Stigmasterol isolated from the ethyl acetate fraction of *Morinda citrifolia* fruit (using the bioactivity-guided method) inhibits α-amylase activity: *In vitro* and *in vivo* analyses

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Abstract. Stigmasterol, a bioactive phytosterol that is present in numerous plants, has been explored for its antidiabetic activity. The present study extracted, fractionated, isolated and characterized the isolate in the ethyl acetate fraction of *Morinda citrifolia* fruit and investigated its potency as an inhibitor of α-amylase. The bioactivity-guided antidiabetic assay revealed that all fractions (A-G) exhibited notable differences with the negative control. However, fraction C reduced blood sugar levels in Swiss-Webster male mice with streptozotocin-induced diabetes by 43.08%, and was further purified to obtain the isolate. The chemical structure of the isolate was determined by employing proton nuclear magnetic resonance (NMR), carbon-13 NMR, distortionless enhancement by polarization transfer and liquid chromatography-mass spectrometry, and was confirmed as stigmasterol. An *in vitro* assay revealed that stigmasterol exhibited potent inhibitory activity against human α-amylase activity with an IC_{50} value of 10.29±0.76 μg/ml, compared to that of the fruit extract and acarbose, which were 14.16±5.72 and 43.37±1.56 μg/ml, respectively. On the whole, the present study demonstrates that both *Morinda citrifolia* fruit extract and stigmasterol isolated from the ethyl acetate fraction of the fruit may have the potential to be developed as an anti-diabetic drugs due to their inhibitory effects on α-amylase activity. However, further studies are required to guarantee its efficacy and safety for humans.

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Key words: diabetes mellitus, hypoglycemic activity, metabolic disease, *Morinda citrifolia*, noni fruit, phytosterols

Introduction

In Indonesia, diabetes mellitus (DM) constitutes a serious health concern. With >10 million diabetic patients, Indonesia was confirmed to have a prevalence rate of 6.2% (1). DM, particularly type 2 DM (T2DM), is associated with the increased production of reactive oxygen species and disruptions in the antioxidant defense system, resulting in oxidative damage. DM is also characterized by an abnormal increase in blood glucose levels and impaired carbohydrate, lipid and protein metabolism due to inadequate insulin secretion and/or action (2). Maintaining stability and reducing blood glucose levels can be accomplished by delaying glucose absorption in the digestive system via the inhibition of carbohydrate hydrolyzing enzymes, such as α-glucosidase and α-amylase. α-amylase hydrolyzes the α-bonds of α-linked polysaccharides, such as starch and glycogen, releasing glucose and maltose into the circulation. It is the most common type of amylase observed in humans and other mammals. The inhibition of α-amylase attenuates the digestion process by reducing starch breakdown in the stomach; hence, it can be utilized as an effective strategy to control hyperglycemic conditions (3).

Stigmasterol, a bioactive phytosterol, has been explored for its antidiabetic activity; e.g., stigmasterol isolated from *Glycine max* oil has been shown to exhibit a weak glucose transporter type 4 (GLUT4) translocation activity in rat skeletal myoblast cells (4). Stigmasterol isolated from *Gelidium spinosum* seaweed has also been found to exhibit antioxidant and α-amylase inhibitory activity (5). In addition, stigmasterol isolated from *Pseuderanthemum palatiferum* leaves has been shown to exert a hypoglycemic effect in rats with streptozotocin (STZ)-induced diabetes (6). Furthermore, stigmasterol and β-sitosterol isolated from banana pseudostem have been found to reduce fasting blood glucose levels in rats with alloxan-induced diabetes (7). Another study demonstrated that stigmasterol isolated from the aqueous ethanol root extract of *Bridelia duvigneaudii* exhibited hypoglycemic activity in albino mice with diabetes induced by oral glucose intake (8). Previously, β-sitosterol and stigmasterol isolated from the roots of *Indigofera heterantha* were measured for their antidiabetic activity on the basis of the glucose uptake in yeast cells (9).

Moreover, the ethanol extract of noni fruit (*Morinda citrifolia*; *M. citrifolia*) was previously shown to decrease blood sugar levels in mice with STZ-induced diabetes (10). Another study also demonstrated that the juice of *M. citrifolia* fruit administered to patients with T2DM resulted in an improvement in blood glucose levels and other pathological parameters (11).

Generally, the identification of plant-based anti-diabetic drugs is challenging; therefore, the present study aimed to extract and isolate the bioactive compound in *M. citrifolia* fruits and to investigate its potency as an α-amylase inhibitor.

