assisted by the SET mechanism (Apak et al., 2008; Özyürek, Güçlü, & Apak, 2011), while the FRAP assay is based on the fact that antioxidant compounds can reduce $Fe(TPTZ)^{3+}$ to $Fe(TPTZ)^{2+}$ (blue complex) (Benzie & Strain, 1999). The results showed that the antioxidant activities of spices detected by the CUPRAC and FRAP assay were consistently higher than those detected by the DPPH assay. It means that spices from Zingiberaceae used in this study have greater antioxidant properties in reduction capacity than free radical scavenging activity. DPPH scavenging activities in this study were found to vary from 8,72 μmol TE g-1 (*C. petiolata* Roxb) to 22,28 μmol TE g-1 (*A. malaccensis* (Burm.f.) Roscoe) with the highest were found in *Z. purpureum* Roscoe and *A. malaccensis* (Burm.f.) Roscoe. There was no significant difference in the value of DPPH scavenging activity between both spices. The highest FRAP antioxidant was recorded for *A. compactum* Sol. ex Maton (559,17 μmol/g), followed by *Z. purpureum* Roscoe (436,67 μmol/g), *A. malaccensis* (Burm.f.) Roscoe (459,17 μmol/g), *B. rotunda* L. Mansf (175 μmol/g) and *Z. aromaticum* Val. (170 μmol/g). Meanwhile, the highest CUPRAC value was recorded for *Z. purpureum* Roscoe (1177,5 μmol/g), followed by *B. rotunda* L. Mansf (850,63 μmol/g), *A. compactum* Sol. ex Maton (721,25 μmol/g), *A. malaccensis* (Burm.f.) Roscoe (673,13 μmol/g), and *C. aeruginosa* Roxb (539,38 μmol/g). It is important to note that *Z. purpureum* Roscoe had the highest amount of TFC (Table 1). Previous research identified several phenolics and flavonoids from this plant with antioxidant activity through nitric oxide generation inhibition, DPPH radical scavenging, tyrosinase inhibitory activities, which include 1-feruloyloxy cinnamic acid, and bisdemethoxycurcumin (Antony et al., 2008). Further observation on the influence of TPC and TFC of the samples to the tested bioactivities, PCA analysis, was conducted (Supplementary file Figure S1). Based on the PCA biplot dan loading plot, it can be concluded that a higher TPC value were closely related to high DPPH, FRAP, and CUPRAC antioxidant activities. High TPC value did not always positively correlate with α-glucosidase inhibitor activity which was indicated with the larger error bar in the loding plot, while TFC showed weaker correlation with all bioactivities tested. From this data, it can also be summarized that samples with high antioxidant activity were not always have high α-glucosidase inhibitor activity.

Volatile profile of spices

Volatiles analysis using SPME-GC/MS resulted in 69 identified compounds (Table 2). Research related to volatile compounds from fresh rhizomes of the Zingiberaceae family with SPME-GC/MS is minimal. Table 2 data was compared with a few previous reports found in the literature. The group of volatile profiles was qualitatively almost similar to those mentioned in previous reports, but the difference was more in the number of individual compounds. In a previous study, volatile components of the two Zingiberaceae spices, *E. cardamomum,* and *A. japonica* were analyzed by HS-SPME/GC–MS (Asakawa et al., 2017). The main volatile components of *A. japonica* consisted of fenchone, eucalyptol, and β-fenchyl acetate. In contrast, in *E. cardamomum*,

Table 1
Total phenolic content, total flavonoid content, α-glucosidase inhibitor, and antioxidant (FRAP, CUPRAC, and DPPH) activities of the spices.

