Analysis of Secondary Metabolites in *Citrus swinglei* Burkill ex Harms (Jeruk Kunci) Essential Oil and Determination of α-Glucosidase Inhibition Activity

Rechia Radatul Putri[1], Armita Harahap[1], Valdy Filando Sardi[1], Rustini[1], and Friardi Ismed[1,2*]

[1]Faculty of Pharmacy
[2]Laboratory of Natural Resources of Sumatra (LBS)
Universitas Andalas
Padang 25163, Indonesia
**friardi@phar.unand.ac.id*

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Abstract

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder associated with persistent hyperglycemia. Inhibitors of α-glucosidase play a crucial role in controlling postprandial blood glucose, but the use of synthetic agents such as acarbose is often limited by gastrointestinal side effects. This study aimed to investigate the secondary metabolite profile of jeruk kunci (Citrus swinglei Burkill ex Harms) essential oil and to evaluate its inhibitory activity against α-glucosidase. C. swinglei essential oil was extracted by steam distillation, yielding 0.135% (w/w) of a colorless oil with a characteristic aroma. The chemical composition was analyzed by gas chromatography-mass spectrometry (GC-MS), while thin-layer chromatography (TLC) combined with bioautography was used for qualitative enzyme inhibition screening. The inhibitory activity of the essential oil was quantitatively determined using a microplate reader assay. GC-MS analysis identified 12 major volatile compounds dominated by monoterpenes, particularly bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl), α-Pinene, β-Pinene, β-myrcene, 1,3-cyclohexadiene, 1-methyl-4-(1-methylethyl), ρ-cymene, D-limonene, γ-terpinene, cyclohexene, 1-methyl-4-(1-methylethylidene), linalool, terpinen-4-ol, and α-terpineol. TLC-bioautography confirmed the presence of active metabolites, indicated by white inhibition zones. The essential oil exhibited significant α-glucosidase inhibition with an IC_{50} value of 230±0.28 µg/mL. These results support the use of Southeast Asian citrus species as candidates for the development of plant-based antidiabetic agents.

***Keywords**:* α-glucosidase inhibition, *Citrus swinglei*, essential oil, GC-MS, TLC-bioautography

1. Introduction

Citrus species (*Citrus* sp.), members of the *Rutaceae* family, represent one of the most economically important fruit crops worldwide due to their broad use in the food, pharmaceutical, and cosmetic industries (Divyasakthi *et al*., 2025). Beyond their fresh fruit value, citrus peels and essential oils are rich in secondary metabolites, including flavonoids, alkaloids, limonoids, coumarins, carotenoids, and phenolic acids, that are known to contribute to diverse biological activities, such as antioxidant, anti-inflammatory, antimicrobial, cardioprotective, and antidiabetic properties (Jacob *et al*., 2023).

Type 2 diabetes mellitus (T2DM) is a major global metabolic disorder characterized by chronic hyperglycemia resulting from impaired insulin secretion or action. Persistent hyperglycemia contributes to oxidative stress through excessive formation of reactive oxygen species (ROS), increasing the risk of both macrovascular and microvascular complications (Chen *et al*., 2025).

Among current therapeutic strategies, α-glucosidase inhibition is a key approach for controlling postprandial hyperglycemia. By blocking the enzymatic hydrolysis of dextrins into glucose in the small intestine, α-glucosidase inhibitors effectively delay carbohydrate absorption and reduce postprandial glucose excursions (Zhang *et al*., 2019). Synthetic agents such as acarbose, metformin, thiazolidinediones, and sulfonylureas have been widely utilized, but their clinical benefits are often limited by gastrointestinal side effects and long-term tolerability issues (Susilawati *et al*., 2023).

To explore potential sources of α-glucosidase inhibitors, the citrus species *Citrus swinglei* Burkill ex Harms (Jeruk kunci in Southeast Asia) represents a promising yet understudied candidate. The species contains a terpenoid-rich essential oil containing limonene, known for its antioxidant, antimicrobial, and potential antidiabetic activities (Elias *et al*., 2020). Although traditionally used for inflammatory respiratory conditions, scientific data on its secondary metabolite profile and α-glucosidase inhibitory potential remain scarce.

