ORIGINAL ARTICLE

Comparative *in Vitro* Antioxidant and Enzymatic Inhibitory Activities of *Zingiber Officinale* Roscoe. And *Cymbopogon Nardus* (L.) Rendle Essential Oils

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ABSTRACT

Introduction: Diabetes mellitus affects over 400 million people worldwide. Due to the side effects of current anti-diabetic drugs, there is growing interest in natural treatments. Antioxidants help prevent diabetic complications, and inhibiting α-glucosidases and α-amylases significantly reduces post-prandial blood glucose levels. This research aims to examine the bioactive compounds in *Zingiber officinale* Roscoe. and *Cymbopogon nardus* (L.) Rendle essential oils using gas chromatography/mass spectrometry (GC/MS) and their antioxidant, as well as α-glucosidase and α-amylase inhibition activities. **Methods:** The chemical composition of the essential oils was determined by GC/MS analysis. Antioxidant activity was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, while enzyme inhibition was assessed using α-amylase and α-glucosidase assays. **Results:** The major phytochemical components of *Z. officinale* essential oil were found to be dodecanoic acid, 1,2,3-propanetriyl ester (37.81%), dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester (9.52%), 3-(octanoyloxy)propane-1,2-diyl bis(decanoate) (4.73%), gingerol (3.62%), 1-(4-hydroxy-3-methoxyphenyl)oct-4-en-3-one (1.67%), linalool (1.46%), geranyl acetate (1.03%), zingiberene (1.08%) and farnesene (0.64%). The major phytochemical constituents in the *C. nardus* are dodecanoic acid, 1,2,3-propanetriyl ester (22.95%), hexadecanoic acid, 2-[(1-oxododecyl)oxy]-1,3-propanediyl ester (2.74%), D-limonene (2.49%), eudesm-11-en-1α-ol (2.01%), α-phellandrene (1.93%), D-citronellol (0.94%), geraniol (0.87%), α-terpineol (0.84%) and citronellal (0.69%). *Z. officinale* oil demonstrated better antioxidant (55.4%, $p< 0.05$), α-amylase (39.7%, $p<0.05$) and α-glucosidase (24.5%, $p<0.05$) inhibitory activity, respectively, compared to *C. nardus* oil. **Conclusion:** This finding indicates that the constituents of *Z. officinale* essential oil may collectively inhibit oxidative stress and enzymes linked to T2DM, and thus potentially help reduce postprandial glucose levels and diabetes complications. Further *in vivo* studies are required to validate these findings.
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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by elevated blood sugar levels, resulting from either insufficient insulin production or ineffective utilisation of insulin by the body (1). DM is generally classified into 2 types, mainly Type 1 DM (T1DM) and Type 2 DM (T2DM). T1DM is an autoimmune disease characterised by pancreatic beta cell destruction and an absolute deficiency of insulin, while T2DM occurs due to a combination of peripheral insulin resistance and inadequate secretory response by pancreatic beta cells (2). In 2022, a staggering 828 million adults worldwide were living with diabetes, marking a sharp increase of 630 million since 1990 (3). This is an alarming trend that underscores a growing global health crisis. In recent years, researchers have explored the roles of oxidative stress in various pathological conditions, including DM (4, 5). Oxidative stress is defined as an imbalance between the production of harmful free radicals and the human body's antioxidants (6). Oxidative stress plays a critical role in the global impact of DM, driving the progression of complications, including both cardiovascular and microvascular issues such as retinopathy, cardiomyopathy, neuropathy, nephropathy,

encephalopathy, and peripheral arteriopathy (7-9).
The complex process of carbohydrate digestion, which contributes to the rise in blood sugar levels, involves several enzymes. α-amylase is an enzyme that is primarily produced in the salivary glands and pancreas, while α-glucosidase is found in the small intestine (10). The breaking down of starch from food sources into oligosaccharides or polysaccharides is catalysed by α-amylase enzymes, followed by α-glucosidase enzymes that further digest the complex carbohydrates into monosaccharides that are easily absorbable in the digestive system (11). By inhibiting these enzymes, it is evident that effective reduction of digestion and absorption of dietary carbohydrates is achieved, leading to improved glycaemic control in diabetic patients, mainly in T2DM patients or those at risk of developing T2DM (12).

