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Essential Oils from *Etlingera elatior* Inflorescence against α-Amylase and α-Glucosidase Inhibition

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ABSTRACT

The increasing number of diabetes mellitus cases every year is alarming for every country. More than 90% of the cases are type-2 diabetes mellitus. The range of diabetes mellitus patients is at a productive age, including in Indonesia. Type-2 diabetes mellitus can be managed by reducing postprandial glucose levels. The approach inhibits α-amylase and α-glucosidase enzymes. A preliminary study on the local edible plants in Solok district, *Etlingera elatior*, showed potential against the α-glucosidase enzyme by TLC-bioautography screening. The essential oils from the inflorescence of *E. elatior* were extracted by hydro-distillation. The analysis of components from the essential oils identified 20 compounds. GC-MS data showed that the highest abundance percentages were 1-dodecanol (39.79%), dodecanal (28.20%), α-pinene (13.11%), decanal (4.16%), and dodecanol acetate (2.75%). The inhibition against the α-glucosidase enzyme obtains the IC_{50} value of 73.95 and 67.55 μg/mL for the essential oils and acarbose, respectively. The inhibition against the α-amylase enzyme obtains the IC_{50} value of 70.87 and 12.28 μg/mL for the essential oils and acarbose, respectively. The essential oils from the inflorescence of *E. elatior* could inhibit α-amylase and α-glucosidase enzymes, even though the potential was lower than that of acarbose as a positive control.

Keywords : Ginger Species, Local Edible Plant, Type-2 Diabetes Mellitus, Volatile Oils

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder due to a lack of insulin response or insulin resistance to regulate blood glucose levels (Ojo et al., 2023). The International Diabetes Federation (IDF) reported that more than 90% of diabetic patients were diagnosed with type-2 diabetes mellitus (T2DM) (Magliano & Boyko, 2021). Indonesia was the fifth country in the world with a high number of people with diabetes mellitus. People with diabetes mellitus need to manage their condition effectively to prevent severe complications. Since Indonesia has rice as its leading food, one strategy to control blood glucose is by reducing the absorption of carbohydrates (Syafni et al., 2021). Some enzymes responsible for carbohydrate metabolism are α-amylase and α-glucosidase (Khoo, 2016; Zeng et al., 2020). By inhibiting this enzyme, the increase of postprandial glucose will be reduced in people with or without diabetes mellitus.

The preliminary study on 14 local edible plants in West Sumatra found that a methanolic extract of torch ginger (*Etlingera elatior*) inhibited the α-glucosidase enzyme (Syafni et al., 2021). The bioautography profile showed inhibition of the extract in the non-polar compounds. Since the compounds in the nonpolar fraction can be included in volatile oil, the study continued to analyze the potential of its essential oils against α-amylase and α-glucosidase enzymes. The antidiabetic aqueous extraction survey has reported its potential (Nor et al., 2020). The previous study on *E. elatior* inflorescence identified stigmasterol as a compound that inhibited α-amylase and α-glucosidase enzymes (Syafni et al., 2023).

Etlingera elatior has been known as the local edible plant in West Sumatra (Syafni et al., 2021). This study approach applies a local edible plant as an herbal medicine. This concept has been discussed, and to get acceptance from professional health care, the research on the targeted plants needs to pursue evidence-based herbal medicine (Carmona & Pereira, 2013).

Herbal medicine has an issue with the multi-target approach and the synergistic effect. Identification of responsible compounds is important in medicinal plants. This approach will lead to standardization of the raw material from plants before continuing to *in vivo*, pre-clinical study, and dosage form production.

This study aimed to identify the potential of essential oils from the inflorescence of *E. elatior* against α-amylase and α-glucosidase enzymes, since there is restricted information on the potential antidiabetic properties of essential oils from this plant. Local people with or without diabetes mellitus can use this plant to prevent and manage the increase in their post-prandial glucose. The study was done *in vitro*, and the composition of secondary metabolites from the essential oils of *E. elatior* inflorescence was analyzed.

MATERIALS AND METHODS

Instruments

The hydro-distillation set with Clavenger-type apparatus contained a still, condenser, and separator. The GC-MS Shimadzu QP 2010 SE was used to analyze the essential oils, the column Rtx-5MS (length 30.0 m, inside diameter 0.25 mm, thickness 0.25 μm). The detector is equipped with mass spectroscopy. A microplate reader (Allsheng®, China) was used to measure α-amylase and α-glucosidase inhibitions.