Monoterpenes present in *Citrus* sp. essential oil exert α-glucosidase inhibition through both active site competition and allosteric modulation. Competitive inhibition arises when metabolites occupy the enzyme's catalytic pocket, preventing substrate binding. In contrast, allosteric interactions induce structural alterations that decrease enzymatic affinity and catalytic turnover.

By inhibiting the hydrolysis of dietary carbohydrates, these metabolites delay glucose release in the small intestine and reduce postprandial glycemic spikes. Such dual inhibitory behavior is consistent with the mechanistic models described in Gupta & Sundaram (2022) and El Aboubi *et al*. (2023).

Given the importance of improving diabetes management through safer, natural therapeutic approaches and the great potential of unexplored local biological resources, this study aims to analyze the secondary metabolites in the essential oil of *C. swinglei* and to determine its inhibitory activity against α-glucosidase as a natural antidiabetic agent. It is hoped that this study will pave the way for the utilization of local citrus species as effective and sustainable raw materials for phytopharmaceuticals.

2. Methodology

2.1 Materials and Apparatus

The study sample comprised ±4 kg of fresh, physiologically mature jeruk kunci (*C. swinglei*) fruits with bright citrus skin and a strong, distinctive smell. To preserve the fruit's physical integrity, harvesting was conducted by hand. All the samples came from plantations in Mangun Jayo, Tebo Tengah District, Tebo Regency, Jambi Province, which is one of the species' natural development centers.

The essential oil was extracted using steam distillation equipment, and additional apparatus included thin layer chromatography (TLC) plates (Merck), a sonicator (Bransonic), micro pipettes and tips (Eppendorf), a microplate reader (ALLSHENG Flex 200), 96-well microplates, a pH meter (Nest), and a GC-MS/MS QP2010 Ultra device (Agilent 7890B/MSD 5977 A, Agilent Technology, Inc., Santa Clara, California, United States). The chemicals used were diethyl ether (Merck), toluene (Merck), formic acid (Merck), methanol (Merck), distilled water, dimethyl sulfoxide (Merck), acetic acid (Merck), silica gel plates 60 F254 (Merck), anisaldehyde-sulfuric acid reagent, citrate reagent, anhydrous sodium sulfate (Merck), 2-naphthyl-α-D-glucopyranoside (Sigma), sodium acetate, (Merck) Fast Blue B salt (Merck), α-glucosidase from *Saccharomyces cerevisiae* (Sigma), and acarbose (Sigma).

2.2 Plant Identification

Plant identification in this study was conducted at the ANDA Herbarium, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang. The plant samples were confirmed as *C. swinglei* (*Rutaceae*).

2.3 Material Processing

Fresh fruits of *C. swinglei* (4000 g) were cleaned to remove adhering debris, sorted, and thoroughly washed with water. The fruits were then chopped into small pieces to increase surface area and improve solvent penetration, thereby facilitating the extraction of essential oils and associated chemical constituents.

2.4 Essential Oil Extraction

Essential oil extraction was performed by steam distillation, following the method of Hien *et al*. (2022) with minor modifications. A total of 4000 g of sorted *C. swinglei* fruits were placed in a 1000 mL round-bottom flask, while 1500 mL of distilled water was added to a separate flask serving as the steam generator. The water flask was heated under thermal insulation to ensure a stable steam flow. Volatile compounds were carried by steam through the condenser and were collected as a distillate. Distillation was conducted for 6 h after the appearance of the first condensate drop and was terminated once the essential oil volume reached a constant value.

The resulting oil-water distillate was separated using a separating funnel. The oil layer was dried with anhydrous sodium sulfate (Na_2SO_4) to remove residual moisture, then filtered under gravity to yield a clear, dehydrated essential oil. The purified oil was stored in the dark in foil-wrapped containers to protect it from photodegradation.