Effective management of T2DM involves both controlling blood glucose levels and mitigating the oxidative stress associated with the disease. The connection between enzyme inhibition and antioxidant activity is particularly relevant in this context, as oxidative stress plays a significant role in the progression and complications of DM. Elevated glucose levels in hyperglycaemic conditions lead to the overproduction of free radicals, which damage cells and tissues. Antioxidants play a crucial role in neutralizing these free radicals, thereby reducing oxidative damage (13). While the inhibition of α-glucosidase and α-amylase primarily targets the regulation of blood glucose, antioxidant properties complement this process by combating the oxidative stress that exacerbates DM-related complications (14). Thus, the dual action of enzyme inhibition and antioxidant activity may offer a synergistic approach to managing DM and its associated oxidative stress.

Recently, the use of plant-derived products to manage DM has increased, particularly in developing countries with limited resources and access to modern treatments (15). In this study, two natural products, namely *Z. officinale* essential oil (ginger oil) and *C. nardus* essential oil (citronella oil), were investigated and compared for their antioxidant, α-glucosidase, and α-amylase inhibiting activities. *Z. officinale* from Zingiberaceae family and *C. nardus* from Poaceae family were chosen based on their previously reported bioactive properties. Moreover, both essential oils have been traditionally used in various cultures for their medicinal and culinary benefits, making them candidates for dietary recommendations (16, 17). The lack of direct comparison between the antioxidant and enzyme inhibition activities of ginger oil and citronella oil in managing T2DM is addressed in this study. While both oils have demonstrated bioactive properties, there is limited research that directly contrasts their effects on oxidative stress and blood glucose regulation through enzyme inhibition, particularly in the context of T2DM management.

By investigating the relationship between the potential enzyme inhibition and antioxidant properties of these plant-derived essential oils, the outcome of this study is anticipated to contribute to the improvement of the quality of life of T2DM patients. These findings may also serve as a foundation for dietary recommendations, providing a complementary approach to managing blood glucose levels and oxidative stress. Ultimately, this could help reduce the burden of complications associated with this prevalent metabolic disorder.

METHODOLOGY

Material

Dimethyl sulfoxide (DMSO), acarbose, 2,2-diphenyl-1-picrylhydrazyl (DPPH), methanol and ascorbic acid were purchased from Sigma-Aldrich, Germany.

Plant collection and preparation of essential oils from plants

The local ginger (*Z. officinale* Roscoe) and citronella (*C. nardus* L. Rendle) were collected from Miri, Sarawak. The plants were sent for identification and authenticated by a botanist from the Herbarium of the National University of Malaysia (UKM), Selangor, Malaysia (Voucher No.: ID113/2024 and ID117/2024). All the plant materials were washed, dried and ground prior to the supercritical fluid extraction (SFE). The ground ginger was extracted at 300 bar and 60˚C (18, 19) while the citronella was extracted at 200 bar and 40˚C (20, 21) using the lab scale supercritical fluid extractor (Spe-ed SFE-2, Applied Separations, USA). Such SFE operating conditions of the samples have been reported to give higher essential oil yields (19, 21).