No	Species	TPC (mg GAE g^{-1})	TFC (mg QE g^{-1})	AGI (%)	DPPH (μmol TE g^{-1})	FRAP (μmol TE g^{-1})	CUPRAC (μmol TE g^{-1})
1	*Curcuma aeruginosa* Roxb	36.9 ± 0.4^{g}	6.8 ± 0.7^{a}	57.8 ± 0.2^{c}	20.8 ± 0.8^{de}	247.5 ± 1.2^{g}	539.4 ± 4.4^{g}
2	*Curcuma zedoria* Roscoe	38.0 ± 0.8^{h}	7.2 ± 0.7^{a}	47.9 ± 5.7^{b}	20.6 ± 0.9^{de}	273.3 ± 2.4^{h}	311.9 ± 2.7^{e}
3	*Zingiber purpureum* Roscoe	40.0 ± 0.4^{i}	144.5 ± 15.0^{f}	58.5 ± 3.5^{cd}	22.2 ± 0.7^{e}	436.7 ± 9.4^{i}	1177.5 ± 1.8^{k}
4	*Zingiber ottensii* Val.	13.1 ± 0.0^{b}	8.0 ± 4.0^{a}	44.3 ± 4.6^{b}	10.5 ± 0.8^{b}	22.5 ± 1.2^{b}	73.1 ± 9.7^{c}
5	*Boesenbergia rotunda* L. Mansf.	17.0 ± 0.3^{d}	19.5 ± 0.9^{bc}	60.8 ± 1.8^{cd}	19.8 ± 0.5^{d}	175.0 ± 4.7^{f}	850.6 ± 11.5^{j}
6	*Curcuma heyneana* Val. & Zijp	25.1 ± 0.1^{e}	43.5 ± 1.9^{d}	48.1 ± 0.7^{b}	20.5 ± 0.6^{de}	138.3 ± 7.0^{e}	227.5 ± 1.8^{d}
7	*Curcuma purpurascens* Blume	$11.0 \pm 0,1^{a}$	77.5 ± 3.5^{c}	64.7 ± 2.0^{de}	10.8 ± 1.0^{b}	77.5 ± 3.5^{d}	346.9 ± 4.4^{f}
8	*Curcuma petiolata* Roxb	11.0 ± 0.2^{a}	5.5 ± 07^{a}	37.7 ± 1.6^{a}	8.7 ± 1.4^{a}	7.5 ± 1.2^{a}	9.4 ± 2.7^{a}
9	*Zingiber aromaticum* Val.	38.3 ± 0.1^{h}	80.1 ± 7.1^{e}	82.0 ± 3.1^{f}	21.7 ± 0.6^{e}	170.0 ± 9.4^{f}	341.3 ± 1.8^{f}
10	*Zingiber zerumpet* L. Roscoe ex Sm.	14.9 ± 0.8^{c}	8.5 ± 3.6^{a}	57.8 ± 0.9^{c}	12.5 ± 1.3^{c}	48.3 ± 2.3^{c}	61.9 ± 0.9^{b}
11	*Amomum compactum* Sol. ex Maton	41.5 ± 0.2^{j}	7.2 ± 1.4^{a}	62.1 ± 0.7^{cd}	20.9 ± 1.5^{de}	559.2 ± 3.5^{k}	721.3 ± 3.5^{i}
12	*Alpinia malaccensis* (Burm.f.) Roscoe	36.1 ± 0.1^{f}	13.0 ± 3.2^{ab}	70.2 ± 0.2^{e}	22.3 ± 0.2^{e}	459.2 ± 13.0^{j}	673.1 ± 2.7^{h}

*Different superscript letters indicate statistical differences according to Duncan's multiple comparison test at $p = 0.05$.

Table 2
Volatile compounds composition of each spices as detected by GC–MS.

