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Preliminary Study: Bioautography Screening on Edible Local Plants with α-Glucosidase Inhibitor

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ABSTRACT
Indonesia is ranked as one of the top ten countries with higher diabetes mellitus cases in 2019. In total, International Diabetes Federation recorded 10.7 million diabetes mellitus cases in 2019. Many researchers reported that diabetes mellitus type 2 could be controlled and managed with diet. Since local food is made from common plants that people can easily find, we collected edible local plats from a small village in Solok Regency and screened their inhibition toward enzyme α-glucosidase. Fourteen local edible plants were collected for the screening of α-glucosidase inhibitors. The plants were macerated with methanol to gain methanolic extract. The extracts were spotted at a concentration of 10 mg/mL with a volume of 8 μL on a silica TLC plat and then eluted with semi-polar mobile phase, chloroform: ethyl acetate: methanol = 65:20:15. Afterward, the TLC plate was sprayed with α-glucosidase inhibitor. The results showed that two samples were inhibited by α-glucosidase. They were rice (*Oryza sativa* L.) and torch ginger (*Etlingera elatior* (Jack) R.M.Sm.). The active spots in rice had rf values at 0.66 and 0.81, while torch ginger had rf values at 0.23, 0.25, and 0.63.

Keywords: *diabetes mellitus type 2, TLC bioautography, rice, local vegetables*

1. INTRODUCTION

Based on International Diabetes Federation (IDF) data, worldwide diabetes mellitus cases reached 463 million in 2019, where the age of patients ranged from 20 to 79 years old [1]. The cases are predicted to be increasing every year in every country [2,3]. Type 2 diabetes mellitus (DM) is more than 90% of all diabetes cases worldwide, including in Indonesia. The management of type 2 diabetes is one of the challenging steps in order to delay its complication. The complication can damage many organs and body functions such as the brain, heart, blood vessels, kidneys, eye, nerves, foot, and also sexual function [4]. Lifestyle and non-pharmacological attention are effective in preventing and developing the burden complications of type 2 DM [5].

The α-glucosidase inhibitor is one of the type 2 DM drug targets that decrease postprandial hyperglycemia [6,7]. This enzyme works in the brush border of the enterocytes that line the intestinal villi by binding to these enzymes preventing the breakdown of complex carbohydrates into absorbable glucose [8,9]. Mechanism of action α-glucosidase inhibitor is a suitable approach for people who consume more carbohydrates in their daily life. The side effect of the α-glucosidase inhibitors is abdominal discomfort because of delayed carbohydrates absorption. However, this side effect is still tolerable.

Using local food in their normal diet can be a possible approach for the management of type 2 DM in developing countries. Many researchers have been reported for applying edible plants toward α-glucosidase inhibitor [10,11]. Most of the Solanaceae family, which are common food around the globe, were reported for their α-glucosidase inhibitor [10]. It has been reported through various methods that vegetables, fruits, spices, and milk have anti-diabetes activities against diabetes mellitus, for example, *Ipomea batatas*, *Momordica charantia*, *Allium cepa ascalonicum*, *Luffa acutangular*, *Cucurbita maxima*, *Mangifera indica*, *Aegle marmelos*, *Curcuma longa*, etc. [10,11,12]. Since the geographical and variety of plants could influence secondary metabolites in many aspects, so the activities of the same plant can be varied in different areas.

In this research, we did screening on local plants that have been consumed for a century. The collected plant extracts were tested using TLC-bioautography for α-glucosidase inhibition. TLC profiles of the plant extracts

were focused on semi-polar compounds that could isolate the responsible compounds for the activity.

2. METHODS

2.1. Plant collection

The collection was conducted in the Batu Banyak Village, Lembang Jaya District, Solok Regency, West Sumatra, Indonesia. Samples were chosen based on the local food that people consume every day. The collected plants were identified by Dr. Nurainas in Herbarium (ANDA) Universitas Andalas.

2.2. Material

Distilled methanol was used to macerate the samples. The macerates were evaporated in a rotary evaporator (BUCHI®). Merck KGaA TLC silica gel 60 F_{254} was applied for TLC profile and bioautography screening.
Chloroform p.a, distilled ethyl acetate, distilled MeOH was used for TLC mobile phase. Methanol (HPLC grade) was used to solve the extracts.
Reagent for visualization of TLC spot was a mixture of EtOH p.a (Merck) : acetic acid p.a (Merck) : anisaldehyde (Merck) = 360:160:12 and then added 2% of sulfuric acid p.a (Merck).
α-Glucosidase from Sigma, 2-naphthyl-α-D-glucopyranoside (Sigma) dissolved in ethanol p.a and the Fast Blue salt B (Sigma) dissolved in distilled water were prepared separately.