GC/MS analysis

The chemical compounds present in the essential oil of ginger and citronella were identified by using the Clarus® SQ 8 GC/MS (PerkinElmer, USA) equipped with an Elite-5ms capillary column (30 m × 0.25 mm i.d. × film thickness 0.25 µm). The detection limit of the machine is 5 ppb. The essential oils were dissolved in methanol. Methanol was used for the blank run. For the ginger oil, the split ratio used was 1:100. The initial oven temperature was set at 50˚C and held for 5 min. It was raised to 200˚C at a rate of 10˚C/min, then to 300˚C at a rate of 5 ˚C/min. The temperature of the injection, ion source and quadrupole were all maintained at 230 ˚C. For citronella oil, the split ratio used was 1:50. The initial oven temperature was 50˚C and then was raised to 300˚C at a rate of 8˚C/min and held for 5 mins. The temperature of the injection, ion source and quadrupole were 250˚C, 200˚C and 300˚C, respectively. The injection volumes of all samples were 1 µL, and helium was used as the carrier gas with a flow rate of 1 mL/min. The identification of constituents was done based on their retention time and mass spectra library search via the National Institute of Standards and Technology (NIST) library. For each sample, the 10 most prominent

compounds were identified and listed in Table I and Table II.

DPPH free radical scavenging assay

The DPPH radical scavenging capacity of the test samples was evaluated according to the method reported previously (22). The initial absorbance of 100 µl of 0.2 mM DPPH in methanol was measured at the wavelength of 517nm on the UV-Vis spectrophotometer (Biobase, BK-EL10C, China). Various concentrations of the test sample in DMSO were added to the methanolic DPPH solution with final concentrations ranging from 0 to 10 mg/ml. The mixed solutions were then allowed to incubate for 30 minutes in the dark, and the change in absorbance at 517 nm was measured again. The negative and positive controls used in this assay are DMSO and ascorbic acid, respectively. Each experiment was repeated thrice. The percentage of radical scavenging capacity (RSC) is calculated using the following formula:

RSC(%)=100%×(ABS control - ABS sample/ABS control)

The determination of the concentration of test samples that caused a 50% reduction of DPPH free radicals (IC_{50}) was done using GraphPad Prism 6.0 by selecting log(inhibitor) vs response for nonlinear regression (23). The results were then expressed as mean ± standard deviation (SD).

α-Amylase and α-Glucosidase inhibition assays

The enzyme inhibitory activity of the essential oils and positive control, acarbose, was assessed using a colorimetric α-amylase (Abcam, UK, Product code: ab283391) and α-glucosidase (Abcam, UK, Product code: ab284520) inhibitor screening kit following the manufacturer's instructions. Briefly, the test samples (ginger oil, citronella oil and acarbose) were prepared by first diluting 100Ч in DMSO and further diluting to 3Ч in the assay buffer. From there, the assay mixture containing 50 µL of the essential oils at a concentration ranging from 0 to 25 mg/mL and 50 µL of the α-amylase or 10 µL of α-glucosidase enzyme was prepared in a 96-well clear microplate. For acarbose, concentrations ranging from 0 to 1000 µg/mL were used. The mixed solution was incubated at room temperature for 10 minutes in the dark. After the incubation, 50 µL of α-amylase/10 µL of α-glucosidase substrate was added to initiate the enzymatic reaction. The absorbance reading was recorded at an optical density of 405 nm in kinetic mode for 20-25 min at room temperature. The reaction system without plant extracts was used as enzyme control, and the system without α-amylase (background control) was used to correct the background absorbance as follows:

Corrected Abs sample = Abs sample – Abs Background control

An additional incubation is carried out using a reaction system containing only DMSO (solvent control). All the tests were conducted three times to obtain triplicate results. The percentage inhibition of enzymes was calculated by the following formula:

Inhibition(%)=((Abs control–Abs sample)/Abs control) x 100

Statistical analysis

The statistical analyses were done using the Two-Way ANOVA test to compare the mean differences between groups, followed by Bonferroni's post-hoc test using IBM SPSS version 21. Differences were considered statistically significant at p-values less than 0.05 ($p < 0.05$).

RESULTS

Compound identification using GC/MS

The phytochemical components in ginger and citronella oils were analyzed using GC/MS by comparing the retention time (minutes), peak area (percentage), and mass spectral fragmentation patterns of the chromatogram peaks with known compounds in the NIST library. The GC/MS chromatograms of ginger and citronella oil are depicted in Fig. 1.