2.5 Metabolite Profile Analysis of C. swinglei Essential Oil

The phytochemical components of *C. swinglei* essential oil were identified by GC-MS using a modified published method (Amawi & Ashmawy., 2026). The helium inlet mode was splitless, with a heater temperature ranging from 0 to 325 °C. The GC oven conditions comprised an initial temperature of 40°C and a 1-minute waiting time.

2.6 α-Glucosidase Inhibitory Activity Assay of C. swinglei Essential Oil

2.6.1 TLC-bioautography Assay

A thin-layer chromatography (TLC) plate (silica gel 60 F254) was spotted with the essential oil solution (1 µg/mL in ethyl acetate) and developed with a mobile phase of toluene, diethyl ether, and formic acid in the ratio 32:8:8 (v/v/v). Next, bioautography screening was performed based on the method proposed by Simões-Pires *et al*. (2009), with modifications involving the use of α-glucosidase enzyme (30 U) dissolved in buffer solution (100 mL, prepared by mixing 10.25 g of sodium acetate in 250 mL of water, then adding 0.1 M acetic acid to adjust to pH 7.5). The solution was then cooled to 4 °C before proceeding to the testing stage. The TLC plate was then dipped into the enzyme solution and incubated for 60 minutes in a box containing a small amount of water. Ensuring no direct contact between the TLC plate and the water, the box was tightly sealed to maintain humidity.

After the incubation process was complete, the plate was dipped into a substrate reagent, designed to detect enzyme inhibitors, consisting of 2-naphthyl-α-D-glucopyranoside (2 mg/mL in ethanol) and fast blue salt (2.5 mg/mL in aquabidest) mixed in a 1:1 ratio. The solution was then sprayed onto the TLC plate; a bright color indicates enzyme inhibition activity, and a dark color indicates no inhibition after a reaction time of 5−10 minutes.

2.6.2 α-Glucosidase Inhibitor Activity Assay

The test to measure α-glucosidase inhibition activity was conducted according to the method described by Yang *et al*. (2015), with some adjustments. The test was carried out in a 96-well microplate using a phosphate buffer solution at pH 6.8 and 37ºC. *C. swinglei* essential oil and the positive control acarbose, dissolved in 10% DMSO and phosphate buffer at pH 6.8, were added to each well (50 µL per well). Additionally, each well contained 100 µL of α-glucosidase enzyme solution at 0.1 U/mL. This reaction mixture was then incubated for 10 min, after which p-nitrophenyl-α-D-glucopyranose solution (50 µL, 5 mM) was added. These components were prepared in 0.1 M phosphate buffer at pH 6.8. After an additional 5-minute incubation, absorbance was measured at 405 nm to assess enzyme activity. All experimental conditions were carefully performed in triplicate to ensure data consistency and reproducibility (Yang *et al*., 2015). Equation 1 provides the quantitative basis for assessing the inhibitory action.

$$Inhibition(\%) = \frac{A-B}{A} x100 \quad (1)$$

where A represents the absorbance without the sample; B is the sample absorbance test

3. Results and Discussion

3.1 Essential Oil Extraction from C. swinglei

Citrus essential oil is a volatile fraction primarily obtained from the outer peel, or flavedo. Citrus peel contains many specialized secretory cavities (oil glands), making it a primary source of aromatic components such as limonene, linalool, citral, and β-pinene. The anatomical structure of citrus peel makes it richer in essential oils than other parts of the fruit (Nuryandani *et al*., 2024).

Meanwhile, citrus fruit juice is predominantly composed of water, sugar, citric acid, vitamins, and other non-volatile compounds. The content of essential oil in the fruit pulp is relatively low because the essential oil storage tissue does not develop in this part. Therefore, if the extraction process is performed on whole citrus fruits, including the peel and pulp, the relative yield of essential oil will be lower (Ayub *et al*., 2025).

In this study, essential oil extraction was performed using 4,000 g of fresh *C. swinglei* fruit. Steam distillation of the plant material produced approximately 5.4 mL of essential oil. This 0.135% yield is lower than yields reported for other citrus species, such as *C. polyandra* (0.2%), *C. sinensis* (0.2%), *C. lemon* (0.27%) (Elias *et al*., 2020), likely due to the use of the whole citrus fruit and the distillation method used. Unlike hydrodistillation, steam distillation uses hot steam that flows through the plant parts, carrying the volatile compounds, which are then recondensed into a two-phase liquid consisting of essential oil and water (hydrosol) (Řebíčková *et al*., 2020).