No	Chemical Compound	Peak Area Percentage (%)											
		ZC	ZA	AM	CP	AC	CA	BR	ZZ	CT	CZ	CH	ZO
Monoterpenes													
1	α-Tricyclene	–	0.52	–	–	–	–	0.43	0.16	0.28	0.14	0.13	–
2	α-Pinene	2.64	5.77	11.05	4.99	2.48	2.17	1.42	2.77	1.47	0.82	4.52	3.64
3	α-Phellandrene	1.14	0.13	9.33	1.18	0.17	0.18	–	1.07	0.06	–	1.36	0.87
4	α-Fenchene	–	0.07	0.07	–	0.14	0.18	0.05	0.07	–	–	–	–
5	Camphene	0.07	17.51	0.97	2.45	0.33	2.07	7.54	4.83	7.43	3.27	2.49	0.31
6	β-Pinene	5.11	0.24	15.77	9.63	4.71	3.3	0.03	0.58	4.57	–	10.71	12.1
7	β-Phellandrene	35.32	0.03	0.43	1.14	1.14	0.78	0.03	1.41	0.11	–	0.57	**27.37**
8	3-Carene	0.01	2.59	0.08	–	0.44	0.02	0.02	6	–	–	0.08	0.07
9	(+)-3-Carene	–	1.99	–	0.11	–	–	–	–	–	–	0.1	0.02
10	D-Limonene	0.74	4.68	5.68	4.19	6.97	1.99	2.93	2.25	1.72	0.24	4.06	1.12
11	β-Myrcene	3.02	1.78	2.35	1.96	1.64	0.78	1.18	1.79	0.95	0.46	1.48	2.21
12	trans β-Ocimene	–	0.25	1.8	0.09	0.03	0.06	4.96	–	–	0.12	0.05	0.15
13	γ-Terpinene	12.09	0.16	0.25	0.16	0.82	0.4	0.35	2.16	0.09	–	0.14	6.19
14	β-Ocimene	0.12	0.09	–	0.08	0.05	0.13	42.07	0.05	0.06	–	0.19	–
15	(Z)-β-Ocimene	0.12	–	2.01	0.04	–	–	–	–	–	0.21	–	1.5
16	α-Terpinolene	–	–	0.28	–	0.41	0.4	0.41	0.28	0.14	–	–	–
17	Fenchone	–	–	4.25	–	6.35	9.0	–	0.08	–	–	–	–
18	Thujone	–	–	–	0.04	–	0.05	–	–	0.05	–	0.03	0.06
19	4-Thujanol	–	–	–	–	0.29	0.42	–	0.17	–	–	–	0.16
20	Linalool	–	10.51	0.18	2.2	1.1	0.22	0.55	2.43	0.18	2	3.68	0.13
21	Pinocarvone	–	–	–	–	0.18	0.07	–	–	–	–	–	0.03
22	Bornyl acetate	0.02	1.14	–	0.1	–	0.08	–	0.48	–	1.68	0.03	0.23
23	Isobornyl acetate	–	0.07	–	0.05	–	–	–	0.04	–	–	0.13	–
24	Fenchol	–	–	0.22	–	0.12	0.17	–	–	–	–	–	–
25	Isoborneol	–	–	–	1.36	–	2.04	–	–	4.33	–	1.79	–
26	Neral	–	–	0.04	–	–	–	0.1	–	–	–	–	0.01
27	α-Terpineol	0.34	0.11	0.53	0.31	0.5	0.77	0.13	0.13	0.11	–	0.54	0.31
Monoterpenoid													
28	Eucalyptol	0.29	4.85	17.26	29.52	42.04	29.95	8.28	5.03	2.14	0.54	33.74	3.04
29	p-Menth-2-en-1-ol	0.42	–	–	–	–	–	–	0.08	–	–	–	0.15
30	*cis*-2-Norbornanol	–	–	0.03	–	0.02	0.03	–	0.02	–	–	–	–
31	*trans*-p-Mentha-2.8-dienol	–	–	–	–	0.05	0.09	–	–	–	–	0.03	–
Terpenes													
32	o-Cymene	3.54	0.87	8.05	0.69	5.18	0.13	0.27	2.81	0.09	–	0.73	1.62
33	Terpinen-4-ol	15.94	0.2	0.44	0.51	0.15	0.41	0.2	2.08	0.4	–	0.68	0.2
34	*endo*-Borneol	0.01	0.84	0.06	0.49	0.03	0.63	0.09	0.46	1.25	0.35	0.62	0.09
Alkanes													
35	(+)-4-Carene	6.2	0.27	0.16	0.06	0.14	0.13	0.11	0.77	0.23	–	0.06	2.57
36	Hexadecane	–	–	–	–	–	–	–	–	–	60.43	–	–
Sesquiterpenes													
37	α-Cubebene	–	–	0.03	0.28	0.17	0.43	–	–	0.04	–	0.28	–
38	ϒ-Muurolene	0.02	–	0.27	0.4	0.02	0.27	–	0.02	0.35	0.06	0.17	0.12
39	Copaene	–	–	–	0.05	0.49	0.04	–	0.12	0.11	–	–	0.13
40	α-Guaiene	0.02	–	–	2.4	0.15	0.14	–	–	2.63	0.24	0.09	0.06
41	α-Guajene	–	–	–	–	–	–	–	–	0.21	0.37	0.12	–
42	ϒ-Elemene	–	–	–	–	–	0.29	–	–	0.03	–	0.07	0.01
43	β-Elemene	–	0.02	0.19	–	–	0.06	–	0.04	0.14	–	0.56	–
44	Caryophyllene	0.05	2.34	0.22	3.32	0.33	2.52	–	4.92	1.27	–	3.22	1.62
45	α-Muurolene	–	–	–	0.02	–	–	–	–	0.1	0.36	–	–
46	Alloaromadendrene	–	–	–	0.1	0.04	0.05	–	–	–	–	0.13	–
47	β-Guaiene	–	–	–	0.26	–	0.75	–	–	0.12	–	–	0.26
48	isoledene	–	–	0.05	0.57	–	0.65	–	–	0.23	–	0.06	–
49	(E)-β-Famesene	0.64	–	0.03	–	–	0.48	–	–	–	–	–	–
50	Humulene	–	14.27	0.26	1.71	0.05	0.04	–	30.96	–	–	–	11.52
51	7-*epi*-α-selinene	–	–	–	–	0.22	–	–	–	0.59	–	0.16	–
52	1.4-Cadinadiene	–	–	–	–	0.57	–	–	0.06	0.25	–	–	–
53	Germacrene D	–	–	0.09	1.24	0.03	4.6	–	–	3.56	0.03	0.25	0.04
54	4.beta.H.11-diene	–	–	–	–	–	–	–	–	1.32	0.06	0.22	–
55	β-Bisabolene	0.04	–	0.05	–	0.08	0.07	–	–	–	–	–	–
56	(-)-β-Cadinene	–	–	0.16	–	0.57	–	–	–	1.7	–	0.32	–
Sesquiterpenoid													
57	3.7(11)-Eudesmadiene	–	–	–	0.03	–	0.05	–	–	0.12	–	0.02	–
58	*trans*-α-Bergamotene	0.1	–	0.69	–	0.01	–	0.05	0.51	–	0.05	–	–
59	(-)-Aristolene	–	–	–	0.88	–	0.05	–	–	0.28	0.21	–	–
Alcohols													
60	2-Heptanol	–	–	–	0.43	–	–	–	–	0.47	–	0.73	–
61	2-Octanol	–	–	–	0.14	–	0.03	–	–	0.18	–	0.22	–
62	2-Nonanol. acetate	–	–	–	0.12	–	0.02	–	–	–	–	0.16	0.01
63	2-Decanol	–	–	–	0.04	–	0.04	–	–	–	–	0.08	–
64	2-Tridecanol	–	–	–	0.16	0.03	–	–	–	–	–	0.22	–
Aldehyde													
65	α-Campholenal	–	0.04	–	0.14	–	–	–	–	–	–	0.12	–