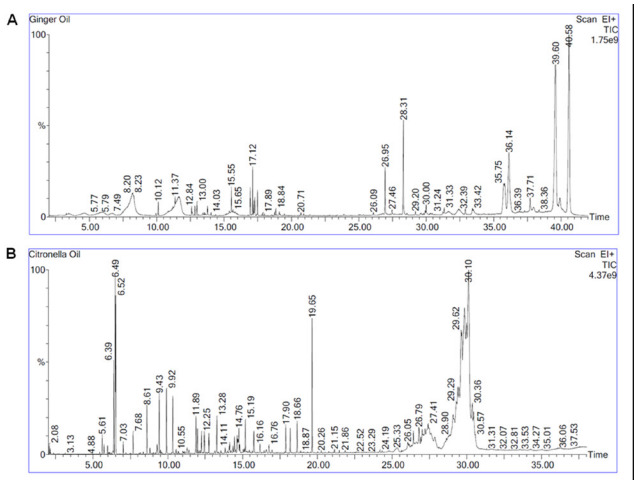

Fig 1. : GC/MS chromatogram showing components of (A) ginger essential oil and (B) citronella essential oil.

The phytochemical components of ginger oil were found to be dodecanoic acid, 1,2,3-propanetriyl ester (37.81%), dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester (9.52%), 3-(octanoyloxy)propane-1,2-diyl bis(decanoate) (4.73%), gingerol (3.62%), 1-(4-hydroxy-3-methoxyphenyl)oct-4-en-3-one (1.67%), linalool (1.46%), geranyl acetate (1.03%), zingiberene (1.08%) and farnesene (0.64%) (Table I).

Table I. The phytochemical constituents identified in the essential oil of *Z. officinale* using GC/MS.

No.	PubChem CID	Compound Name	Molecular formula	Molecular weight	Retention time (min)	Peak Area (%)
1	33979	Dodecanoic acid, 1-(hydroxymethyl)-1,2 - ethanediyl ester	$C_{27}H_{52}O_5$	456.7	8.230	9.523
2	6549	Linalool	$C_{10}H_{18}O$	154.25	11.366	1.463
3	1549026	Geranyl acetate	$C_{12}H_{20}O_2$	196.29	15.545	1.029
4	521253	Zingiberene	$C_{15}H_{24}$	204.35	17.118	1.083
5	5362889	cis-6-Farnesene	$C_{15}H_{24}$	204.35	17.489	0.635
6	9794897	1-(4-Hydroxy-3-methoxyphenyl)oct-4-en-3-one	$C_{15}H_{20}O_3$	248.32	26.952	1.667
7	442793	Gingerol	$C_{17}H_{26}O_4$	294.4	28.312	3.617
8	15904519	3-(Octanoyloxy)propane-1,2-diyl bis(decanoate)	$C_{31}H_{58}O_6$	526.8	35.823	4.725
9	10851	Dodecanoic acid, 1,2,3-propanetriyl ester	$C_{39}H_{74}O_6$	639.0	39.604	20.306
10					40.594	17.501

The phytochemical constituents detected in the citronella oils are dodecanoic acid, 1,2,3-propanetriyl ester (22.95%), hexadecanoic acid, 2-[(1-oxododecyl)oxy]-1,3-propanediyl ester (2.74%), D-limonene (2.49%), eudesm-11-en-1α-ol (2.01%), α-phellandrene (1.93%), D-citronellol (0.94%), geraniol (0.87%), α-terpineol (0.84%) and citronellal (0.69%) (Table II).

Table II. The phytochemical constituents identified in the essential oil of *C. nardus* using GC/MS.