3.2 Thin-layer Chromatography-bioautography

Thin-layer chromatography (TLC) analysis of *C. swinglei* essential oil was performed using silica gel 60 F254 as the stationary phase and a toluene:diethyl ether:formic acid (32:8:8) mobile phase resulted in clear component separation. Visualization under UV light at 365 nm revealed a

single fluorescent spot (Figure 1b) with an Rf value of 0.45, while most components were not detectable at 254 nm (Figure 1a). Following derivatization with anisaldehyde-sulfuric acid (ANS), the chromatographic spots became clearly visible with distinct color variations, indicating the predominance of non-aromatic and semi-aromatic secondary metabolites, particularly terpenoid compounds.

The specific color responses observed after ANS spraying reflect the presence of carbonyl functional groups and conjugated double-bond systems that form new chromophores through condensation reactions upon heating (Puspawati *et al*., 2016). Fluorescence at 365 nm further suggests that the major constituents of *C. swinglei* essential oil possess extended conjugation, a characteristic feature of oxygenated monoterpenes commonly associated with the biological activity of citrus essential oils (Agarwal *et al*., 2022).

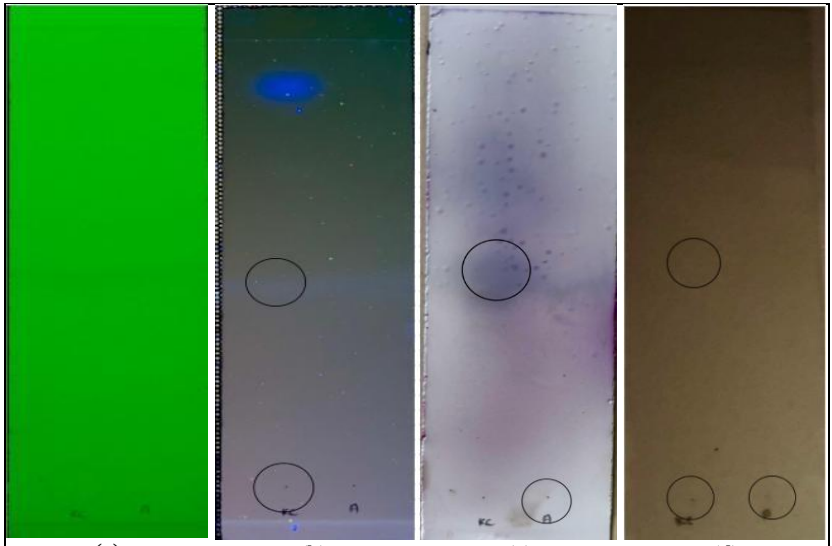

Figure 1. TLC-Bioautographic profile of essential oil from jeruk kunci (*C. swinglei*) using the mobile phase toluene : diethyl ether : formic acid (32:8:8). KC = jeruk kunci essential oil, A=acarbose. Chromatograms involving UV 254 nm (a), UV 365 nm (b), anisaldehyde-sulfuric acid (c), the α-glucosidase enzyme (d)

α-glucosidase bioautography of *C. swinglei* essential oil revealed the formation of distinct white inhibition zones on the TLC plate, indicating the presence of constituents α-glucosidase bioautography of *C. swinglei* essential

oil revealed the formation of distinct white inhibition zones on the TLC plate, indicating the presence of constituents capable of suppressing α-glucosidase activity. These inhibition zones arise from blocked hydrolysis of chromogenic substrates, such as p-nitrophenyl-α-D-glucopyranoside or 2-naphthyl-α-D-glucopyranoside, thereby preventing the formation of colored products in regions containing active compounds (Simões-Pires *et al*., 2009). In contrast, areas lacking inhibitory activity developed uniform coloration due to normal enzymatic reactions. Although the observed inhibition zones were relatively faint (Figure 1d), likely attributable to the low concentration of active compounds or the volatile nature of monoterpenes, this finding constitutes the first direct experimental evidence of α-glucosidase inhibitory activity in *C. swinglei* essential oil using a TLC-based bioautographic approach. The results are consistent with inhibitory characteristics previously reported for citrus essential oils (Agarwal *et al*., 2022).