Materials

Inflorescence of torch ginger (*E. elatior*) was purchased in a traditional market in Guguak, Solok District, West Sumatra, Indonesia. The α-glucosidase assay used substrate enzyme from *Saccharomyces cerevisiae* (Sigma-Aldrich Chemical), p-nitrophenyl-α-D-glucopyranoside. The α-amylase assay used α-amylase enzyme from *Bacillus licheniformis* (Sigma), soluble starch (Merck) as a substrate, 3,5-dinitrosalicylic acid (DNSA) (Himedia), and aquabidest. The dimethylsulfoxide (DMSO) and phosphate buffer solution (PBS) were used to dissolve and dilute the sample, enzyme and substrate. Acarbose (Sigma) was used as a positive control.

Extraction of Essential Oils

The sample of five kg of torch-ginger inflorescence was chopped, and the essential oils were extracted by hydro-distillation. The extraction of essential oils was done for six hours after the first drop of condensed water appeared during the extraction process. Then, the essential oils were collected and separated from water. The separated essential oils were added with sodium sulphate anhydrous to remove water from the oils. Isolated essential oils were stored in a dark bottle at 4ºC for further use (Susanti et al., 2013).

Analysis of Essential Oils using Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS Shimadzu runs start and end time from 0.00 to 19.00 min, a scan speed of 1250, and start and end m/z from 40.00 to 400.00. Analytical condition: injector temperature 200ºC, oven temperature programmed from 60 to 200ºC at the rate 10ºC; carrier gas helium, pressure 36.1 kPa, total flow 101.2 mL/min, column flow 0.75 mL/min, linear velocity 31.6 cm/sec, purge flow 3.0 mL/min; split ratio 130.0, the identification of the components was performed by comparison of their retention times and mass on computer matching against commercial (WILEY) library.

Determination of α-Glucosidase Inhibition Activity

Inhibition of enzyme α-glucosidase (from *Saccharomyces cerevisiae*, Sigma-Aldrich Chemical) was measured as previously described by Yang et al. (Simões-Pires et al., 2009; Syafni et al., 2023; Yang et al., 2015) with some modification. Samples were dissolved in DMSO with reaction concentrations of 250, 125, 62.5, 31.25, and 15.63 μg/mL in a phosphate buffer solution (PBS) mixture and DMSO 1%. Acarbose was prepared at the same concentration as in previous research (Syafni et al., 2023).

The sample and acarbose (each 50 μL, triplicate) were placed into 96-well plates, mixed with 50 μL enzyme (0.26 U/mL) and incubated for 10 min at room temperature, 27ºC. Afterwards, a 100 μL substrate (p-nitrophenyl-α-D-glucopyranoside dissolved in PBS pH 6.9) was added and incubated for 20 min at room temperature. Control was divided into sample control and buffer control; sample control contained the sample without enzyme, and buffer control contained the buffer without enzyme. All sample and control absorbances were measured at 405 nm in a microplate reader. The following equation calculates the percentage of inhibition :

Inhibition percentage (%) = (1-As/Ac) x 100%

As = absorbance of sample – control sample absorbance

Ac = (absorbance of mixture PBS, enzyme and substrate) – (absorbance of mixture PBS and substrate)

Determination of α-Amylase Inhibition Activity

The α-amylase inhibition was measured using a microplate reader following the

Wickramaratne method and previous research at room temperature (Miller, 1959; Syafni et al., 2023; Wickramaratne et al., 2016). The sample and acarbose were prepared similarly to the α-glucosidase inhibition activity preparation and concentration. Each sample was taken in 100 μL in triplicate and then incubated with the enzyme (10 U/mL) for 10 minutes. Afterwards, 10 μL of starch 1% dissolved in PBS was added and incubated for three minutes. The reaction was stopped by adding 100 μL DNSA after incubating and heating at 105ºC for 20 minutes; the last 500 μL was aquabidest. The measurement was done at 540 nm. The sample control was a sample without enzymes. The blank is used with PBS's enzyme and substrate (Ac). The following formula calculates the percentage of inhibition:

Inhibition percentage (%) = (1-As/Ac) x 100%

RESULTS AND DISCUSSION

Essential oils from torched ginger

Hydro-distillation of five kg of inflorescence of torch ginger gained 3 mL of essential oils after adding sodium sulphate anhydrous. The yield of essential oils from torch ginger was meagre, with a colourless liquid. This is also supported by some research on fresh flowers of torch ginger with a yield of 0.086% (Susanti et al., 2013). However, the yield of essential oil from the dried flower was reported to be 0.67%, with white or colourless oil (Wijekoon et al., 2013).