(*continued on next page*)

Table 2 (*continued*)

No	Chemical Compound	Peak Area Percentage (%)											
		ZC	ZA	AM	CP	AC	CA	BR	ZZ	CT	CZ	CH	ZO
Ketones													
66	2-Nonanone	–	–	–	0.84	0.05	0.62	–	–	0.72	–	1.31	0.02
67	2-Decanone	–	–	–	0.04	–	0.06	–	–	0.08	–	0.07	–
68	(+)-2-Bornanone	–	1.75	0.56	3.16	0.43	7.39	5.95	0.81	13.84	1.81	5.21	–

eucalyptol was also the most eminent one, followed by β-fenchyl acetate, and α-fenchene Another study that also explored volatile compounds of *A. zerumbet* leaves extract using HS-SPME/GC–MS indicated that the major compound of this species is eucalyptol, followed by p-cymene and humulene (Chen et al., 2014).

The distribution of volatile compound groups of each spice used is summarized in Fig. 1A. It can be seen that monoterpenes were the predominant volatiles in *Z. purpureum, A. malaccensis, Z. ottensii,* and *B. rotunda*. Alkanes were the highest in *C. zedoria* and *C. petiolata*, while sesquiterpenes were the highest in *Z. zerumpet*. Other compounds such as sesquiterpenoids, alcohols, aldehydes, and ketones were found in relatively lower amounts in all spices. PCA was also conducted to obtain an overview of the 12 spices classification based on the differences and similarities of their volatiles profiles (Fig. 1B and 1C). The unsupervised multivariate data analysis, such as PCA, is used as the first step quality assessment of the multivariate model. When the PCA showed a reasonable pattern and the value of R^2Y and Q^2 was higher than 0.4, it indicated the model's reliability, and further analysis using other multivariate data analysis methods can be conducted (Worley & Powers, 2016). PCA of the volatiles data resulted in 5 components (PCs) which explain 89.8% of the total variation. In the PCA score plot of the first two components (PC1 = 32.1%, PC2 = 26.7%), at least 6 clusters were observed (Fig. 1B). *C. zedoria, C. petiolata, Z. aromaticum, and Z. purpureum* were clustered separately, while the other eight spices were in the same group. It indicated that the eight spices (*Z. ottensii, C. aeruginosa, C. purparescens, A. malaccensis, B. rotunda,* and *C. heyneana*) had almost similar volatiles composition. From the loading plot (Fig. 1C), it can be observed that these spices were predominated by several markers, which include fenchone, α-terpinolene, α -terpinene, 2-heptanol, 2-nonanone, eucalyptol, and β-ocimene. *C. zedoria* was characterized by volatiles such linalool, α-guaiene, (+)-2-bornanone, hexadecane, and 2-decanone. Discriminating volatiles for *C. petiolata* and *Z. aromaticum* were bornyl actetate and *cis*-2 norbornanol, respectively. *Z. zerumpet* was discriminated from others as a result of predominant α-terpineol, isoborneol, neral, humulene, and (E)-β-famesene. Lastly, discriminating volatiles for *Z. purpureum* included β-guaiende, 3-carene, pinocarvone, thujone, α-cubebene, and β-myrcene.

Orthogonal projection to the least square analysis

Next, we used another multivariate data analysis method, OPLS, to correlate the volatiles profile of the spices with the bioactivity tested. The aims were to discover volatiles strongly associated with the respective activity. In this multivariate model, sample classification is not based on differences or similarities in volatiles profile but rather on the bioactivity value input as a response variable. We first conducted OPLS to identify compounds responsible for α-glucosidase inhibitor activity. The OPLS score plot, S-plot, the result of permutation test to validate the model, Y-related profile plot, and VIP plot are presented in Fig. 2A–2E, respectively. This OPLS had excellent performance with R^2Y = 0.9 and Q^2Y = 0.7. Model validation using permutation test at 100 permutations resulted in the excellent performance in which the value of R^2Y and Q^2Y of permutated models (green circles and blue squares located in the left lower part of the plot, respectively) were always lower than the original model (green circle and blue square located in the right upper part of the plot, respectively) (Fig. 2C) (Eriksson et al., 2013).