3.3 α-glucosidase Inhibitor Activity Assay on Essential Oil from C. swinglei

The α-glucosidase enzyme inhibition activity assay was performed using a microplate reader, measuring the absorbance of the enzymatic reaction product. The synthetic substrate commonly used is p-nitrophenyl-α-D-glucopyranoside (pNPG) or its derivatives. The hydrolysis reaction catalyzed by the α-glucosidase enzyme produces p-nitrophenol (Pnp), which is yellow and has an absorbance peak (λ max) at 405 nm under basic conditions (San *et al*., 2021). The absorbance data was analyzed to calculate the percentage of enzyme activity inhibition. The IC_{50} value was determined as the concentration of essential oil required to inhibit 50% of enzyme activity.

Table 1. IC_{50} value of *C. swinglei* essential oil and positive control (acarbose) as α-glucosidase inhibitors

Sample	IC_{50} Value (µg/mL)	RIC_{50}
C. swingle essential oil	230±0.28	0.85
Acarbose (Positive control)	196.48±3.29	-

Based on the results in Table 1, the essential oil from *C. swinglei* has inhibitory activity against α-glucosidase with an IC_{50} value of 230±0.28 µg/mL, while the positive control (acarbose) showed an IC_{50} value of 196.48±3.29 µg/mL. Comparing this result with those of different citrus species, such as Buddha's hand citrus (Dang *et al*., 2016) and lemon peel (El Aboubi *et al*., 2023), with IC_{50} values of 412.2 and 2467.62 µg/mL, respectively, indicates that *C.*

swinglei has stronger inhibitory activity. Meanwhile, a comparison of the IC_{50} values of the essential oil and the positive control (acarbose) showed no significant difference, with an RIC_{50} value of 0.85. RIC_{50} is the ratio of the IC_{50} values of the positive control and the sample, expressed in µg/µg (no units) (Kong *et al*., 2018). For *C. swinglei*, RIC_{50} 0.85 means that 0.85 acarbose is equivalent to 1 µg *C. swinglei* essential oil. At a test concentration of 250 µg/mL, both *C. swinglei* essential oil and the acarbose control were able to produce measurable inhibition of the α-glucosidase enzyme, further supporting the inhibitory potential of this essential oil. Therefore, it can be concluded that the essential oil from *C. swinglei* has the potential for α-glucosidase inhibitory activity.

3.4 Secondary Metabolite Profile of C. swinglei Essential Oil

Phytochemical analysis by GC-MS was conducted to identify the constituents of the essential oil of *C. swinglei*. The identification process was performed by comparing the mass spectra of each chromatographic peak with reference data available in the NIST mass spectral library, allowing accurate matching of fragment patterns. The GC-MS analysis identified 12 main constituents, which were further characterized by mass spectrometry.

As shown in Table 2, the main components identified in *C. swinglei* essential oil are terpenoids. In the composition of essential oils, the most abundant terpenoids are C_{10} monoterpenes. In GC analysis, compounds with lower boiling points reach the detector more quickly due to their greater volatility, resulting in shorter retention times. The retention time of each compound is determined by its boiling point.

As shown in Table 2, the essential oil of *C. swinglei* is characterized by a predominance of hydrocarbon monoterpenes, while monoterpene alcohols occur at comparatively low levels.

The GC-MS analysis reported by El Aboubi *et al*. (2023) demonstrated that lemon peel essential oil is predominantly composed of hydrocarbon monoterpenes, with D-limonene as the major constituent (53.44%), followed by *β*-pinene (18.29%) and γ-terpinene (12.84%). Additional constituents, including p-cymene, α-pinene, *β*-myrcene, and small amounts of oxygenated monoterpenes, such as linalool, terpinen-4-ol, and α-terpineol, were detected in lower proportions. This strong predominance of C_{10} monoterpenes reflects

the typical phytochemical profile of citrus essential oil, characterized by a high abundance of volatile bioactive metabolites.