2.3. Extraction process

All collected plants were freshly chopped into small pieces. Briefly, 50 g of each sample was macerated in methanol for three days and repeated three times. Each combination macerate was evaporated *in vacuo* to gain dry extracts. The extracts were stored in glass vials at 5°C before doing the extract screening.

2.4. TLC Bioautography method

Each extract was dissolved in methanol to gain a concentration of 10 mg/mL and spotted 8 µL on a TLC plate. The mobile phase was mixture of $CHCl_3$:EtOAc:MeOH = 65:20:15.
Bioautography for α-glucosidase inhibitor was adopted from Simoes-Piere, et al., 2009 [13].

3. RESULTS AND DISCUSSION

Fourteen samples (Table 1.) have been collected from the Batu Banyak Village which most people here are working as a farmer. The plants were collected based on the part that people consume in their daily life.

TLC-bioautography screening was tested for α-glucosidase inhibitor with established protocol [13]. The optimized mobile phase was used to target semi-polar compounds based on a semi-polar eluent that showed an appropriate TLC profile of extract screening. Each TLC plate for phytochemical and α-glucosidase bioautography was eluted at the same chamber. The TLC plates were observed under UV wavelength at 254 and 360 nm (Figure 1 and 2). In order to observe invisible compounds under UV-Vis light, an anisaldehyde sulfuric-acid reagent was used to visualize the compounds [35]. This reagent gives purple color for triterpenoid and dark bluish-green color for some possible compounds. The dark bluish-green color can indicate a mixture of a group of compounds such as allylic alcohols, monoterpenes, amines aldehydes, ketones, carbohydrates, and esters [36,37]. The UV inactive compounds from the TLC profile were detected and depicted in Figure 3.

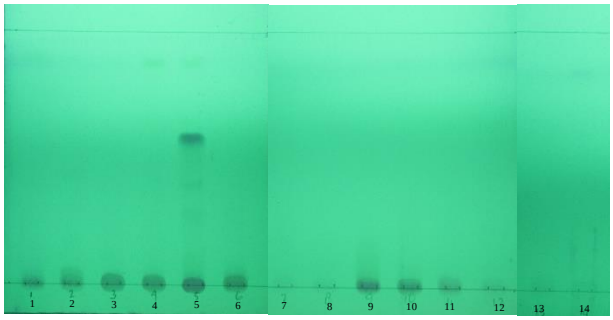

Figure 1. TLC-plate of 14 extracts observed under UV λ 254 nm, sample numbering based on table 1.

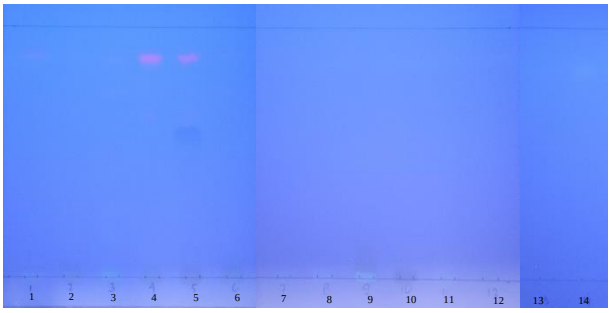

Figure 2. TLC-plate of 14 extracts observed under UV λ 360 nm, sample numbering based on table 1.