Materials and methods

Chemicals. The solvents used for the extraction and isolation process were 96% ethanol (Brataco Chemica, http://bratachem.com/), n-hexane (MilliporeSigma), ethyl acetate (MilliporeSigma), methanol (MilliporeSigma) and chloroform (MilliporeSigma). Thin layer chromatography (TLC) was carried out using pre-coated silica gel 60 F254 plates with a thickness of 0.20 mm (MilliporeSigma). The human α-Amylase Inhibitor Screening kit (cat. no. K482-100, BioVision, Inc.), which hydrolyzes the synthetic substrate and yields a chromophore, was used for *in vitro* testing. Nuclear magnetic resonance (NMR) analyses, which included proton (^{1}H)-NMR, carbon-13 (13C)-NMR and distortionless enhancement by polarization transfer (DEPT) were performed using deuterated solvents (acetone-d6, CD_3OD, and/or $CDCl_3$) on 400 mHz NMR (JNM-ECZ500R/S1, JEOL, Ltd.) with tetramethylsilane (MilliporeSigma) as an internal reference.

Plant materials. The fresh ripe fruits were harvested in August, 2019 (outside temperature, 25 to 30˚C) at Kendari (Google coordinates: -4.045723429369156; 122.57746055261806), Southeast Sulawesi, Indonesia. The fruits were taxonomically identified at the College of Life Sciences, Bandung Institute of Technology (Bandung, Indonesia), and were confirmed as *M. citrifolia* L. (sample no. 66/I1.CO2.2). The characteristics of the plant samples matched those described in the flowering plants' taxonomic references (12-14).

The fruits were washed under tap water to separate any soil, dust, and other foreign inorganic matter, and were cleaned using standard pharmacognosy laboratory procedures, e.g., medicinal plant materials should be entirely free from visible signs of contamination by molds or insects and other animal contamination, including animal excreta. No abnormal odor, discoloration, slime, or signs of deterioration should be detected (15). The clean fruits were dried at 40˚C without being exposed to sunlight. The dried samples were mashed using a pestle in a white porcelain mortar and sealed in a plastic container before being further processed.

Extraction and isolation. Dried *M. citrifolia* coarse powder fruit (3,064 g) was macerated with 20 liters of 96% ethanol three times for 24 h to obtain 540 g extract (yield, 17.62% w/w). The extract was then thickened using a vacuum rotary evaporator (Buchi) and further diluted in ethyl acetate and divided into soluble (338 g or 62.59%) and insoluble (338 g or 37.40%) partitions. The insoluble ethyl acetate partition contains polar molecules (glycosides) that can disrupt the isolation process.

The ethyl acetate extract was then fractionated using vacuum liquid chromatography apparatus (consisting of a glass Buchner filter funnel and a 10-cm length glass column connected to a vacuum pump) using 21 mobile phases or eluents (the volume of each solvent is 150 ml) i.e., n-hexane, a combination of n-hexane and ethyl acetate in the ratios of (9:1), (8:2), (7:3), (5:5), (3:7), (2:8), ethyl acetate and methanol, on a 10-cm column with a silica gel stationary phase (Silica gel 60 Merck® for column chromatography; CAS no. 7631-86-9; MilliporeSigma) (250 g), yielding 21 fractions observed in the chromatogram pattern using thin layer chromatography. The fractions with the same stain pattern were merged based on the chromatograms and were tested for their hypoglycemic activity following the bioactivity guide method.

Bioactivity-guided in vivo anti-diabetic assay. A total of 30 male Swiss-Webster mice (*Mus musculus*), weighing 20-30 g, were adapted to a controlled temperature condition at 28-30˚C under a 12-h dark/light cycle (light was turned on from 6 a.m. to 6 p.m.), with daily standard food and water freely available, for 1 week prior to treatment. The health and behavior of the mice were observed daily and their cages were cleaned every 2 days to remove the feces and urine. No animals were found dead during the acclimatization and the *in vivo* experiments. Animal handling, maintenance and euthanasia procedures were performed as approved by the Ethics Committee of Halu Oleo University, Kendari, Indonesia. Following 1 week of acclimatization, the mice were fasted for 18 h and their fasting blood glucose levels were measured using a glucometer (ACCU-CHEK Inform II, Roche Diagnostics). All the mice were then administered STZ at a dose of 65 mg/kg body weight intraperitoneally to induce diabetes; this was followed by a period of modification (16). STZ [chemical name, 2-deoxy-2-(3-(methyl-3-nitrosourea)-D -glucopyranose] is produced by *Streptomycetes acromogenes*. Following an intraperitoneal or intravenous administration, this antibiotic enters the pancreatic β-cell through the Glut-2 transporter and induces the alkylation of DNA (17).

At 48 h following the STZ administration, the blood glucose levels of the mice were measured by obtaining blood from the tail vein, and mice with blood glucose levels >200 mg/dl were randomly assigned to 9 groups, with 3 mice in each group in one cage, as follows: The negative control group (treated with a suspension of sodium carboxymethylcellulose 0.5%); the drug control group (treated with exogenous insulin 1 IU/kg body weight subcutaneously); and seven treatment groups of fractions A-G (150 mg/kg body weight) in a suspension of sodium carboxymethylcellulose 0.5% by oral administration using oral gavage feeding every afternoon at 2 p.m. for 14 consecutive days.

