[IJC] Editor Decision

From: Tri Joko Raharjo (trijr_mipa@ugm.ac.id)

To: etihayati74@yahoo.com

Date: Thursday, December 6, 2018 at 11:43 AM GMT+7

Ferlinahayati Ferlinahayati:

We have reached a decision regarding your submission to Indonesian Journal of Chemistry, "[illegible]-Glucosidase Inhibitory and A Leptospermone Derivative from Rhodomyrtus tomentosa".

Our decision is: Revisions Required

Comments of the reviewers can be seen in the bottom part of this email. The revised paper has to be completed with responds for the reviewer's comments, point by point, in the beginning pages of the paper, and the revised parts should be indicated with different color of letters or author's comments. The revised paper has to be resubmitted in the system within three weeks.

Thank you for your intending to contribute the journal and giving us to read your work.

Best regards,

Tri Joko Raharjo
Laboratory of Organic Chemistry, Department of Chemistry, Universitas Gadjah Mada
trijr_mipa@ugm.ac.id

Reviewer A:

Additional Comment::
1. This compound is not new, it should be written reference of the comparison compounds.
2. The value of the coupling constant (J) on the H-NMR to be explained
3. It would be better, when using proof reader

Reviewer B:
• The significance and objective of this study have been explained clearly in the introduction. However, there is no originality stated in introduction. It would be better if the authors could confirm the originality of this study on introduction part.
• and at discussion section, discussion of inhibitory activity must be added more, compare with other papers.
• Others, please check the manuscript.

Reviewer C:

Additional Comment::

Indonesian Journal of Chemistry
https://jurnal.ugm.ac.id/ijc
Indexed by SCOPUS since 2012

Re: [IJC] Editor Decision

From: Tri Joko Raharjo (trijr_mipa@ugm.ac.id)

To: etihayati74@yahoo.com

Date: Monday, January 7, 2019, 9:32 AM GMT+7

Please your answer to reviewer's comments as well as submit your revised paper through Indo J Chem OJS system as you did for paper sbmission

On Wed, Dec 26, 2018 at 6:57 AM ferlina hayati <etihayati74@yahoo.com> wrote:

Dear Editor,

Thank you to for reviewing my paper.
I have been resubmitted the revised paper to the system as well as my responds for the reviewer's comment in the separated paper

Here in I also attach the revised paper and my reponds for the reviewer's.

Best regards,

Ferlinahayati
Chemistry department, FMIPA, Sriwijaya University
On Thursday, December 6, 2018, 11:43:08 AM GMT+7, Tri Joko Raharjo <trijr_mipa@ugm.ac.id> wrote:

Ferlinahayati Ferlinahayati:

We have reached a decision regarding your submission to Indonesian Journal of Chemistry, "-Glucosidase Inhibitory and A Leptospermone Derivative from Rhodomyrtus tomentosa".

Our decision is: Revisions Required

Comments of the reviewers can be seen in the bottom part of this email. The revised paper has to be completed with responds for the reviewer's comments, point by point, in the beginning pages of the paper, and the revised parts should be indicated with different color of letters or author's comments. The revised paper has to be resubmitted in the system within three weeks.

Thank you for your intending to contribute the journal and giving us to read your work.

Best regards,

Tri Joko Raharjo
Laboratory of Organic Chemistry, Department of Chemistry, Universitas Gadjah Mada
trijr_mipa@ugm.ac.id

Reviewer A:

Additional Comment::
1. This compound is not new, it should be written reference of the comparison compounds.
2. The value of the coupling constant (J) on the H-NMR to be explained
3. It would be better, when using proof reader

Reviewer B:

• The significance and objective of this study have been explained clearly in the introduction. However, there is no originality stated in introduction. It would be better if the authors could confirm the originality of this study on introduction part.

• and at discussion section, discussion of inhibitory activity must be added more, compare with other papers.

• Others, please check the manuscript.