The range of the distillation process was also different; the fresh flowers were distilled for 8 hours, and the dried flowers were only 2-3 hours. However, a different extraction method of essential oils from torch ginger was done by supercritical CO_2 extraction (Marzlan et al., 2020). Variations in the extraction method and the long time of extraction impact the yield and composition of the essential oils in torch ginger inflorescence (Marzlan et al., 2020; Susanti et al., 2013; Wijekoon et al., 2013). Several compounds, including the five more amounts from the essential oils of torch ginger, were 1-dodecanol, dodecanal, α-pinene, decanal, and dodecanol acetate, with percentages of 39.79, 28.20, 13.11, 4.16, and 2.75 %, respectively. The result of the GC-MS analysis of essential oils is presented in Figure 1 and Table 1.

Another method with the hydro distillation of fresh samples reported dodecanal as the highest percentage at 6.87%, followed by 1-decanol (16.27%), n-dodecyl acetate (16.40%), cis-9-tetradecen-1-ol (16.29%), and 1-hexadecanol (16.34%) (Susanti et al., 2013). The dried flower with the same extraction method showed the composition of compounds 1-dodecanol, dodecanal, dodecanoic acid, hexadecane-1-ol, trans-9-dodecan-1-yl acetate with a percentage of 25.17, 17.5, 20.40, 12.73, 8.51%, respectively (Wijekoon et al., 2013). The primary composition of essential oils with supercritical CO_2 extraction was the same as water distillation, 1-dodecanol (23.895). However, the following substances had some different percentages as follows: lauryl acetate (21.51), cis-9-tetradecenoic acid, heptyl ester (7.96), pimelic acid, hexyl octadecyl ester (7.66), tetradecanol (6.45), (E)-9-tetradecen-1-ol acetate (5.19) (Marzlan et al., 2020).

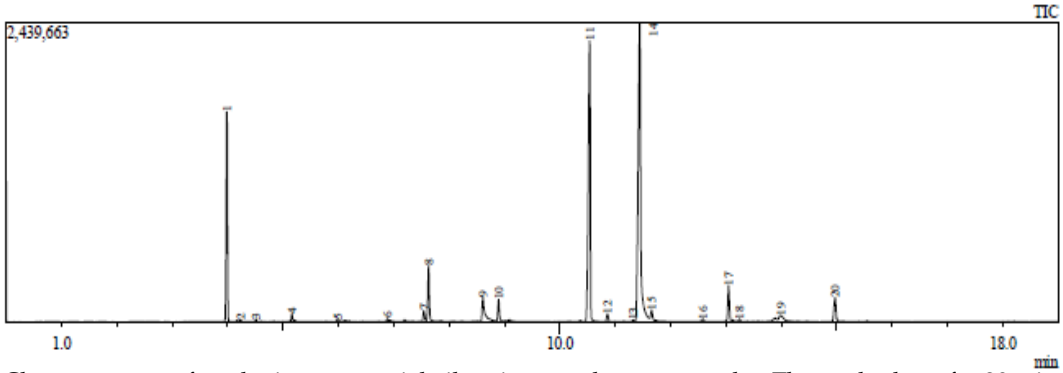

Figure 1. Chromatogram of torch ginger essential oils using gas chromatography. The method ran for 20 minutes, and the peak showed the abundance of separate essential oil components

Result of α-glucosidase and α-amylase inhibition activity

The activity of essential oils from the inflorescence of torch ginger is reported to have antibacterial, antioxidant, and photo-protective potential (Khor et al., 2017; Marzlan et al., 2020; Susanti et al., 2013; Vairappan et al., 2012; Wijekoon et al., 2013). The antibacterial activity of essential oils from the inflorescence of torch ginger showed less inhibition toward Gram-negative bacteria than Gram-positive bacteria (Susanti et al., 2013; Wijekoon et al., 2013). Essential oils from the flower of torch ginger inhibited Gram-negative bacteria *Klebsiella pneumoniae* with a MIC value of 1.56 mg/mL (Wijekoon et al., 2013). The dried sample of essential oils reported no inhibition against yeast and fungi

compared to fresh flower extraction (Susanti et al., 2013; Wijekoon et al., 2013). Essential oils from the inflorescence of torch ginger showed inhibition against Gram-positive bacteria such as *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Listeria monocytogenes*. The antibacterial activity of essential oils from the inflorescence of torch ginger indicated its potential as a food preservative against foodborne pathogenic bacteria. Several compounds responsible for antimicrobial activity in essential oils from the inflorescence of *E. elatior* are α-pinene (Salehi et al., 2019). The compounds that were reported as antifungal were dodecanol (Davis-Hanna et al., 2008). Essential oils from *E. elatior* inflorescence have limited reports on the bioactivity as antidiabetics instead of its extract.