The blood glucose levels of the animals were measured on days 1, 3, and 7 after treatment. The reduction in blood glucose levels was calculated using the following formula:

$$\text{Reduction in blood glucose level} = \frac{\textit{Initial Glucose level} - \textit{Glucose level after treatment}}{\textit{Initial Glucose level}} \times 100$$

The results of the present study are expressed as the mean ± SEM. At the end of the study period, the mice were sacrificed using isoflurane 2% inhalation 1 liter/minute for >4 h following a procedure described elsewhere with a

modification of the dose (18). Animal death was verified by the absence of respiration and heartbeat for a period of >5 min.

Purification and isolation of the compound from the active fractions with hypoglycemic activity. Active fractions with hypoglycemic activity were further purified using a radial chromatographic method (The Chromatotron™ model 7924T), followed by ^{1}H-NMR, 13C-NMR, and DEPT analyses using the JEOL JNM-ECZ500R/S1 (JEOL, Ltd.) instrument, and were validated using liquid chromatography-mass spectroscopy (LC-MS Waters ACQUITY UPLC I-Class in tandem with the Xevo G2-X2 Quadrupole Time-of-Flight Mass Spectrometer; Waters Corporation), to elucidate the chemical structure of the isolate.

In vitro α-amylase inhibitory activity assay. The α-amylase inhibitory activity of *M. citrifolia* fruit extracts and the isolate were assayed using the α-Amylase Inhibitor Screening Kit (BioVision Inc.). Acarbose provided in the enzyme kit (BioVision Inc.) was employed as the control drug and dimethyl sulfoxide (DMSO) as a blank solvent. Each sample was dissolved in DMSO to generate a concentration of 150, 75, 37.5, 18.75, and 9.375 ppm for each sample. A total of 50 μl of the sample (5x) was added to designated wells of a clear 96-well microplate. The preparation of the reagents and the procedure of this assay were performed by following the instructions provided with the kit. The microplate was incubated at 37˚C for 25 min before being measured at 405 nm.

The α-amylase inhibitory activity was calculated using the following equation:

$$\text{The α-amylase inhibitory} = \frac{Abs\ control - Abs\ sample}{Abs\ control} \text{ x } 100\ \%$$

The concentration of sample required to inhibit 50% of α-amylase activity under the above condition was defined as the IC_{50} value. The α-amylase inhibitory activity of the samples was calculated, and its IC_{50} values were determined using GraphPad Prism software.

Ethical considerations. Animal handling, maintenance and euthanasia procedures were performed as approved by the Research Ethics Committee, Halu Oleo University, Kendari, Southeast Sulawesi, Indonesia (document no. 1404/UN29.20/PPM/2020).

Results

Thin-layer chromatography technique. The 21 fractions were observed in the chromatogram pattern using thin-layer chromatography (Fig. 1). The fractions with the same stain pattern were merged based on the similarity of the chromatograms, yielding 7 fractions (A-G). These fractions (A-G) were tested for their hypoglycemic activity following the bioactivity guide method.

Bioactivity-guided in vivo antidiabetic assay. To determine the active fraction of *M. citrifolia* fruit, fractions A-G were assayed for their hypoglycemic activity in Swiss-Webster mice using the glucose tolerance method. The results are presented in Table I. The findings demonstrated that the control drug (exogenous insulin) and all fractions exhibited notable differences (95% CI) compared with the negative control (Na-CMC). Fraction G (48.10%), fraction C (43.08%) and fraction E (41.27%) exhibited the most prominent suppressive effects on blood sugar levels.

Purification and isolation of the compound from the active fractions with hypoglycemic activity. By considering the TLC pattern compared to a previous study (19) and the hypoglycemic activity of the fraction, fraction C was selected and proceeded to be purified using a radial chromatographic method and yielded eight sub-fractions (C1-C8). The C1 sub-fraction exhibited a single spot (isolate), which was further characterized using ^{1}H-NMR, 13C-NMR, DEPT spectroscopy and LC-MS.

Structure elucidation of the isolate. According to the ^{1}H-NMR spectrum data, the molecular structure of the C1 isolate consists of 48 protons, four of which have relatively large chemical shifts of 5.34, 5.14, 5.00 and 3.51 ppm, indicating that the protons may have low electron density. In addition, the similarity of the coupling constant (J=15.5 Hz) values between the protons at the chemical shifts of 5.00 and 5.14 ppm indicate that these two protons are in trans-position. Furthermore, proton stacking with very large integration is observed in the ^{1}H-NMR spectrum and the multiplicity data (*m, br d* and *br s*), that indicate the number of neighboring protons or the position of the protons are quite close together. This confirms the structure of the C1 isolate may belong to the steroid class.