Reviewer C:

Additional Comment::

Indonesian Journal of Chemistry
https://jurnal.ugm.ac.id/ijc
Indexed by SCOPUS since 2012

α-Glucosidase Inhibitory and A Leptospermone Derivative from *Rhodomyrtus tomentosa*

ABSTRACT

One of the treatments for diabetes mellitus disease is to control blood sugar level using an inhibitor of α-glucosidase enzyme. The methanol extracts of the fruit, stem, and leaves of *Rhodomyrtus tomentosa* were found significant in inhibiting α-glucosidase (IC_{50} 20.57, 20.36 and 43.99 μg/mL respectively). The ethyl acetate and butanol fractions from the methanol extract of *R. tomentosa* fruit exhibited the potent inhibition (IC_{50} 13.49 and 19.29 μg/mL) compare to acarbose and *n*-hexane fraction (IC_{50} 383.68 and 1175.16 μg/mL). A leptospermone derivative, rhodomyrtosone D was isolated from the ethyl acetate fraction of *R. tomentosa* fruit. The structure of rhodomyrtosone D was identified base on spectroscopic analysis, as well as comparing with literature data. The α-glucosidase inhibition of rhodomyrtosone D (IC_{50} 110.45 μg/mL) was 3.5 fold more potent than acarbose. Thus, *R. tomentosa* plant could be potential as a natural resource of α-glucosidase inhibitor.

Keywords: α-glucosidase, *Rhodomyrtus tomentosa*, antidiabetic, rhodomyrtosone D, ethyl acetate fraction

ABSTRAK

Salah satu penanganan diabetes mellitus adalah dengan mengontrol kadar gula darah menggunakan penghambat kerja enzim α-glukosidase. Ekstrak metanol buah, batang dan daun *R. tomentosa* menunjukkan penghambatan α-glukosidase yang signifikan (IC_{50} 20,57; 20,36 dan 43,99 μg/mL). Fraksi etil asetat dan butanol yang diperoleh dari ekstrak metanol buah R. tomentosa menunjukkan penghambatan yang potensial (IC_{50} 13,49 dan 19,29 μg/mL) dibandingkan dengan akarbosa dan fraksi *n*-hexane (IC_{50} 383,68 and 1175,16 μg/mL). Suatu turunan leptospermon yaitu rhodomyrtoson D telah diisolasi dari fraksi etil asetat buah *R. tomentosa*. Struktur senyawa rhodomyrtosone D ditetapkan berdasarkan analisis spektroskopi dan membandingkan dengan literatur. Penghambatan α-glukosidase dari rhodomyrtoson D menunjukkan 3,5 kali lebih kuat dibandingkan dengan akarbosa. Dengan demikian, tumbuhan *R. tomentosa* berpotensi sebagai sumber alami penghambat enzim α-glukosidase.

Kata kunci: α-glukosidase, *Rhodomyrtus tomentosa*, antidiabetes, rhodomyrtosone D, fraksi etil asetat.

Commented [f1]: Reviewer : Exract (Family)?
Author : has ben deleted

Commented [A2]: *Rhodomyrtus tomentosa*
Author : has been revised

Commented [A3]: Fractions
Author : has been added

Commented [A4]: The structure of rhodomyrtosone D was…
Author : has been added

Commented [A5]: *R. tomentosa* plant could be……..
Author : has been added

Commented [A6]: .
Author : has been added

Commented [A7]: senyawa rhodomyrtoson D
Author : has been added

Commented [A8]: menunjukkan 3,5 kali…….
Author : has been added

Commented [A9]: Tumbuhan R. tomentosa berpotensi……
Author : has been revised

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorder, in which there are high blood sugar levels (hyperglycemia) over a prolonged period [1]. It will happen if the pancreas does not produce enough insulin that is able to convert sugar into energy, or the body's cells do not respond well to the insulin produced. Some serious complication of hyperglycemia such as cardiovascular disease, damage to the eyes, atherosclerosis, and chronic kidney disease (nephropathy) can also occur [2]. The control of blood sugar level by inhibition of carbohydrate-hydrolyzing enzymes in the digestive organ is believed to be important in hyperglycemia treatment [1]. The α-glucosidase, an enzyme in the small intestine is responsible for the degradation of carbohydrate. The α-glucosidase inhibitor will interfere with the digestion of carbohydrate and thereby reduce the postprandial glucose level and insulin responses in a diabetic patient [2-3]. Acarbose, miglitol, and voglibose have been found as an α-glucosidase inhibitor and currently clinically used to control blood glucose of diabetic patients [4]. However, they have been caused serious gastrointestinal side effects. Nowadays, natural resources have received tremendous attention as a therapeutic agent in the inhibition of α-glucosidase and have shown very promising biological activity.