Table 2. Results of essential oil component analysis of *C. swinglei*

Number	Retention time (Min)	Chemical components	Molecular formula	Chemical class	Area (%)
1.	5.970	Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)	$C_{10}H_{16}$	Hydrocarbons	1.00
2.	6.106	α-Pinene	$C_{10}H_{16}$	Hydrocarbons	3.03
3.	6.854	*β*-Pinene	$C_{10}H_{16}$	Hydrocarbons	2.58
4.	6.991	*β*-Myrcene	$C_{10}H_{16}$	Hydrocarbons	2.85
5.	7.481	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)	$C_{10}H_{16}$	Hydrocarbons	0.57
6.	7.609	ρ-Cymene	$C_{10}H_{14}$	Hydrocarbons	9.47
7.	7.688	D-Limonene	$C_{10}H_{16}$	Hydrocarbons	53.34
8.	8.145	γ-Terpinene	$C_{1f0}H_{16}$	Hydrocarbons	24.02
9.	8.586	Cyclohexene, 1-methyl-4-(1-methylethylidene)	$C_{10}H_{16}$	Hydrocarbons	1.42
10.	8.782	Linalool	$C_{10}H_{18}O$	Alcohols	0.54
11.	10.094	Terpinen-4-ol	$C_{10}H_{18}O$	Alcohols	0.77
12.	10.314	*α*-Terpineol	$C_{10}H_{18}O$	Alcohols	0.43

A similar pattern was observed in the GC–MS analysis of the essential oil of *C. swinglei*, in which hydrocarbon monoterpenes constituted the predominant chemical class. D-Limonene was identified as the major component (53.34%), followed by γ-terpinene (24.02%) and p-cymene (9.47%). In contrast, oxygenated monoterpenes, including linalool (0.54%), terpinen-4-ol (0.77%), 1,3-cyclohexadiene, 1-methyl-4-(1-methylethyl) (0.57%), and α-terpineol (0.43%), were detected only in minor amounts. Overall, these results confirm that hydrocarbon monoterpenes represent the dominant secondary metabolites in the essential oil of *C. swinglei*.

Differences in analyte retention times are attributed to interactions between analyte molecules and the nonpolar stationary phase of the gas chromatography column: more polar compounds elute earlier, while nonpolar compounds are retained longer. The resulting chromatogram reflects the sample's chemical composition, with peak intensities proportional to the relative abundance of each component. Mass spectrometric analysis of *C. swinglei* essential oil yields characteristic ion fragments with specific mass-

to-charge (*m/z*) ratios that facilitate molecular structure elucidation. Electron impact ionization produces molecular ions and fragment ions, with the molecular ion peak corresponding to the neutral molecular weight of the parent compound, typically appearing at the high mass end of the mass spectrum (Bizzo *et al*., 2023; Koljančić *et al*., 2023; Ranjani *et al*., 2023).

Figure 2 shows the chemical structures of each component of *C. swinglei* essential oil.