Furthermore, the 13C-NMR spectrum of the C1 isolate shows the 29 carbons that make up the structure. The DEPT technique reveals that the isolate possesses three quaternary carbons, 11 methines, 9 methylene and 6 methyls. Carbons with chemical shifts >95.0 ppm belong to the olefinic carbon (alkene) or Csp^2 carbon. One olefinic quaternary carbon indicates a chemical shift of 140.9 ppm, while three olefinic methine carbons indicate chemical shifts of 121.9, 129.4 and 138.5 ppm, respectively. These four olefinic carbons enable the formation of two double bonds.

By considering the ^{1}H-NMR, 13C-NMR and DEPT data, the molecular formula of the C1 isolate is $C_{29}H_{48}O$ with double bond equivalence (DBE)=6, of which 2 come from the alkene group created by 4 Csp^2 atoms and the other 4 are from cyclic carbon.

By comparing the NMR data with a Wiley library database., the hypothesized chemical structure of the C1 isolate is stigmasterol (Fig. 2). Further verification was obtained by comparing the C1 isolate data with the reference (20) (presented in Table II).

The estimated chemical structure of the C1 isolate was validated by LC-MS/MS electrospray ionization. According to the LC-MS/MS data, the C1 isolate exhibited $[M+H]^+$ ion at m/z 413.38, with molecular ion fragmentation at m/z 395.37, 351.31, 273.22, 271.21 and 255.21. The $[M+H]^+$ ion at m/z 413.38 represents the first ionization of the C1 isolate with a positive ion (H^+). $[M+H]^+$ ion at m/z 413.38 undergoes fragmentation by releasing H_2O molecules as confirmed by the presence of the m/z 395.37 peaks or the m/z 273.22 to the m/z 255.21. The ^{1}H-NMR spectrum of the C1 isolate is presented in Fig. 3. Additionally, bond breaking occurs in the branching area, such as C17 and C20 bonds or C24 and C27 bonds, since

Table I. Hypoglycemic activity assay of *Morinda citrifolia* fractions using the glucose tolerance test method.

Groups	Fasting blood glucose level (mg/dl)	Blood glucose level after induction (mg/dl)	Blood glucose level following treatment (mg/dl)				Percentage reduction of blood glucose level
			120 min	Day 1	Day 3	Day 7	
Negative control	76.00±15.52	193.30±7.63	196.67±20.21	229.30±18.82	262.60±40.62	312.60±54.04	-
Control drug	75.60±17.09	220.6±45.56	N/A	220.30±101.74	152.60±24.58	107.60±3.78	51.22
Fraction A	83.6±11.37	192.60±5.50	165.33±32.38	110.00±9.84	121.30±13.31	101.30±6.65	31.75
Fraction B	91.00±11.26	189.60±9.50	191.33±78.62	165.60±12.58	139.30±5.03	78.30±55.33	24.08
Fraction C	97.30±2.08	189.00±7.21	107.67±17.62	133.30±26.27	158.00±42.50	130.30±27.50	43.08
Fraction D	74.00±12.16	222.60±58.44	112.67±14.47	272.30±102.64	97.00±4.35	92.00±14.52	38.80
Fraction E	62.30±13.65	218.60±20.74	121.67±21.36	350.00±1.00	195.00±111.7	170.30±81.37	41.27
Fraction F	88.00±12.12	262.30±66.90	138.33±6.66	158.60±53.40	133.00±14.17	120.30±19.75	29.90
Fraction G	76.00±15.71	301.00±90.70	103.33±14.47	106.30±10.11	112.30±13.61	94.60±9.86	48.10