Karamunting is locally named (Sumatera island) for *Rhodomyrtus tomentosa* and belonging to Myrtaceae family. This plant is an evergreen shrub which is native to Southern Asia and Southeast Asia and is widely distributed in Indonesia. *R. tomentosa* is widely used as traditional medicines to treat a variety of disease caused by bacteria such as diarrhea, dysentery and urinary tract infections [5-6]. In addition, its ripe fruits are used to boost the immune system [7]. Biologically, ethanolic extract of *R. tomentosa* fruits possesses potent antioxidant activities on DPPH radical scavenging activity, reducing power as well as inhibition of lipid peroxidation activity [8] Furthermore, some extract of this plants were reported as antibacterial and anti-hepatitis properties [9]. Chemically, various secondary metabolites have been reported such as polyketide, flavonoids, anthocyanins, stilbenoids, and triterpenoids. Rhodomyrtone, a phloroglucinol polyketide from *R. tomentosa* have displayed significant antibacterial activities against Gram-positive bacteria and suggested as a new candidate as a natural antibacterial drug [10]. Meanwhile, tomentosone A, a hexacyclic phloroglucinol was reported as antimalarial against chloroquine-resistant and sensitive strains of *Plasmodium falciparum*. Resveratrol and piceatannol, a stilbenoid compound has been characterized from this plant [7]. A stilbenoid compound from *Syagrus romanzoffiana* was reported as a potential hypoglycemic agent. In a search for potential α-glucosidase inhibitor from natural resources, we have been investigated the ability of *R. tomentosa* plant to inhibit the activity of the α–glucosidase enzyme as well as to isolate the bioactive compound. One active compound, rhodomyrtosone (**1**) was isolated and its α-glucosidase inhibition was determined. The following describes the outcomes of these efforts.

Commented [A10]: The α–.....

Author: has been added

Commented [A11]:

Author : has been revised to " antimalarial"

EXPERIMENTAL SECTION

.

Materials

Rhodomyrtus tomentosa (fruits, leaves, and stem) were collected from Inderalaya, Ogan Ilir, South Sumatera. The plant was identified at Herbarium Anda, Department of Biology, University of Andalas. The solvents (methanol, *n*-hexane and ethyl acetate) were a technical quality that is distilled while *n*-butanol and dimethylsulfoxide (DMSO) were pro analysis (p.a) from Merck. The α-glucosidase (from *Saccharomyces cerevisiae*) and *p*-nitro-phenyl-α-D-glucopyranoside were purchased from Sigma-Aldrich. Bovine serum albumin (BSA) was purchased from Merck. Silica gel 60G (Merck) was used for vacuum liquid chromatography and silica gel 60 PF_{254} (Merck) was used for radial chromatography. TLC analysis was performed on Kieselgel 60 GF_{254}, 0.25 mm aluminum plate (Merck) and visualized with cerium sulfate.

Commented [A12]: The α–...

Author : Has been added

Instrumentation

Incubator Biosan PST-60HL was used for sample incubation process. The absorbance of *p*-nitrophenol was measured by a Tecan Infinite F50 Microplate reader. UV spectrum was recorded with Shimadzu UV-1240 spectrophotometer. IR spectrum was determined using KBr pellets on a Perkin Elmer FTIR Spectrum One spectrophotometer. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded with Agilent DD2 spectrometer, using residual and deuterated solvent peaks as reference standards.

Commented [A13]: C-NMR (100 MHz)?

Author : has been revised to "^{1}H-NMR (500 MHz) and ^{13}C-NMR (100 MHz)

Procedure

Extraction of Sample

As much as 100 gr of each the dried powdered sample (fruits, leaves, and stem) of *R.tomentosa* were extracted by maceration method using methanol (400 mL) as the solvent at the room temperature. The maceration process was carried out three times @ 24 hours. The methanol solvents were evaporated in under reduce pressure to give a crude extracts of methanol of fruit, leaves, and stem (4.6, 4.2 and 3.9 g respectively). The crude of methanol extract of fruit was partitioned successively with *n*-hexane, ethyl acetate, and *n*-butanol and produce of each fraction after the solvent was evaporated.

Commented [A14]: As much as 100 g ……? mix of fruts, leaves, and stem

Author : has been revised to "As much as 100 gr of each"

Commented [A15]: times @ 24 h.