No.	PubChem CID	Compound Name	Molecular formula	Molecular weight	Retention time (min)	Peak Area (%)
1	440917	D-Limonene	$C_{10}H_{16}$	136.23	6.489	2.491
2	7460	6-Phellandrene	$C_{10}H_{16}$	136.23	6.524	1.932
3	7794	Citronellal	$C_{10}H_{18}O$	154.25	8.610	0.687
4	17100	α-terpineol	$C_{10}H_{18}O$	154.25	9.431	0.840
5	101977	D-citronellol	$C_{10}H_{20}O$	156.26	9.92	0.938
6	637566	Geraniol	$C_{10}H_{18}O$	154.25	10.332	0.867
7	68738243	56,76H,10a-Eudesm-11-en-1a-ol	$C_{15}H_{26}O$	222.37	19.654	2.014
8	10851	Dodecanoic acid, 1,2,3-propanetriyl ester	$C_{39}H_{74}O_6$	639.0	30.115	20.325
9	545681	Hexadecanoic acid, 2-[(1-oxododecyl)oxy]-1,3-propanediyl ester	$C_{47}H_{90}O_6$	751.2	30.362	2.741
10	10851	Dodecanoic acid, 1,2,3-propanetriyl ester	$C_{39}H_{74}O_6$	639.0	30.459	2.621

Antioxidant activity

The highest total antioxidant activity among the test samples studied was demonstrated by ginger oil with IC_{50} values of 2.5 ± 0.3 mg/ml, followed by citronella oil with IC_{50} of 6.1 ± 1.8 mg/ml (Table III). To confirm the validity of the DPPH assay, ascorbic acid was used as the positive control. Ascorbic acid yielded an IC_{50} value of 2.9 ± 0.3 µg/ml. Comparatively, ginger oil demonstrated 55.4% ($p<0.05$) better antioxidant activity compared to citronella oil.

Table III. Inhibition concentration 50 (IC_{50}) values for DPPH radical scavenging activity and enzyme inhibitory effects of the essential oils in comparison to positive controls

Test samples	IC_{50} (mean ± SD)		
	DPPH	α-amylase	α-glucosidase
Ginger oil	2.5 ± 0.3 mg/mL	13.5 ± 2.7 mg/ml	11.4 ± 1.3 mg/ml
Citronella oil	6.1 ± 1.8 mg/mL	22.4 ± 1.8 mg/ml	15.1 ± 0.9 mg/ml
Ascorbic acid	2.9 ± 0.3 µg/mL	-	-
Acarbose	-	312.1 ± 22.7 µg/ml	431.5 ± 31.2 µg/ml

Inhibitory effects against α-amylase and α-glucosidase enzymes

Both ginger and citronella oils demonstrated dose-dependent inhibition of the α-amylase and α-glucosidase enzymatic activities (Fig. 2). Ginger oil showed higher α-amylase inhibitory activity (IC_{50} = 13.5 ± 2.7 mg/ml) than citronella oil (IC_{50} =22.4 ± 1.8 mg/ml) (Table III). The IC_{50} of ginger oil for α-amylase inhibitory activity was significantly ($p<0.05$) different from that of citronella oil. The findings for α-glucosidase enzyme inhibition activity are of a similar manner, whereby ginger oil showed higher α-glucosidase inhibitory activity (IC_{50}=11.4 ± 1.3 mg/ml) than citronella oil (IC_{50}=15.1 ± 0.9 mg/ml) (Table III). Comparatively, ginger oil demonstrated 39.7% and 24.5% better α-amylase and α-glucosidase inhibitory activity, respectively, compared to citronella oil.