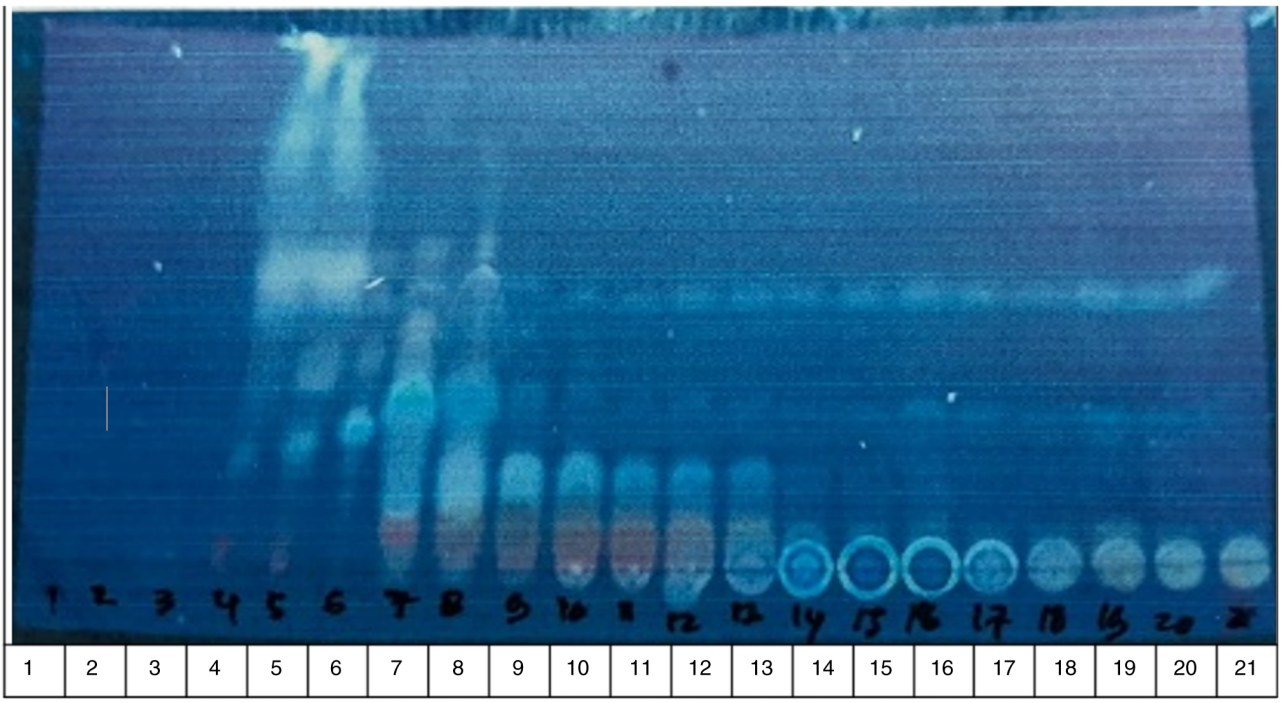

Figure 1. Thin layer chromatography chromatograms of 21 fractions of *Morinda citrifolia* fruit extract.

secondary ion radicals and secondary carbocations are more stable than the primary form (branching effect). The proposed mechanism of fragmentation is presented in Fig. 4. Thus, it was concluded that the molecular weight of the C1 isolate is 412.37.

In vitro α-amylase inhibitory activity assay. M. citrifolia fruit extract exhibited potent inhibitory activity against α-amylase with an IC_{50} value of 14.16±5.72 ppm. Similarly, stigmasterol (the C1 isolate) exhibited a prominent inhibitory activity against α-amylase with an IC_{50} value of 10.29±0.76 ppm which is more potent than that of acarbose, a known inhibitor of the enzyme,with an IC_{50} value of 43.37±1.56 ppm. The results are presented in Table III.

Discussion

The present study successfully isolated a phytosterol, stigmasterol, from the active sub-fraction C1 of *M. citrifolia* fruit, by following a bioactivity-guided method. Chemically, there are no differences in the structure of stigmasterol extracted from various plants, the differences are only in the amount.

The intravenous injection of the 65 mg/kg dose of STZ in adult Wistar rats may cause the enlargement of the pancreas followed by the deterioration of β-cells and induces experimental DM condition (21). The subcutaneous injection of exogenous insulin at 1 IU/kg has been reported to significantly reduce high blood glucose levels in rats with STZ-induced diabetes (22-24); thus, this drug was used as the control in the

Table II. Comparison of the ^{1}H-NMR and C13-NMR data for the C1 isolate and stigmasterol.

	C1 isolate		Stigmasterol (16)	
Carbon no.	δ_C 125 MHz (ppm)	δ_H (ΣH, m, J=Hz) 500 mHz (ppm)	δ_C 125 MHz (ppm)	δ_H (ΣH, m, J=Hz) 600 mHz (ppm)
1	37.4		37.6	
2	32.1		32.1	
3	71.9	3.51 (1H, m)	72.1	3.51 (1H, tdd, J=4.5; 4.4; 3.8 Hz)
4	42.5		42.4	
5	140.9		141.1	
6	121.9	5.34 (1H, m)	121.8	5.31 (1H, t, J=6.1 Hz)
7	32.1		31.8	
8	31.8		31.8	
9	50.2		50.2	
10	36.7		36.6	
11	21.2		21.5	
12	39.9		39.9	
13	42.4		42.4	
14	56.9		56.8	
15	24.3		24.4	
16	28.4		29.3	
17	56.1		56.2	
18	40.7		40.6	
19	23.2	0.91 (3H, d, J=7)	21.7	0.91 (3H, d, J=6.2 Hz)
20	138.5	5.00 (1H, dd, J=8.5; 15.5)	138.7	4.98 (1H, m)
21	129.4	5.14 (1H, dd, J=8.5; 15.5)	129.6	5.14 (1H, m)
22	46.0		46.1	
23	26.2		25.4	
24	12.0	0.83 (3H, m)	12.1	0.83 (3H, t, J=7.1 Hz)
25	29.3		29.6	
26	20.0	0.82 (3H, m)	20.2	0.82 (3H, d, J=6.6 Hz)
27	19.6	0.80 (3H, m)	19.8	0.80 (3H, d, J=6.6 Hz)
28	18.9	0.67 (3H, s)	18.9	0.71 (3H, s)
29	12.2	1.00 (3H, s)	12.2	(3H, s)