Author : has been added

Commented [A16]: Extracts

Author : has been revised

In-vitro α-glucosidase inhibition assay

The α-glucosidase assay has been performed using the spectrophotometric method as previously described [2, 11, 12] with slight modification. As much as 10 μL of the sample at various concentrations was added with 55 μL of 50 mM phosphate buffer (pH 6.8) and 10 μL of 10 mM *p*-

Commented [A17]: As much as 10……

Author : has been added

nitrophenyl-α-D-glucopyranoside as the substrate. After preincubated for 5 min at 37 °C, 25 µL of 0.1 U/mL α-glucosidase (in the phosphate buffer pH 6.8 containing 0.1 mg/mL bovine serum albumin) was added. The mixture was then incubated for 30 min at 37 °C. After that, the stopped solution (100 µL of 200 mM Na_2CO_3) was added to the mixture. The absorbance of the *p*-nitrophenol released due to hydrolysis of the substrate by the α- glucosidase was measured by microplate reader at 405 nm. The blank solution was prepared by replaced sample solution by DMSO. Acarbose (Glucobay®) is used as a positive control. The percentage inhibition of α-glucosidase was calculated using the following equation: Inhibition % = [1-(A_{sample} / A_{blank})] x 100. The IC_{50} was calculated by linear regression equation analysis between concentration and percentage inhibition.

Commented [A18]: µL
Author : has been revised

Commented [A19]: µL
Author : has been revised

Extraction and Isolation

The dried fruits (2 kg) was extracted with methanol (3 x 7 L, 24 h each) by maceration method. The methanol extract was concentrated under reduced pressure to give 1.1 L syrup which was suspended in distilled water. This suspension was partitioned successively with *n*-hexane, ethyl acetate, and *n*-butanol to afford *n*-hexane, ethyl acetate and *n*-butanol fractions. The ethyl acetate fraction (15 g) was fractionated by vacuum liquid chromatography on silica gel 60 G, eluting with *n*-hexane-ethyl acetate system with increment ethyl acetate gradually (9:1→8:2→7:3→6:4→4:6 →2:8→1:9→0:10, each 150 mL) to give 8 fractions (A-H). Fraction C (374 mg) was further separated by radial chromatography over silica gel 60 PF_{254} (1 mm), eluted with n-hexane-ethyl acetate gradually (85:15→80:20→75:25→70:30→60:40→50:50) to yield a leptospermon derivative **1** (8.9 mg). ~~Subfraction C2 yielded a leptospermone derivative 1 (8.9 mg).~~

Commented [A20]: Fruits (2kg) was….
Author : has been revised

Commented [A21]: Fractions
Author : it has been revised

Commented [A22]: detail?
Author : has been revised and state clearly

RESULTS AND DISCUSSION

The α-Glucosidase inhibition of extracts and fractions

Commented [A23]:
Author : has been added with "the"

The extraction of three parts of karamunting (*R. tomentosa*) plants namely fruit, stem, and leaves) produced methanol extract of 4.6%, 3.9%, and 4.2% respectively. All of these extracts were tested for α-glucosidase inhibitory using *p*-nitrophenyl-α-D-glucopyranoside as the substrate and acarbose as the reference or positive control. The methanol extract from the stem and fruit have the similar ability to inhibit α-glucosidase activity (IC_{50} 20.36 and 20.57 µg/mL). Both of these extracts demonstrated two times more potent than the leaves methanol extract (IC_{50} 43.99 µg/mL) (Figure 1). All three methanol extracts possessed high potency in inhibiting α-glucosidase compare to the reference drug, acarbose (IC_{50} 383.68 µg/mL) (Table 1). Previously, it has been reported that ~~by Lai et al,~~ *R. tomentosa* fruit contains stilbenoid compound, such as resveratrol, and piceatannol [7]. These stilbenoids showed the more potent inhibition of α-glucosidase activity with IC_{50} 91 and 60 mg/mL respectively than acarbose (IC_{50} 247 µg/mL) [4]. Therefore, it is

Commented [A24]: Previosly, ….
Author: has been added with ","

Commented [A25]: [no.?]
Author : has been revised with numbering

assumed that the inhibition of α-glucosidase in this plant is due to the content of the stilbenoid compound.

Base on its inhibition of α-glucosidase, the methanol extract of fruits was partitioned into *n*-hexane, ethyl acetate, and butanol. Ethyl acetate fraction had the highest α-glucosidase inhibitory (IC_{50} 13.49 μg/mL than butanol fraction (IC_{50} 19.29 μg/mL) due to its phenolic content, meanwhile, the *n*-hexane fraction was not as potent as α-glucosidase inhibitory (IC_{50} 1175.16 μg/mL) (Table 1 and Figure 1).