OPLS score plot showed a clear classification between spices with low, medium, and high α-glucosidase inhibitor activity (Fig. 2A). The

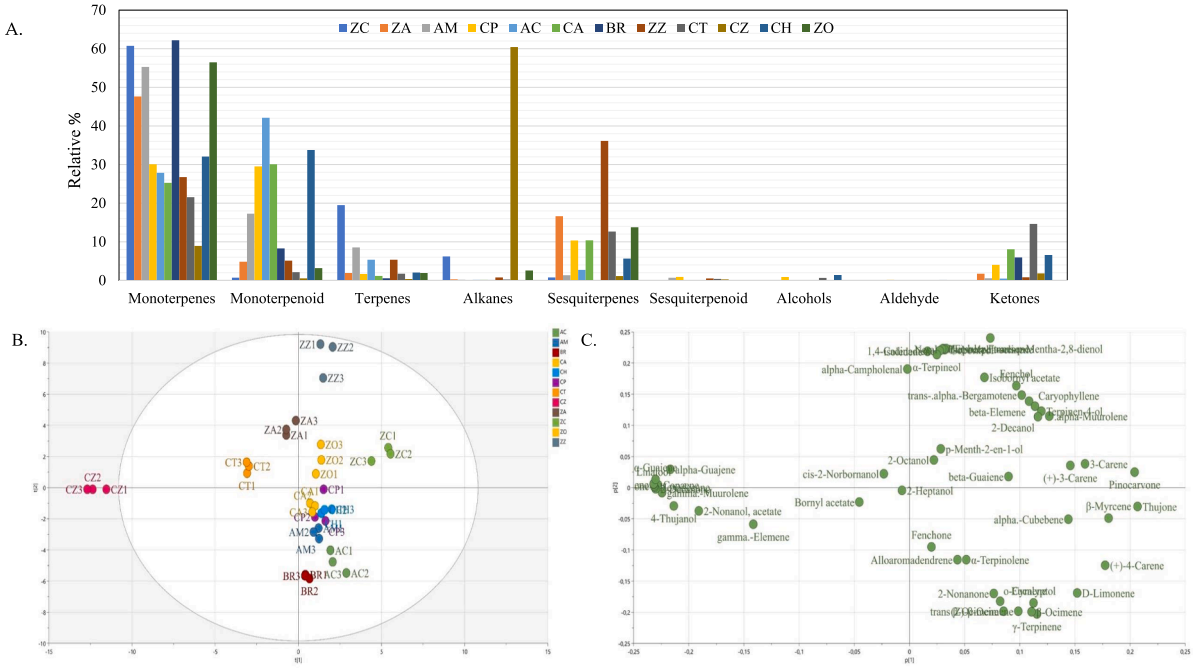

Fig. 1. A. Distribution of the group of volatile compounds in the tested spices B. PCA score plot C. PCA loading plot of the 12 spices' volatiles data obtained from GC–MS measurement (R^2X = 0.89 and Q^2Y = 0.82).