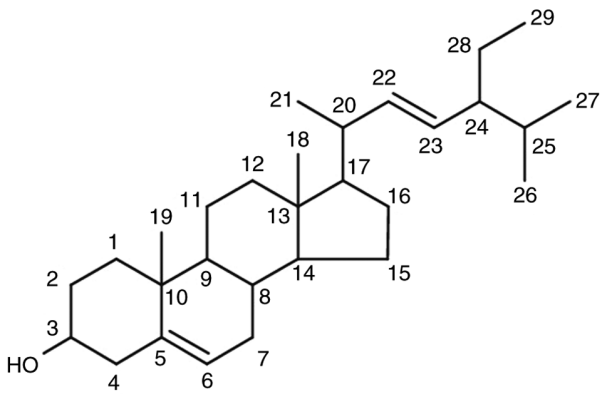

Figure 2. Chemical structure of the C1 isolate (stigmasterol).

present study. In the present study, both the origin extract and the stigmasterol isolate exhibited potent inhibitory activity against α-amylase compared to that of acarbose.

The digestion of dietary carbohydrates is associated with the elevation of postprandial blood glucose levels. Thus, limiting the activity of carbohydrate digestive enzymes in the intestinal tract is considered an important strategy. α-amylase is the key enzyme that catalyzes the hydrolysis of carbohydrates into smaller units, e.g., glucose (25).

The inhibitory activity towards α-amylase is categorized as very active with an IC_{50} value ≤25 μg/ml; active with an IC_{50} value >25 and ≤50 μg/ml; less active with an IC_{50} value >50 μg/ml and ≤100 g/ml; and inactive with an IC_{50} value IC_{50} >100 g/ml (26).

The findings of the present study confirmed that stigmasterol exhibited a potent inhibitory activity against α-amylase; the extract was categorized as very active (IC_{50} ≤25 g/ml) and acarbose was in the active category (IC_{50} value >25 g/ml and ≤50 g/ml).

α-Amylase inhibitors are also known as starch blockers due to their ability to prevent or attenuate the absorption of starch by inhibiting the hydrolysis of 1,4-glycosidic

Table III. *In vitro* α-amylase inhibitory activity of *M. citrifolia* extract and stigmasterol

Sample	IC_{50} value (ppm)	Regression equation and correlation coefficient (r^2)
M. citrifolia extract	14.16±5.72	y=0.01679 x + 49.92 r^2=0.8164
Stigmasterol (C1 isolate)	10.29±0.76	y=0.01993 x + 50.41 r^2=0.8817
Acarbose	43.37±1.56	y=0.11730 x + 42.77 r^2=0.9248