Table 1. Inhibitory effect of the extract, fraction and compound on α-glucosidase activity

Extract/compound	**Inhibitor concentration (IC_{50}, μg/mL)**
MeOH extract of the leaves	43.99
MeOH extract of the stem	20.36
MeOH extract of the fruit	20.57
n-hexane fraction of the fruit	1175.16
Ethyl acetate fraction of the fruit	13.49
Butanol fraction of the fruit	19.29
Compound 1	110.45
Acarbose	383.68

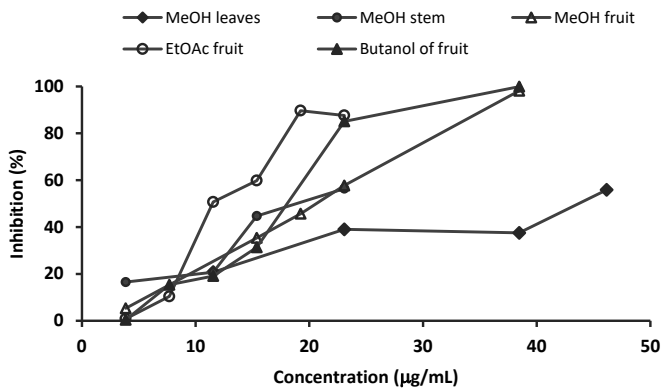


Figure 1. Effect of extracts and fractions on the inhibition of α-glucosidase

Isolation and structural elucidation

The sequential partition to the methanol crude extract of *R. tomentosa* fruits (87 g) yielded *n*-hexane, ethyl acetate and butanol fraction of 1.7%, 20.5%, and 0.5% respectively. Ethyl acetate fraction with the highest α-glucosidase inhibition was chromatographed over silica gel with some chromatographic technique to afford a leptospermone derivative **1.**

Compound **1** was isolated as a white powder with m.p. 120-121 ^{o}C. The UV spectrum in methanol showed the maximum absorption at 242 nm which indicated the presence of α,β carbonyl unsaturated. The IR spectrum displayed absorption for the isolated carbonyl group at 1715 cm^{-1} as well as conjugated carbonyl group at 1678 and 1663 cm^{-1} which consisted to UV spectrum. In addition, there is absorption for C-H aliphatic group at 2976 and 2941 cm^{-1}. The ^{13}C-NMR (125 MHz, $CDCl_3$) was showed the presence of 14 signal. Two of the signal confirmed the existence of the isolated and conjugated carbonyl at pada δ_C 212,2 ppm and δ_C 192,2 ppm respectively. In addition, ^{13}C-NMR displayed the presence of five other quarternary carbon signal (δ_C 175.5 (oxy-carbon), 128.3, 113.2, 56.6, and 45.3 ppm), two signal for methine carbon (δ_C 46.6 and 34.5 ppm), and five signal for methyl carbon (δ_C 25.9, 24.5, 24.0, 22.4 and 15.6 ppm). Considering the intensity of quarternary carbon signal at 128.3 ppm with the six other quarternary carbon (included the carbonyl) which has a ratio of 1:2, indicating that the six quarternary carbon is equivalent to twelve carbon. Furthermore, the five methyl carbon signals have an intensity ratio of 2: 1 with a carbon methine signal at δ_C 34.5 ppm, consequently each of these methyl signals is identical for 2 methyl carbon (there are a total of 10 methyls). Based on this, compound **1** actually has 25 carbon atoms. The ^{1}H-NMR (500MHz, $CDCl_3$) spectrum exhibited the presence of a singlet signal of methine proton at δ_H 4.67 ppm. The spectrum also indicated the presence of an isopropyl unit with the appearance of a doublet signal at δ_H 1.00 ppm (6H, *d*, *J* = 6.9 Hz, 2xCH_3) which is adjacent to the methine proton at δ_H 2.35 ppm (1H, *sept*, *J* = 6.9 Hz). These constant coupling value indicates that the both signals are correlated to each other as vicinal aliphatic protons. In addition, there are three singlet signals at δH 1.41 (12H), 1.32 (6H) and 1.25 ppm (6H) which indicate the presence of 8 methyl groups. The HMBC correlation revealed a correlation of both methyl on a geminal dimethyl group (δ_H 1.25 and 1.32 ppm) to the isolated and conjugated carbonyl group (δ_C 212.2 and 192.2 ppm) as well as correlation of both methyl on another geminal dimethyl group to the isolated carbonyl (δ_C 212.2 ppm) and oxy-carbon (δ_C 175.5 ppm). These explained that both of geminal dimethyl are α position in β-triketone unit. Based on the previous NMR data, there is actually two symmetrical unit of β-triketone. Furthermore, the correlation between of proton δ_H 4.67 ppm to isopropyl unit (δ_C 34.5 ppm) and oxy-carbon (δC 175.5 and 128.3 ppm) indicating that the isopropyl group was an adjacent bis-furan ring and the bis-furan ring was integrated with the β-triketone unit. According to these spectral studies and comparing to the those of reported literature [13], the structure of compound **1** was established as rhodomyrtosone D. This compound has been previously reported from *R. tomentosa* leaves [13].