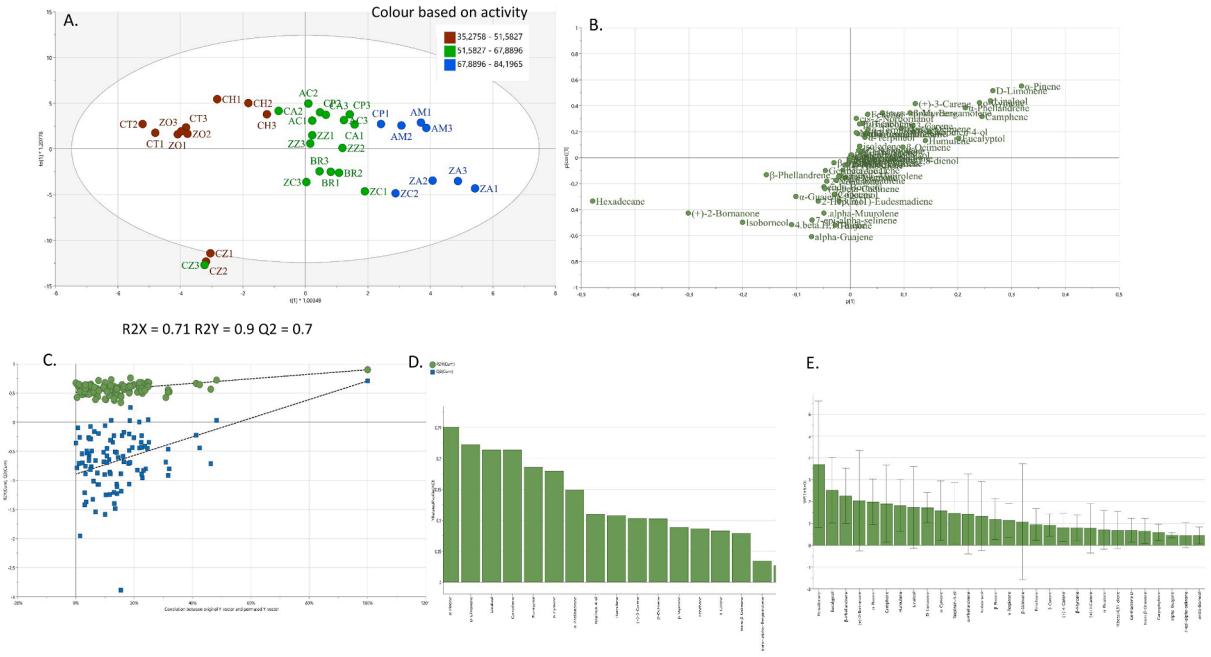

Fig. 2. OPLS Analysis to analyze α-glucosidase inhibitor compounds ($R^2X = 0.71$, $R^2Y = 0.9$ $Q^2Y = 0.7$). A. OPLS score plot showed samples classification based on the α-glucosidase inhibitor activity. Color differences from red, green, and blue represent lower (35%–50% inhibition), medium (51%–67% inhibition), and high (68%–84% inhibition) α-glucosidase inhibitor activity B. OPLS S-plot to determine volatiles associated with the α-glucosidase inhibitor activity C. Permutation test plot D. Y-related profile plot, only some volatiles with the highest positive value was shown E. VIP plot, only volatiles with VIP value higher than 0.9 and the error bars did not touch the x-axis were selected. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

strongest discriminating volatiles among the most active and the least active group can be identified from the S-pot (Fig. 2B). Volatiles, which are the markers for the active groups, were located in the corresponding position with the score plot on the right side of the S-plot. Thus, it can be summarized that α-pinene, D-limonene, linalool, and o-cymene were the compounds that positively correlated with the α-glucosidase inhibitor activity. Another way to quantitatively identify these active compounds is to use the VIP value and Y-related profile value, which can be identified from the Y-related profile plot (Fig. 2D) and VIP plot (Fig. 2E). In this case, the higher α-glucosidase inhibitor activity was pointed by the higher percentage value, which means we had to focus on the volatiles which had high and positive Y-related profile value and high VIP value (VIP always had a positive value, in this study only volatiles with VIP value higher than 0.9 and the error bars did not touch the axis were considered as significant).

With the same OPLS analysis protocol, compounds strongly correlated with antioxidant activity (DPPH, FRAP, and CUPRAC) were identified and summarized in Table 3. The respective OPLS score, S-Plot, permutation, Y-related, and VIP plots for identifying compounds with strong correlations with DPPH, FRAP, and CUPRAC analysis were available as supplementary data (Figure S2, Figure S3, and Figure S4, respectively). It is notable that several compounds consistently appeared to have a strong correlation with all tested bioactivity but with different levels of VIP value. For example, eucalyptol was the strongest correlation with α-glucosidase inhibitor, DPPH, and CUPRAC antioxidant, respectively. The same case for α-phellandrene and α-pinene. o-Cymene was strongly correlated with the four tested bioactivities with the highest correlation with FRAP antioxidant, followed by α-glucosidase inhibitor, CUPRAC antioxidant, and DPPH antioxidant. Fenchone also strongly correlated with all activities with FRAP, DPPH, CUPRAC antioxidant, and α-glucosidase inhibitor, respectively. Conversely, several compounds only showed a strong correlation with one activity. For example, camphene, humulene, and linalool only positively correlated with α-glucosidase inhibitor activity. Similarly, γ-terpinene, (+)-4-carene, and *trans*-β-ocimene were associated with CUPRAC antioxidants.

Despite limited information on the antidiabetic and antioxidant data

Table 3
Summary of volatile compounds with significant positive correlation with α-glucosidase inhibitor and antioxidant activities of the spices.

No.	Compounds	Group	Activity	VIP
1.	Eucalyptol	Monoterpenoid	α-glucosidase inhibitor	2.5
2.	α-Phellandrene	Monoterpene		2.3
3.	α-Pinene	Monoterpene		2.0
4.	Camphene	Monoterpene		1,9
5.	Humulene	Alkane		1.8
6.	D-Limonene	Monoterpene		1.7
7.	Linalool	Monoterpene		1.7
8.	o-Cymene	Terpene		1.6
9.	Terpinen-4-ol	Terpene		1.4
10.	Fenchone	Monoterpene		0.9
No.	Compounds	Group	Activity	VIP
1.	Eucalyptol	Monoterpenoid	Antioxidant (DPPH)	2.9
2.	Fenchone	Monoterpene		1.6
3.	Terpinen-4-ol	Terpene		1.5
4.	o-Cymene	Terpene		1.3
5.	α-Phellandrene	Monoterpene		1.2
6.	D-Limonene	Monoterpene		1.1
7.	α-Pinene	Monoterpene		1.1
No.	Compounds	Group	Activity	VIP
1.	o-Cymene	Terpene	Antioxidant (FRAP)	2.1
2.	Fenchone	Monoterpene		1.9
3.	β-Pinene	Monoterpene		1.4
4.	D-Limonene	Monoterpene		1.3
5.	α-Phellandrene	Monoterpene		1.2
6.	α-Pinene	Monoterpene		0.9
No.	Compounds	Group	Activity	VIP
1.	Terpinen-4-ol	Terpene		3.4
2.	γ-Terpinene	Monoterpene	Antioxidant (CUPRAC)	1.7
3.	Eucalyptol	Monoterpenoid		2.1
4.	o-Cymene	Terpene		1.3
5.	(+)-4-Carene	Alkane		1.2
6.	Fenchone	Monoterpene		1.1
7.	trans β-Ocimene	Monoterpene		1.0