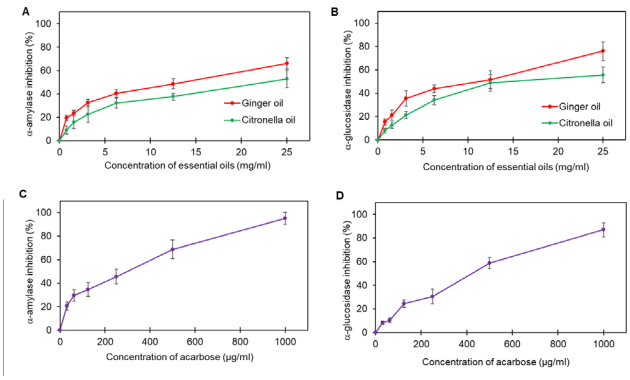

Fig 2. : Enzyme inhibitory effects of ginger and citronella essential oils at different concentrations (0 – 25 mg/mL) against (A) α-amylase and (B) α-glucosidase. Acarbose, a known pharmacological inhibitor of α-glucosidase and α-amylase was used as the positive control. Enzyme inhibitory effects of acarbose at different concentrations (0 – 1000 μg/mL) against (C) α-amylase and (D) α-glucosidase were included to ensure reliability of the results. Results were presented as mean ± SD of three independent experiments (n = 3).

DISCUSSION

The phytochemical components of ginger oil extracted via the SFE method in this study are mainly sesquiterpenes, monoterpenes and esters. This is concurrent with the findings of previous studies where ginger oil obtained with a supercritical CO_2 extraction method contains major substances such as zingiberene, sesquiphellandrene, farnesene, geranial, bisabolene and eudesmol (24). The phytoconstituents of the citronella oil also align well with the findings of previous studies. For instance, Chanthai et al. reported that geraniol, geranial, neral, α-copaene, citronellol, γ-cadinene, δ-cadinene, eugenol and β-caryophyllene are the main components of citronella oil, (25) while Rastuti et al. identified citronellal, limonene, and geraniol as the major components (26). However, a different study investigating the essential oil extracted from fresh ginger rhizomes through the hydrodistillation method revealed a distinct profile, with zingiberene, valencene, β-funebrene, selina-4(14),7(11)-diene, citronellyl n-butyrate, β-phellandrene, camphene and α-pinene as major components (27). This variation highlights the influence of the extraction method on the chemical composition of ginger oil. This finding is supported by studies on peppermint leaves extraction, which showed that hydrodistillation primarily yielded volatile compounds, while SFE effectively extracted terpenoids and other lipophilic compounds, enhancing the bioactivity of the extracts (28).

Ginger oil demonstrated superior antioxidant activity to citronella oil. The bioactive compounds such as gingerol, linalool, zingiberene, and farnesene in ginger oil are anticipated to have contributed to its antioxidant activity (29-32). Moreover, a study has reported that diethylnitrosamine pre-injected rats supplemented with ginger oil showed improvement in antioxidant activity, by lowering the serum malondialdehyde level and increasing the serum activities of enzymatic antioxidants such as catalase and GSH-Px and non-enzymatic antioxidants such as the total antioxidant capacity (33). The component of citronella oil, particularly citral, is reported to contribute to its free radical scavenging activity (34). A study reported that the fractions of citronella oil containing a higher percentage of geraniol (47.03-62.41%) demonstrated the most potent antioxidant activity (26). The weaker antioxidant activity of citronella oil in this study could be attributed to the lower geraniol content (0.87%) detected via GC/MS. Several studies have also reported that the antioxidant activity of plant-derived essential oils may not only be influenced by one bioactive compound but is a synergistic effect of several compounds (35, 36).

The inhibition of α-amylase and α-glucosidase plays a crucial role in modulating postprandial glucose levels in patients with T2DM. α-amylase, secreted by the salivary glands and the pancreas, catalyzes the hydrolysis of α-1,4 glycosidic bonds in polysaccharides such as starch and glycogen. This produces smaller oligosaccharides and maltose (10). The inhibition of α-amylase reduces the initial breakdown of complex carbohydrates in the digestive tract, leading to a slower release of maltose and dextrins (10). Subsequently, α-glucosidase, located in the brush border of the small intestine, facilitates the hydrolysis of these oligosaccharides into absorbable monosaccharides, such as glucose. The inhibition of α-glucosidase delays the conversion of disaccharides and oligosaccharides into glucose, leading to reduced glucose absorption and a more gradual rise in blood sugar levels (11). This inhibitory mechanism effectively controls postprandial hyperglycemia by lowering the rate at which glucose enters systemic circulation, reducing insulin demand, and improving insulin sensitivity over time. The therapeutic relevance of such inhibition is evident in the clinical use of acarbose, which inhibits both α-amylase and α-glucosidase (37). However, acarbose is known to induce various adverse effects such as hypoglycaemic episodes, nausea, vomiting,