Commented [A26]: check….

Author : yes, that is correct

Commented [A27]: check….

Author : yes, that is correct

Commented [A28]: ?

Author : has been explained

Commented [A29]: ?

Author: has been explained

Commented [A30]: new compound?, reference?

Author : has been revised and reference was added

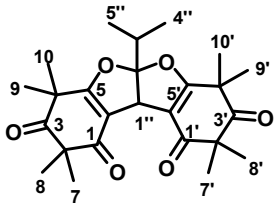


Figure 2. Structure of compound **1** (rhodomyrtosone D)

Table 2. NMR data of compound **1** in $CDCl_3$

No	Compound 1		
	δC[a]	δ_H (ΣH, *mult*, J_{Hz})[b]	HMBC (H→C)
1 (1')	192.2	-	-
2(2')	56.6	-	-
3(3')	212.2	-	-
4(4')	45.3	-	-
5(5')	175.5	-	-
6(6')	113.2	-	-
7(7')	25.8	1.25 (6H, *s*)	C-3(3'), C-1 (1'), C-2(2'), C- 8(8')
8(8')	22.4	1.32 (6H, *s*)	C-3(3'), C- 1 (1'), C- 2(2'), C- 7(7')
9(9')	24.0	1.41 (6H, *s*)	C-3(3'), C-5(5'), C-4(4'), C- 9(9'), C-10(10')
10(10')	24.5	1.41 (6H, *s*)	C-3(3'), C-5(5'), C-4(4'), C- 9(9'), C-10(10')
1''	46.6	4.67 (1H, *s*)	C-5(5'), C- 2'', C- 6(6'), C- 3''
2''	128.3	-	-
3''	34.5	2.35 (1H, s*ept*)	C-2'', C- 1'', C- 4'', C- 5"
4'', 5"	15.6	1.00 (6H, *d*, 6.9)	C-2'', C- 3'', C- 4', C- 5"

[a] 125 MHz
[b] 500 MHz

The isolated compound **1** (rhodomyrtosone D) was examined for α-glucosidase inhibitory activity with concentration range about 30.77 to 0.24 μg/mL. The α- glucosidase inhibitory effect of rhodomyrtosone D (17.7% at 30.77 μg/mL) seems higher than the acarbose (8.54 % at 30.77 μg/mL). Using the extrapolation method to linear regression, the IC_{50} of rhodomyrtosone D on inhibiting α-glucosidase was 110.45 μg/mL.

CONCLUSION

In summary, the leaves, the stem, and the fruit of *R. tomentosa* plant were potential as a source of a natural antidiabetic, especially from the ethyl acetate fraction of fruit. A bioactive compound, rhodomyrtosone D was isolated from the fruit of *Rhodomyrtus tomentosa* and showed higher α-glucosidase inhibition than acarbose.

Commented [A31]: ?

Author : has been revised

ACKNOWLEDGMENTS

The author would like thanks to Dr. Nurainas, M.Si from Herbarium Anda, Andalas University for identification of plant specimen. We are grateful to the Ministry of Research, Technology and Higher Education for research grants through PD-UPT scheme (No: 093/SP2H/LT/DPRM/IV/2018).