of these volatiles, the bioactivity given by the OPLS model agreed with some previous research on similar bioactivity of volatiles compounds found in different plants. α-Pinene was previously reported to have mild hypoglycemic activity in diabetic mice (Özbek & Yılmaz, 2017). Eucalyptol found in rosemary essential oil was shown to have medium DPPH antioxidant activity (Nie et al., 2020). Terpinen-4-ol identified in sweet ginger (*Alpinia coriandriodora* D. Fang) essential oil was reported to have strong DPPH antioxidant activity followed by eucalyptol and α-phellandrene (Dong et al., 2020). Most studies on antidiabetic and antioxidative herbs and spices were conducted using essential oils. Therefore, molecular docking analysis was conducted to support further the results given by the multivariate data analysis. For α-glucosidase inhibitor activity, although all ten compounds had binding energy close to Acarbose as the native ligand, the order of compounds based on the activity was slightly different from those obtained from OPLS modeling (Table 4). Based on this molecular docking analysis, the most active volatiles was humulene, followed by terpinen-4-ol, fenchone, and eucalyptol.

This study provided important basic information that can be used to consider when these minor spices will be developed as functional food. However, this study is still preliminary. Many factors may influence the composition of phytochemicals of spices, thus their health benefits, for example, soil condition, climate, storage, and processing methods. A recent study reported that Chinese prickly ash peels (*Zanthoxylum bungeanum* Maxim.) collected from 26 locations in eight Chinese provinces had different volatile compositions, which impacted their aroma description. Peels from the southwest and northwest regions have an aromatic flavor due to higher levels of limonene and linalool. In contrast, samples from the North, East, and Central China have a spicy flavor due to higher levels of β-myrcene and (E)-ocimene (Zheng et al., 2021). A decrease in volatile compounds of red pepper flake during 3 months was reported in another study (Korkmaz, Atasoy, & Hayaloglu, 2020). These volatiles composition differences may affect the medicinal properties of the studied spices, although these two studies did not discuss this topic. Diverses effect of boiling to several obesity-related bioactivities of ten spices from Srilanka has been studied (Fernando et al., 2019), but in contrast with previous studies, the changes in volatiles profile was not reported. Depending on spices type, boiling was shown to increase, decrease, or not affect lipase inhibitor, amylase inhibitor, glucosidase inhibitor, and antioxidant activity of the studied spices. These previously reported data should be considered in designing future research direction for these twelve Zingiberaceae spices. Another important point is the effective dose when the spices are consumed at normal daily use. Our study was conducted in vitro, to investigate whether similar effects will be obtained in a more complex system such as in the human body needs further investigation.

The molecular interaction between the active compounds and the enzyme was analyzed further using ligplot software (EMBL-EBI, Cambridgeshire, UK). It was found that the standard drug, Acarbose, formed a hydrogen bond with Arg1510 and His1584 of the enzyme site. Interaction between the enzyme and Acarbose was also strengthened by hydrophobic interaction between Acarbose and Asp1157, Tyr1251, Asp1279, Ile1280. Acarbose was previously reported as a competitive inhibitor (Ren et al., 2011). This ability was also verified using reaction kinetics of enzyme and docking analysis (Proença et al., 2017; Syabana et al., 2022). In our study, the output of the ligplot showed that all tested ligands bound to the enzyme mostly through the hydrophobic interaction. Only linaool, terpinen-4-ol, and fenchone had both H-bond and hydrophobic interaction with the enzyme. All the tested ligands are bound to the same amino acids of enzyme site as those of Acarbose, which indicated that those tested ligands also attached to the active site of the enzyme. A study on the enzyme's kinetic reaction is required to confirm this.

Table 4
The results of molecular docking analysis of the potential active compounds using α-glucosidase as protein receptor.