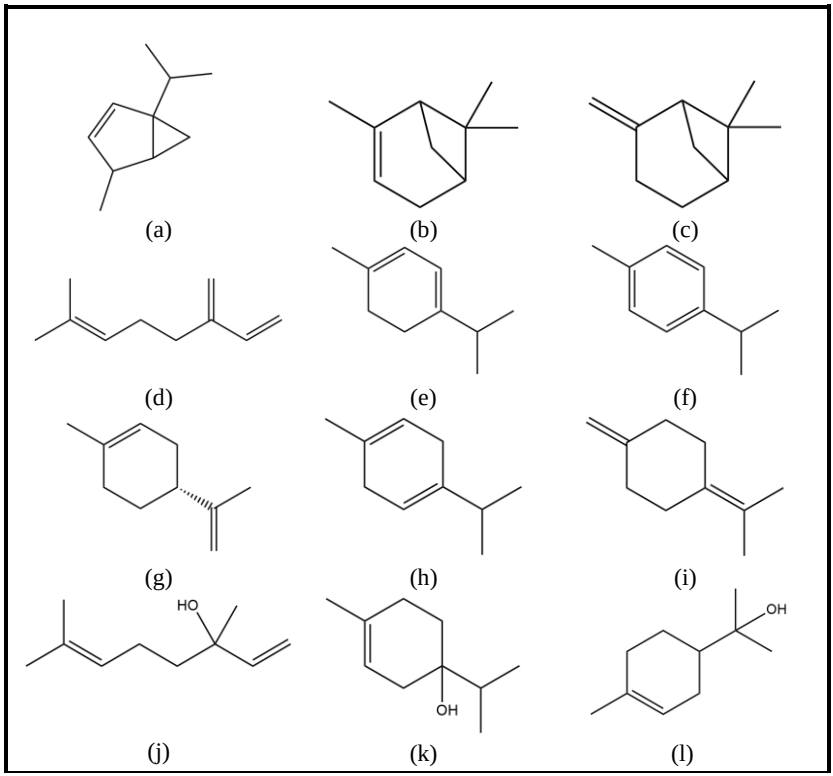

Figure 2. The structure of *C. swinglei* essential oil constituents: bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl) (a); α-Pinene (b); *β*-Pinene (c); *β*-myrcene (d); 1,3-cyclohexadiene, 1-methyl-4-(1-methylethyl) (e); ρ-cymene (f); D-limonene (g); γ-terpinene (h); cyclohexene, 1-methyl-4-(1-methylethylidene) (i); linalool (j); terpinen-4-ol (k); and α-terpineol (l)

The systemic bioavailability of monoterpene-rich essential oils is generally low due to their physical and metabolic characteristics. Monoterpenes are highly volatile and strongly lipophilic, characteristics that facilitate rapid

passive diffusion across the gastrointestinal epithelium but contribute to substantial pre-systemic loss. As demonstrated by ADME predictions, these compounds exhibit high gastrointestinal absorption but only moderate oral bioavailability, largely due to extensive first-pass metabolism in both the intestinal mucosa and the liver (Hosseini *et al*., 2024). After absorption, monoterpenes are rapidly oxidized by cytochrome P450 enzymes and subsequently conjugated via glucuronidation or sulfation, forming hydrophilic metabolites that are readily excreted in urine (Zhao *et al*., 2021). As a consequence, only a small fraction of the parent monoterpenes reaches systemic circulation, resulting in low plasma concentrations and short residence times. These pharmacokinetic characteristics are highly relevant when interpreting the α-glucosidase inhibitory activity observed in vitro, as the biological potency demonstrated under controlled laboratory conditions may not fully translate into in vivo activity due to rapid metabolism, high volatility, and limited systemic exposure (Gonçalves *et al*., 2025). Therefore, while the essential oil shows promising α-glucosidase inhibition, its potential therapeutic impact after oral administration must be evaluated within the context of these biopharmaceutical constraints, underscoring the need for formulation strategies that enhance monoterpene stability and systemic bioavailability.

4. Conclusions and Recommendations

This study demonstrates that the essential oil of *C. swinglei* is characterized by a chemical profile dominated by hydrocarbon monoterpenes, with major constituents including α-pinene, *β*-pinene, *β*-myrcene, *p*-cymene, *D*-limonene, γ-terpinene, and related monoterpene derivatives. GC–MS analysis, supported by TLC bioautography, confirmed the presence of biologically active secondary metabolites. Evaluation of α-glucosidase inhibitory activity revealed that the essential oil of *C. swinglei* exhibited moderate inhibitory potency, with an IC_{50} value of 230 ± 0.28 µg/mL. These findings indicate that *C. swinglei* essential oil represents a promising natural source of α-glucosidase inhibitor candidates with potential relevance for the development of plant-based antidiabetic agents. However, given the moderate level of enzyme inhibition observed, further studies are warranted. Future work should focus on fractionation, isolation, and characterization of the principal bioactive constituents to identify the compounds responsible for the observed antidiabetic activity and to better evaluate their pharmacological potential.

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