Table 1. List of collected plant

No.	Latin name (Family)	Part of the plant	Secondary metabolite	Ref
1.	*Monochoria vaginalis* (Burm. f.) C. Presl ex Kunth (Pontederiaceae)	young leaves	(-)-loliolide, 3-oxo-α-ionol, dehydrovomifoliol, 24-methylenelophenol, 4α-methyl-5α-ergosta-7,24(28)-diene-3β,4β-diol, stigmast-4-en-3-one, 6β-hydroxystigmast-4-ene-3-one, cyclolauden-3β-ol, vomifoliol, (20*R*,24*R*)-campest-5-ene-3β,4β-diol, (10*Z*)-1-(2,6-dihydroxyphenyl)octadec-10-en-1-one.	[14]
2.	*Limnocharis flava* L. (Limnocharitaceae)	young leaves	not well reported	
3.	*Solanum torvum* Sw. (Solanaceae)	fruit	tyrosol, 3-hydroxybenzoic acid, sesamol, 4-hydroxybenzoic acid, protocatechuic aldehyde, 2-hydroxybenzoic acid, 4-ethylcatechol, estragole, anethole, pelargonidin 3-O-rutinoside, isorhoifolin, rhoifolin, arctigenin, epirosmanol, avenanthramide 2f, phlorin, eugenol, 2-methoxy-5-prop-1-enylphenol	[15]
4.	*Enhydra fluctuans* Lour. (Asteraceae)	young leaves	4',5,6,7-tetrahydroxy-8-methoxyisoflavone-7-O-β-D-galactopyranosyl-(1→3)-O-α-D-xylopyranosyl-(1→4)-O-α-l-rhamnopyranoside	[16]
5.	Toona sureni Merr. (Meliaceae)	young leaves	Surenone, surenon, tetranortriterpenoid, methyl gallate, surenolacton	[17], [18], [19]
6.	*Paederia* sp. (Rubiaceae)	young leaves	Iridoid glucosides	[20], [21]
7.	*Luffa acutangular* (L.) Roxb. (Cucurbitaceae)	fruit	2,3-dihydro,3,5-dihydroxy-6-methyl-(4H)-pyran-4-one, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, (3b, 20R)-cholest-5-en-3-ol, 9,12,15-octadecatrienoic acid methyl ester, citronellyl tiglate, ascorbic acid, carotene	[22]
8.	*Momordica charantia* L. (Cucurbitaceae)	fruit	Steroid charantin, charantoside VI, kuguaglycoside C, goyaglycoside D, charantagenins D, charantadiol A, goyaglycoside b, charantagenins E, momordicosides, momordicoside K, acylglucosylsterols, 7-oxostigmasta-5,25-diene-3-O-β-D-glucopyranoside, stigmasta-7,25(27)-dien-3β-ol, 3β,25-dihydroxy-5β,19-epoxycucurbita-6(23E)-diene, linolenoylglucopyranosylclerosterol, α-eleostearic acid, phenolic acids, flavonoids, alkaloid fraction,	[23], [24]
9.	*Leucaena leucocephala* (Lam.) de Wit (Fabaceae)	fruit	Mimosine, chatechin, flavonoids	[25]
10.	*Etlingera elatior* (Jack) R.M.Sm. (Zingiberaceae)	inflorescence	Essential oils from group of terpene hydrocarbons, oxygenated compounds	[26]
11.	*Sauropus androgynus* (L.) Merr. (Phyllantaceae)	young leaves	(+)-isolariciresinol 3α-O-β-glucopyranoside, (_)-isolariciresinol 3α-O-β-glucopyranoside, (+)-syringaresinol di-O-β-glucopyranoside, liriodendrin, guanosine, corchoionoside C, megastigmane glucose sauroposide, kaempferol glycosides, essential oils	[27], [28]
12.	*Musa paradisiaca* L. (Musaceae)	inflorescence	(24R)-4α,14α,24-trimethyl-5α-cholesta-8,25(27)-dien-3β-ol	[29]
13.	*Leucocasia gigantea* (Blume) Schott (Araceae)	petiole	α-amyrin, β-amyrin, monoglyceryl stearic acid, penduletin	[30]
14.	*Oryza sativa* L. (Paoceae)	fruit	Oryzabran A, oryzabran B, oryzabran C, oryzabran D, cycloartenol, 24-methylene cycloartenol, β-sitosterol, campesterol, tricin, tricinin, ferulic, coumaric, sinapic, protocathecuic, chlorogenic, hydroxybenzoic, vanillic, syriginic, caffeis, gallic acid, tocotrienol (vitamin E), γ-sitosterol, momilactone A and B	[31], [32], [33], [34]

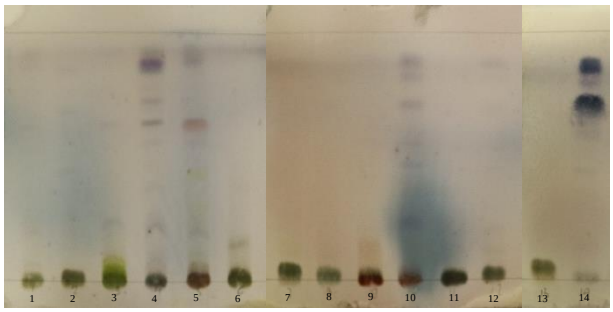

Figure 3. Visualization of TLC-plate of 14 extracts with anisaldehyde reagent, number based on table 1.