abdominal discomfort, weight gain, and diarrhoea (38). These adverse effects are mainly attributed to the non-selective inhibition of α-amylase and α-glucosidase, leading to undigested carbohydrates reaching the colon, where they are fermented by bacteria, producing gas and causing gastrointestinal symptoms (39). Both essential oils used in this study exhibited superior inhibition of the α-glucosidase enzyme compared to α-amylase, in contrast to the positive control acarbose, which demonstrated stronger inhibition of α-amylase activity. By selectively inhibiting α-glucosidase over α-amylase, glucose absorption can be specifically targeted, thus reducing the risk of hyperglycemia after meals with minimal interference to the initial carbohydrate breakdown (40, 41). This distinction suggests that the essential oils may offer a more favourable profile in mitigating the primary drawbacks associated with acarbose (41, 42).

Overall, the ginger oil was more effective in inhibiting the activity of α-amylase and α-glucosidase, therefore probably contained potentially potent compositions for treating T2DM than the citronella oils. The results obtained are also consistent with the previous study that reported the shielding effect of 6-gingerol, which is an important phytoconstituent of ginger, demonstrated protection against hepatic inflammation, which is the underlying pathogenesis of insulin resistance and T2DM (43). Another study reported that the same phytoconstituent reduced renal dysfunction biomarkers in streptozotocin-induced diabetic animals, suggesting a probable renal protective property (44). The GC/MS analyses of ginger oil used in this study revealed the presence of gingerol (3.62%), which may have contributed to the potent enzyme inhibitory activities. Moreover, the presence of D-limonene and linalool was correlated with the α-glucosidase inhibitory activity (45). At the molecular level, inhibitors of these enzymes typically bind to the active site of the enzymes, blocking substrate access. For α-amylase, inhibitors often interact with key amino acid residues like Arg195, Asp197, Glu233, His299 and Asp300 at the catalytic site, disrupting the enzyme's ability to cleave α-1,4 glycosidic bonds in starches (42). An in silico study revealed that α-terpineol forms a hydrogen bond with Asp197 of α-amylase, aligning with its observed *in vitro* α-amylase inhibition (46). Another study reported that citronellol, a component detected in the citronella oil, interacted with Arg195 and Asp300 at the α-amylase catalytic site (47). α-glucosidase inhibitors often bind to the active site of α-glucosidase with a stronger affinity than the natural carbohydrate-substrate complex. This results in competitive inhibition of α-glucosidase activity, reducing the hydrolysis of carbohydrates and limiting glucose absorption (48, 49). The catalytic site of α-glucosidase contains key amino acid residues such as Asn258, Asp327, Ile143, and Asp382 (49). Conventional hydrogen bonding to these residues often contributes to α-glucosidase inhibition, similar to acarbose (49).

Essential oils extracted from other plants in the Zingiberaceae and Poaceae family have also been reported to possess significant enzyme inhibitory potential, owing to their phytochemical profiles. For instance, studies have shown that the essential oils extracted from the seeds of *Aframomum melegueta* and *Aframomum danielli*, containing mainly oxygenated monoterpenes, possess α-amylase inhibitory potential, though their potency was not as strong as acarbose (50). A study comparing the bioactivity of essential oils from Zingiber *atroporphyreus* and *Warburgia schmidtii*, both from Zingiberaceae family, reported that essential oils extracted from Z. *atroporphyreus* exhibited higher inhibitory potential against α-amylase, which is likely attributed to the presence of monoterpenes in their chemical profile. Both essential oils, however, showed minimal to no inhibition against α-glucosidase (51). *Cymbopogon pendulus* essential oils with high concentrations of polyphenolics have been reported to possess high α-amylase inhibitory activity (52). Thus, it is worth noting that a key challenge associated with the practical application of ginger and citronella essential oils lies in the inherent variability of their composition and bioactive compound content. The chemical profiles of plant-derived oils are influenced by various factors, including the genetic origin of the plants, the specific plant parts used for extraction, environmental conditions, and the time of harvesting (53, 54). For ginger and citronella oils to be consistently effective, standardized production methods, rigorous quality control measures, and advanced analytical techniques are required to ensure their composition remains stable. This will aid in the formulation of more reliable and effective treatments using ginger and citronella oils.