No.	Compounds	Binding energy (kcal/mol)	Constant inhibitor (μM)	H-bond	Hydrophobic interaction
1.	Eucalyptol	−5.85	53.63	–	Tyr1251, Asp1279, Ile1280, Ile1315, Trp1355, Trp1418, Asp1420, Met1421, Arg1510, Asp1526, Phe1559, His1584
2.	α-Phellandrene	−5.75	61.04	–	Tyr1251, Asp1279, Ile1280, Ile1315, Trp1355, Trp1418, Asp1420, Met1421, Trp1523, Asp1526, Phe1559, His1584
3.	α-Pinene	−5.75	61.23	–	Tyr1251, Asp1279, Ile1315, Trp1355, Trp1418, Asp1420, Met1421, Arg1510, Trp1523, Asp1526, Phe1559, His1584
4.	Camphene	−5.67	69.53	–	Tyr1251, Asp1279, Ile1315, Trp1418, Asp1420, Met1421, Arg1510, Trp1523, Asp1526, Phe1559, His1584
5.	Humulene	−6.99	7.52	–	Asp1157, Tyr1251, Asp1279, Ile1315, Trp1355, Trp1418, Asp1420, Met1421, Arg1510, Trp1523, Asp1526, Phe1559, Tyr1560, His1584
6.	D-Limonene	−5.62	76.39	–	Tyr1251, Asp1279, Ile1280, Ile1315, Trp1355, Trp1418, Asp1420, Met1421, Trp1523,

(*continued on next page*)

Table 4 (*continued*)

No.	Compounds	Binding energy (kcal/mol)	Constant inhibitor (μM)	H-bond	Hydrophobic interaction
7.	Linalool	−5.21	152.38	Asp1279, His1584	Asp1526, Phe1559, His1584 Tyr1251, Ile1315, Trp1355, Trp1418, Asp1420, Met1421, Asp1526, Phe1559, Phe1560, Thr1586
8.	o-Cymene	−5.06	195.65		Tyr1251, Asp1279, Ile1280, Ile1315, Trp355, Trp1418, Asp1420, Arg1510, Trp1523, Asp1526, Phe1559, His1584
9.	Terpinen-4-ol	−6.26	25.78	Asp1279, His1584	Tyr1251, Ile1280, Ile1315, Trp1355, Trp1418, Asp1420, Met1421, Arg1510, Trp1523, Asp1526, Phe1559
10.	Fenchone	−6.13	32.38	His1584	Tyr1251, Asp1279, Ile1315, Trp1418, Asp1420, Met1421, Arg1510, Trp1523, Asp1526, Phe1559
11.	Acarbose	−10.11	0.039	Arg1510, His1584	Asp1157, Tyr1251, Asp1279, Ile1280, Trp1355, Trp1418, Asp1420, Met1421, Trp1523, Asp1526, Phe1559

Conclusions

In this study, volatile compounds associated with antidiabetes and antioxidant activity of 12 minor Zingiberaceae spices were succesfully identified using SPME-GC/MS combined with OPLS analysis. Eucalyptol was identified as the compound with the strongest correlation with AGI and DPPH antioxidant activities. o-Cymene and terpinen-4-ol had the strongest correlation with FRAP and CUPRAC antioxidant activities, respectively. The result of antidiabetic compounds identification was in line with the results of molecular docking analysis with α-glucosidase as a protein target. Ten compounds that showed strong association with AGI activity in OPLS analysis, also had binding energy close to Acarbose as AGI reference drug. This study provided important data on the volatile compounds found in underutilized spices that are linked to their health-promoting properties. Information on the compound responsible for the bioactivity is critical to ensure quality consistency when these spices are developed as a functional food. However, the results of this study need to be confirmed by in vivo or clinical studies, not only to determine the effective dose but also the toxicity, since excessive spices consumption can pose a health risk. There is also a need for research on the numerous aspects that influence the variability of bioactive chemicals in spices, such as geographical origin, post-harvest, and processing methods.

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CRediT authorship contribution statement

Fitra Tunnisa: Methodology, Investigation, Formal analysis, Writing – original draft. **Didah Nur Faridah:** Supervision, Writing – review & editing. **Ani Afriyanti:** Investigation, Writing – review & editing. **Dian Rosalina:** Investigation, Writing – review & editing. **Mohamad Ana Syabana:** Visualization, Software, Writing – review & editing. **Noviyan Darmawan:** Project administration, Writing – review & editing. **Nancy Dewi Yuliana:** Conceptualization, Funding acquisition, Supervision, 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2022.100285. This study is available at the NIH Common Fund's National Metabolomics Data Repository (NMDR) website, the Metabolomics Workbench, https://www.metabolomicsworkbench.org where it has been assigned Study ID ST002102. The data is temporarily available at: http://dev.metabolomicsworkbench.org:22222/data/DRCCMetadata.php?Mode=Study&StudyID=ST002102&Access=OlmD4720. The DOI for this study is: https://doi.org/10.21228/M8P427. The study is scheduled to be released on 2023-02-24 (YYYY-MM-DD)

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