REFERENCES

1. Yin, Z., Zhang, W., Feng, F., Zhang, Y., and Kang, W., 2014, α-Glucosidase Inhibitors Isolated from Medicinal Plants, *Food Science, and Human Wellness*, 3, 136-174.
2. Choudhary, M.I., Adhikari, A., Rasheed, S., Marasini, B.P., Hussain, N., Kaleem, W.A., and Rahman, A., 2011, Cyclopeptyde Alkaloid of *Ziziphus oxyphylla* Edgw as Novel Inhibitors of α-Glucosidase Enzyme and Protein Glycation, *Phytochemistry Letters*, 4, 404-406.
3. Lam. S.H., Cheng, J.M., Kang, C.J., Chen, C.H., and Lee, S.S., 2008, α-Glucosidase Inhibitors from the Seed of *Syagrus romanzoffiana*, *Phytochemistry*, 1173-1178.
4. Zhang, A.J., Rimando, A.M., Mizuno, C.S., and Mathews, S.T., 2017, α-Glucosidase Inhibitory Effect of Resveratrol and Piceatannol, *The Journal of Nutritional Biochemistry*, 47, 86-93.
5. Lavanya, G., Voravuthikunchai, S.P, and Towatana, N.H., 2012, Acetone Extract from *Rhodomyrtus tomentosa*: A Potent Natural Antioxidant, *Evidence-Based Complementary and Alternative Medicine,* 2012, Article ID 535479, 1-8.
6. Limsuwan, S., Kayser, O., and Voravuthikunchai, S.P., 2012, Antibacterial Activity of Rhodomyrtus tomentosa (Aiton) Hassk. Leaf Extract against Clinical Isolates of *Streptococcus pyogenes*, *Evidence-Based Complementary and Alternative Medicine,* 2012, Article ID 697183, 1-6.
7. Lai, T.N.H., Herent, M.F., Quetin-Leclercq, J., Nguyen, T.B.T., Larondelle, Y., Andre, C.M., and Rogez, H., 2013, Piceatannol, a Potent Bioactive Stilbene, as Major Phenolic Component in *Rhodomyrtus tomentosa*, *Food Chemistry*, 138: 1421-1430
8. Wu, P., Ma, G., Li, N., Deng, Q., Yin, Y., and Huang, R., 2015, Investigation of In Vitro and in Vivo Antioxidant Activities of Flavonoids Rich Extract from the Berries of *Rhodomyrtus tomentosa* (Ait.) Hassk., *Food Chemistry*, 173, 194-202.
9. Hiranrat, A., Mahabusakaram, W., Carrol, A.R., Duffy, S., and Avery, V.M., 2012, Tomentosones A and B, Hexacyclic Phloroglucinol Derivatives from the Thai Shrub *Rhodomyrtus tomentosa*, *J. Org. Chem*., 77, 680-683.
10. Limsuwan, S., Trip, E.N., Kouwen, T.R.H.M., Piersma, S.,Hiranrat, A., Mahabusakaram, W., Voravuthikunchai, S.P., Dijl, JM., and Kayse, O., 2009, Rhodomyrtone, A new Candidate as Natural Antibacterial Drug from *Rhodomyrtus tomentosa*, *Phytomedicine*, 16, 645-651.

11. Yang, Z., Wang, Y., Wang, Y., and Zhang, Y., 2012, Bioassay-Guided Screening and Isolation of α-glucosidase and Tyrosinase Inhibitors from Leaves of *Morus alba*, *Food Chemistry*, 617-625.
12. Anisah, L.N., Syafii, W., Pari, G., and Sari, R.K., 2018, Antidiabetic Activities and Identification of Chemical Compound from Samama (*Anthocephalus macrophyllus* (Roxb) Havil), *Indones. J. Chem*, 18(1), 66-74.
13. Hiranrat, A., and Mahabusakaram, W., 2008, New Acylphloroglucinols from the Leaves of *Rhodomyrtus tomentosa*, *Tetrahedron*, 64, 11193-11197.

α-Glucosidase Inhibitory and A Leptospermone Derivative from *Rhodomyrtus tomentosa* Extract

ABSTRACT

One of the treatments for diabetes mellitus disease is to control blood sugar level using an inhibitor of α-glucosidase enzyme. The methanol extracts of the fruit, stem, and leaves of *R. tomentosa* were found significant in inhibiting α-glucosidase (IC_{50} 20.57, 20.36 and 43.99 μg/mL respectively). The ethyl acetate and butanol fraction from the methanol extract of *R. tomentosa* fruit exhibited the potent inhibition (IC_{50} 13.49 and 19.29 μg/mL) compare to acarbose and *n*-hexane fraction (IC_{50} 383.68 and 1175.16 μg/mL). A leptospermone derivative, rhodomyrtosone D was isolated from the ethyl acetate fraction of *R. tomentosa* fruit. The structure was identified base on spectroscopic analysis, as well as comparing with literature data. The α-glucosidase inhibition of rhodomyrtosone D (IC_{50} 110.45 μg/mL) was 3.5 fold more potent than acarbose. Thus, the plant could be potential as a natural resource of α-glucosidase inhibitor