The TLC bioautography of collected samples gave white spots in the cycle of the TLC plate, which indicated α-glucosidase inhibition (Figure 4). The active spots were exhibited by samples number 10 and 14 on TLC plates, which are *Etlingera elatior* and *Oryza sativa.* All active spots were invisible under UV-Vis light. Visualization with anisaldehyde-sulfuric acid reagent *Etlingera elatior* was detected with three purple spots with rf values at 0.23, 0.25, and 0.63. Rice extract showed a dark bluish-green color with rf values at 0.66 and 0.81.

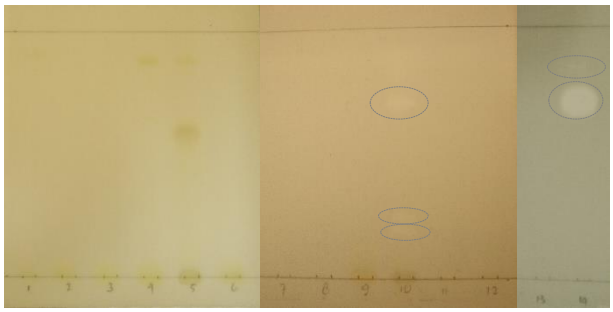

Figure 4. TLC bioautography assay of 14 extracts displayed white spots indicating inhibition toward α-glucosidase in sample numbers 10 and 14.

A review on candidate plants for diabetes treatments reported 27 potential families as bioactive secondary metabolites against α-glucosidase inhibitors [38]. The result of extract screening showed that 2 out of the 27 known plant families inhibited the enzyme. Both of them were Zingiberaceae for *E. elatior* and Poaceae for *O. sativa*. The TLC visualization for active compounds of both plants indicated the presence of triterpenoids and or steroids. Hence, most of the potential group of compounds toward α-glucosidase inhibitor was from the terpene group [38,39]. For instance, akebonic acid is from triterpenoids, taxane type diterpenoids, and abietane type diterpenoids are from diterpenoids. The next potential group of compounds for α-glucosidase inhibitor was flavones, diarylheptanoids, and coumarins followed as an α-glucosidase inhibitor with percentages of 7, 6, and 6, respectively [38].

E. elatior is native to Malaysia and Indonesia, where people consume young inflorescences as a vegetable. It has been reported for antioxidant, antimicrobial, anticancer, antihyperglycemic, antihyperuricemic, anti-inflammatory activities [40,41,42]. Chemical substances from *E. elatior* consist of glycoside and aglycon flavonoids, phenols, saponins, tannins, steroids, and terpenoids. It also possesses essential oil that is potential for preservative [43,44]. By having α-glucosidase inhibition screening and the possibility of terpenoids as active compounds, there would be a possibility for continuing to isolate the active substances.

Rice was first reported in 1986 by Japanese researchers to have possessed hypoglycemic substances, glycan group of compounds [31,45]. Different types of compounds from rice, triterpene alcohols, and sterols were then informed to decrease postprandial hyperglycemia *in vivo* and in humans [32,33]. The γ-oryzanol is known as a compound that is responsible for some activities of the rice [34]. However, the result of the 14 extracts screening showed that the rice sample was invisible under UV-Vis. It was possible that the compounds were a part of the triterpenoid group. Rice samples were collected without following the refining process by milling, and it was one of the favorite rice variants in Solok district with the local name “bareh sokan”. Continuation of this investigation is still needed since the active compounds in the selected samples are not defined yet.

The report on antidiabetic plant extract from some selected plants was reported based on the results of different methods. One of the well-known plants is a fruit called bitter melon, known in Latin as *Momordica charantia*; it was reported for *in vivo* insulin enhancement [23]. Since the antidiabetic drugs have various mechanisms of action [46], there is a possibility of negative results if applying different assay screening.

4. CONCLUSION

Etlingera elatior showed inhibition toward α-glucosidase, although the rice was observed to have more significant white spots on the TLC plate. The group of compounds that gave the activity was predicted as terpenes, triterpenoids, more specifically for *E. elatior*, and a mixture of compounds for the rice. Further

research is needed to identify the responsible compounds for α-glucosidase inhibitors.

AUTHORRS' CONTRIBUTIONS

NS performed sample collection, extraction, TLC, analyzed the data and wrote the manuscript. NA performed bioautography for the α-glucosidase inhibitor. FI and DPP supervised the project. All authors have checked and agreed the manuscript to pulish.

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