While ginger and citronella essential oils hold promise for therapeutic use, their potential toxicity and safe dosage levels must be carefully determined, particularly when used in T2DM or other health-related applications. Several studies have reported the *in vivo* oral toxicity profiles of plant-derived essential oils. For instance, male and female Wistar rats that were administered with ginger oil at various doses by oral gavage for 90 days showed no deaths or significant growth differences upon treatment. Blood tests, pathology, and histology showed no harmful effects. The highest dose tested (500 mg/kg bw per day) was identified as the no observed adverse effect level (NOAEL) (55). In an OECD-compliant study, Sprague Dawley rats were administered citronella oil at various doses, with the highest observed NOAEL for systemic toxicity at 708 mg/kg bw/day in males and 771 mg/kg bw/day in females, and a NOAEL for developmental toxicity at 88 mg/kg bw/day, with no significant adverse effects (56). Another study reported that *Cymbopogon giganteus* essential oil showed no mutagenicity and caused no mortality or harmful effects in acute and subchronic toxicity tests at all tested concentrations in male and female Wistar rats. However, while doses below 0.5% v/v showed

minimal acute pulmonary toxicity, higher doses were toxic (57). Although the oral intake of essential oil is largely harmless, it is also concerning that essential oils rich in monoterpenes, when taken in high doses, have been reported to cause reproductive hormone modulation, nephropathy, abortifacient effects, as well as teratogenic and neurotoxic properties (58). Therefore, it is critical to establish the optimal therapeutic dose range and determine maximum tolerable levels for these oils, ensuring they can be safely consumed. The absence of *in vivo* validation, crucial for confirming the efficacy and safety of the essential oils, is a limitation in this study. While *in vitro* findings provide valuable insights into enzyme inhibition, *in vivo* studies would help determine pharmacokinetic and pharmacodynamic properties as well as the potential systemic effects of the essential oil.

CONCLUSION

In conclusion, the results obtained in this study revealed that ginger oil possesses better antioxidant capabilities as well as better α-amylase and α-glucosidase inhibitory activities than citronella oil. These results suggest that ginger oil could be a potential candidate for further therapeutic development as a dietary supplement. It is anticipated that the constituents of ginger oil, such as gingerol, linalool, zingiberene, and farnesene contributed collectively to its inhibitory potential against enzymes linked to T2DM and oxidative stress and thus could be more effective in reducing postprandial glucose levels and complications of diabetes. However, to fully harness its therapeutic potential, bioactivity-guided investigations are necessary to isolate the specific lead compounds responsible for its antidiabetic effects. These investigations will also help to elucidate the underlying mechanisms of action, offering insights into both upstream and downstream pathways involved in glucose regulation. Further *in vivo* studies are essential to confirm these findings and explore the broader pharmacological properties of ginger oil in managing diabetes. *In vivo* studies using a diabetes rodent model such as STZ- or Alloxan-induced diabetic rats/mice model to assess the effects on postprandial glucose levels and metabolic markers would provide valuable insights into the effectiveness of the essential oils as antidiabetic agents, as well as their long-term safety profile, mechanism of action and establishment of optimal dosage. These findings could form the basis for clinical trials, which would be the next step in validating the therapeutic potential of ginger oil in human subjects. Such research could pave the way for the development of new therapeutic formulations or dietary interventions aimed at improving glycaemic control, ultimately offering complementary yet practical applications for diabetes management, especially in resource-limited settings.

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