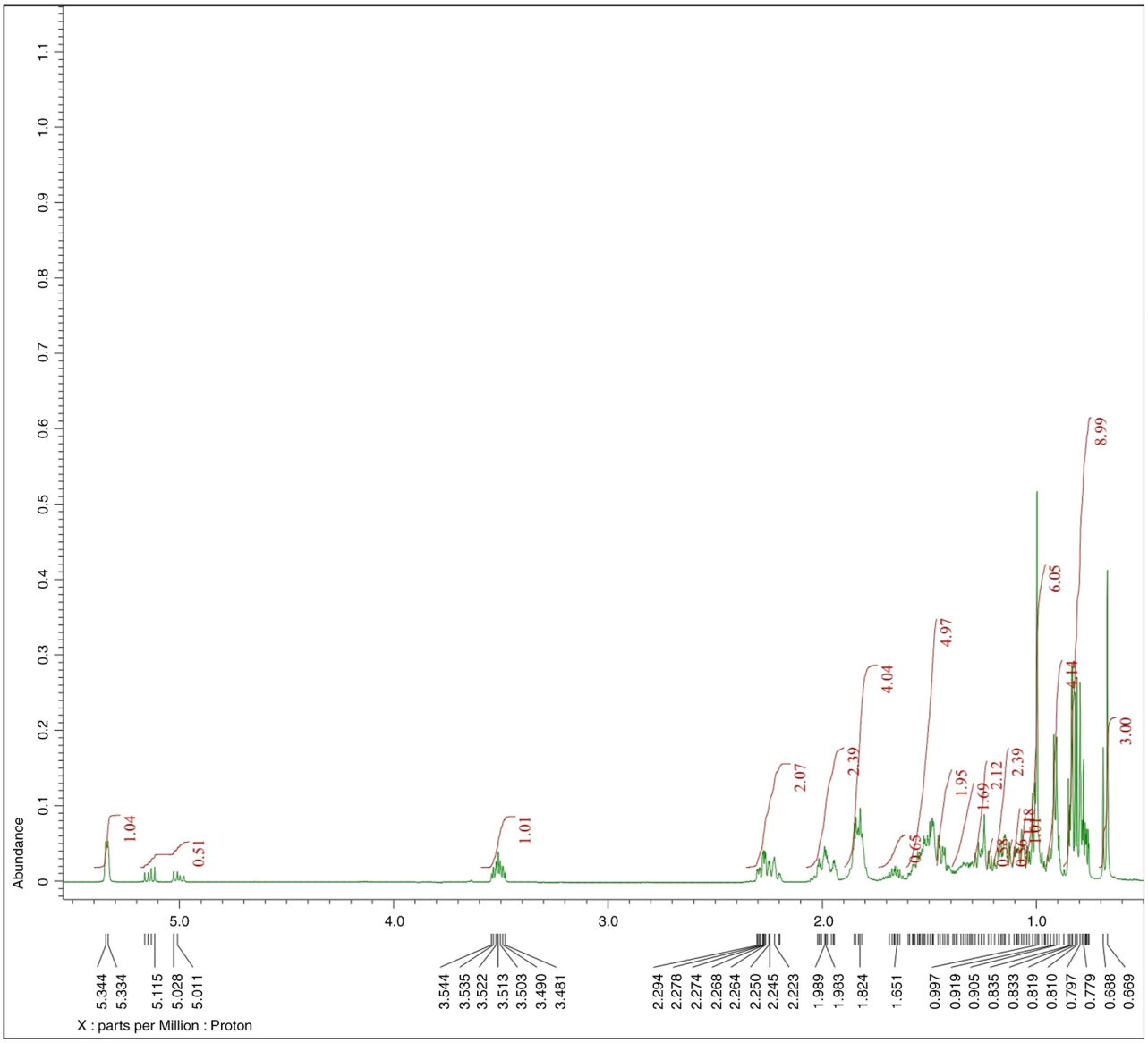

Figure 3. The proton nuclear magnetic resonance spectrum of the C1 isolate (stigmasterol).

linkages of starch and other oligosaccharides into maltose, maltotriose and other simple sugars (27). These findings are relevant to an initial study by the authors that confirmed the molecular interaction of stigmasterol towards α-amylase with a stable complex and higher affinity compared to that of acarbose (28).

In addition to being an inhibitor of α-amylase, stigmasterol has been reported to have anti-diabetic properties through a variety of mechanisms. *In vivo* investigations using animals have revealed that stigmasterol has the ability to lower glucose, urea and creatinine levels. The administration of stigmasterol isolated from plant extracts induces the release of insulin