Table 1. The Torch Ginger Essential Oil Components

Peak	Retention Time (Rt)	Similarity Index (SI)	Area %	Compound Name
1	3.987	99	13.11	α-pinene
2	4.232	94	0.16	Verbenene
3	4.510	95	0.21	2-β-pinene
4	5.162	96	0.55	Limonene
5	5.993	85	0.26	α-terpinolene
6	6.886	96	0.23	Camphor
7	7.543	97	0.90	α-terpineol
8	7.629	98	4.16	Decanal
9	8.610	98	2.56	1-decanol
10	8.893	98	1.62	2-undecanone
11	10.538	96	28.20	Dodecanal
12	10.86	96	0.67	Trans-caryophyllene
13	11.315	92	0.2	α-humulene
14	11.442	98	39.79	1-dodecanol
15	11.656	81	0.75	d-nerodiol
16	12.584	95	0.20	Nerolidol B (cis or trans)
17	13.046	95	2.75	Dodecanol acetate
18	13.24	77	0.27	Ketene dimers
19	13.985	96	0.85	Tetradecanol
20	14.968	91	2.54	Zerumbone
Total			**100**	

Table 2. The IC_{50} value of inhibition against the enzymes α-glucosidase and α-amylase

No.	Sample	α-glucosidase inhibitor (μg/mL)	α-amylase inhibitor (μg/mL)
1	Essential oils	73.95	70.87
2	Acarbose	67.55	12.28

A study on essential oils from the inflorescence of torch ginger showed inhibition against α-glucosidase and α-amylase enzymes with the IC_{50} value of 73.95 and 70.87 μg/mL, respectively (Table 2). Essential oils from *E. elatior* inflorescence had lower inhibition towards acarbose against both testing enzymes. The potential of torch ginger flower as an antidiabetic was reported, and aqueous extraction was done to extract the targeted compounds from the sample (Nor et al., 2020).

The aqueous extract was tested against α-glucosidase and α-amylase enzymes (*in vitro*) and *in vivo* using a type-2 diabetes mellitus rat model. Both methods possessed the potential to be antidiabetic and maintain postprandial glucose levels. The possible compounds that are responsible for the activity are flavonoids and quercetin. However, potential essential oils as antidiabetics have been reported from clove, peppermint, lavender, oregano, *Nigella sativa*, *Thymus kotschyanus, Salvia officinalis, Citrus aurantifolia*, black pepper, rosemary, and many more (Bungau et al., 2023).

Most essential oils were tested using an enzymatic assay against the α-glucosidase and the α-amylase enzymes. The study of essential oils from *Nigella sativa* and *Cuminum cyminum* has been conducted in a clinical study of type-2 diabetes mellitus patients. For the case of essential oils from the florescence of *E. elatior*, there was a limited report on its inhibition against α-glucosidase and α-amylase

enzymes. The possibility compounds in essential oils of *E. elatior*, such as *α*-pinene, were reported in mice.

The major compound and its derivatives from the essential oils of *E. elatior* inflorescence need to be measured because of the lack of data. The concentration of *α*-pinene of 0.25 mL/kg showed reduced fasting blood glucose levels in diabetic mice during the observation from 1 to 24 hours compared to control and Glibenclamide (Özbek & Yılmaz, 2017). Current research also reported the potential of hypoglycemia of *α*-pinene in diabetic rats.

This research was also supported by experiments in HepG2 cells and murine skeletal muscle cells (Choghakhori et al., 2024; Feriotto et al., 2024). A study on HepG2 cells showed the synergic effect of *α*-pinene in decreasing inflammation and oxidative stress, similar to a study in rats (Choghakhori et al., 2024; Özbek & Yılmaz, 2017). Murine skeletal muscle cells express the glucose transporter of glucose in the cells and are responsible for glucose uptake into insulin-sensitive cells (Feriotto et al., 2024).

CONCLUSION

Essential oils from the inflorescence of *Etlingera elatior* showed their potential to inhibit *α*-glucosidase and *α*-amylase enzymes. The responsible compounds in the essential oils for the *in vitro* activity were probably *α*-pinene. However, there is limited reporting on the antidiabetic effects of the primary compound derived from the essential oils of *E. elatior* inflorescence.

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