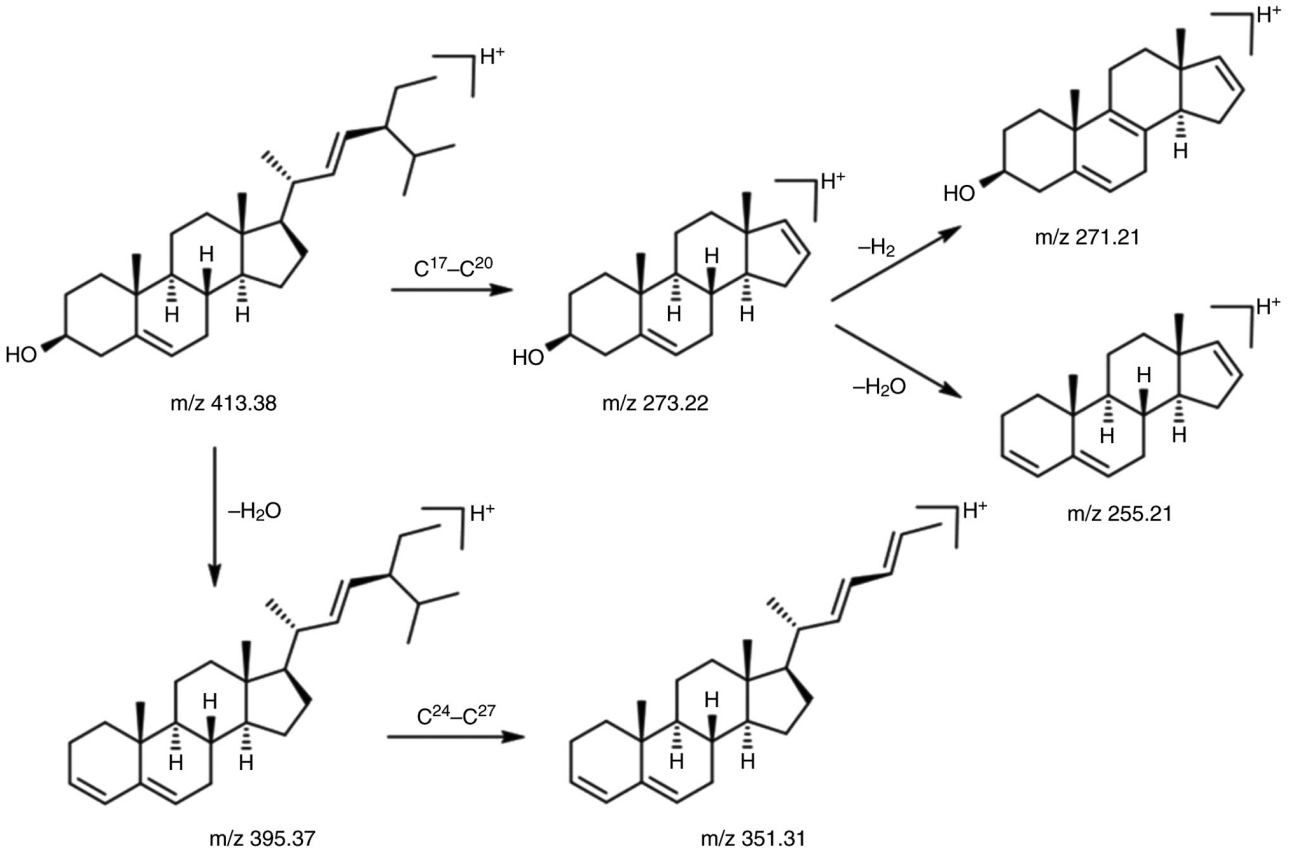

Figure 4. The proposed mechanism of fragmentation of the C1 isolate.

from pancreatic α-cells, resulting in an anti-hyperglycemic effect (5,6). In another study, stigmasterol was demonstrated to exert an anti-diabetic effect in mice with alloxan-induced diabetes (7).

Stigmasterol has been reported to have two mechanisms for lowering glucose levels *in vivo*, the first of which is to reduce intestinal glucose absorption or to increase in glycolytic and glycogenic systems with a subsequent decrease in glycogenolysis and gluconeogenesis pathways. The second mechanism includes the activation or repair of α-cells, followed by insulin release or insulin receptor stimulation (2,4,29). Stigmasterol has also been found to have the ability to operate on the GLUT4 receptor of the glucose transporter, including enhanced translocation and expression of GLUT4 (4). Moreover, a treatment of stigmasterol was reported could prevent early apoptosis, elevated total insulin, and improved insulin secretion in cells exposed to glucolipotoxicity (30). Stigmasterol isolated from the bark of *Butea monosperma* has been shown to reduce blood glucose levels accompanied by elevated plasma insulin levels in diabetic mice (31).

Although both *M. citrifolia* fruit extract and stigmasterol isolated from the ethyl acetate fraction of the fruit may have the potential to be developed as an α-amylase inhibitor, further studies on the underlying mechanisms are warranted. The present study did not evaluate the effects of the extract and/or stigmasterol on the signaling pathway of insulin, such as the ERK/MAPK pathway and/or IRS/PI3K/AKT pathway, that play a crucial role in the glucose, protein and lipid metabolism; thus, this was a limitation of the present study.

The present study performed the extraction, bioactivity-guided fractionation, isolation and characterization of stigmasterol in *M. citrifolia* fruits and examined its potency as an inhibitor of α-amylase. To the best of our knowledge, the present study is the first report the successful isolation of stigmasterol from *M. citrifolia* fruits. In conclusion, both the fruit extract and the stigmasterol isolate demonstrated a potent inhibitory activity against α-amylase compared to acarbose. However, further studies are required to evaluate the effects of the extract and/or stigmasterol on the signaling pathway of insulin, such as the ERK/MAPK pathway and/or IRS/PI3K/AKT pathways in order to guarantee its efficacy and safety in humans.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the first author on reasonable request.

Author's contributions

SAS, IS and JL were equally responsible for the conception and design of the study. NL was responsible for the formal analysis and the curation of the data. SAS, IS and JL confirm the authenticity of all the raw data. NL contributed to the writing of the original version of the manuscript. JL was responsible for reviewing and revising the manuscript. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Animal handling, maintenance and euthanasia procedures were performed as approved by the Research Ethics Committee, Halu Oleo University, Kendari, Southeast Sulawesi, Indonesia (document no. 1404/UN29.20/PPM/2020).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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