Keywords: α-glucosidase, *Rhodomyrtus tomentosa*, antidiabetic, rhodomyrtosone D, ethyl acetate fraction

ABSTRAK

Salah satu penanganan diabetes mellitus adalah dengan mengontrol kadar gula darah menggunakan penghambat kerja enzim α-glukosidase. Ekstrak metanol buah, batang dan daun *R. tomentosa* menunjukkan penghambatan α-glukosidase yang signifikan (IC_{50} 20,57; 20,36 dan 43,99 μg/mL). Fraksi etil asetat dan butanol yang diperoleh dari ekstrak metanol buah R. tomentosa menunjukkan penghambatan yang potensial (IC_{50} 13,49 dan 19,29 μg/mL) dibandingkan dengan akarbosa dan fraksi *n*-hexane (IC_{50} 383,68 and 1175,16 μg/mL). Suatu turunan leptospermon yaitu rhodomyrtoson D telah diisolasi dari fraksi etil asetat buah *R. tomentosa*. Struktur senyawa ditetapkan berdasarkan analisis spektroskopi dan membandingkan dengan literatur. Penghambatan α-glukosidase dari rhodomyrtoson D 3,5 kali lebih kuat dibandingkan dengan akarbosa. Dengan demikian, tumbuhan ini berpotensi sebagai sumber alami penghambat enzim α-glukosidase.

Kata kunci: α-glukosidase, *Rhodomyrtus tomentosa*, antidiabetes, rhodomyrtosone D, fraksi etil asetat.

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorder, in which there are high blood sugar levels (hyperglycemia) over a prolonged period [1]. It will happen if the pancreas does not

produce enough insulin that is able to convert sugar into energy, or the body's cells do not respond well to the insulin produced. Some serious complication of hyperglycemia such as cardiovascular disease, damage to the eyes, atherosclerosis, and chronic kidney disease (nephropathy) can also occur [2]. The control of blood sugar level by inhibition of carbohydrate-hydrolyzing enzymes in the digestive organ is believed to be important in hyperglycemia treatment [1]. α-glucosidase, an enzyme in the small intestine is responsible for the degradation of carbohydrate. The α-glucosidase inhibitor will interfere with the digestion of carbohydrate and thereby reduce the postprandial glucose level and insulin responses in a diabetic patient [2-3]. Acarbose, miglitol, and voglibose have been found as an α-glucosidase inhibitor and currently clinically used to control blood glucose of diabetic patients [4]. However, they have been caused serious gastrointestinal side effects. Nowadays, natural resources have received tremendous attention as a therapeutic agent in the inhibition of α-glucosidase and have shown very promising biological activity.

Karamunting is locally named (Sumatera island) for *Rhodomyrtus tomentosa* and belonging to Myrtaceae family. This plant is an evergreen shrub which is native to Southern Asia and Southeast Asia and is widely distributed in Indonesia. *R. tomentosa* is widely used as traditional medicines to treat a variety of disease caused by bacteria such as diarrhea, dysentery and urinary tract infections [5-6]. In addition, its ripe fruits are used to boost the immune system [7]. Biologically, ethanolic extract of *R. tomentosa* fruits possesses potent antioxidant activities on DPPH radical scavenging activity, reducing power as well as inhibition of lipid peroxidation activity [8] Furthermore, some extract of this plants were reported as antibacterial and anti-hepatitis properties [9]. Chemically, various secondary metabolites have been reported such as polyketide, flavonoids, anthocyanins, stilbenoids, and triterpenoids [7-11]. Rhodomyrtone, a phloroglucinol polyketide from *R. tomentosa* have displayed significant antibacterial activities against Gram-positive bacteria and suggested as a new candidate as a natural antibacterial drug [10]. Meanwhile, tomentosone A, a hexacyclic phloroglucinol was reported as antimalaria against chloroquine-resistant and sensitive strains of *Plasmodium falciparum*. Resveratrol and piceatannol, a stilbenoid compound has been characterized from this plant [7]. A stilbenoid compound from *Syagrus romanzoffiana* was reported as a potential hypoglycemic agent. However, there is no literature on the α-glucosidase inhibitory of *R.tomentosa* and its bioactive chemical compound. In a search for potential α-glucosidase inhibitor from natural resources, we have been investigated the ability of *R. tomentosa* plant to inhibit the activity of the α–glucosidase enzyme as well as to isolate the bioactive compound. One active compound, rhodomyrtosone (**1**) was isolated and its α-glucosidase inhibition was determined. The following describes the outcomes of these efforts.

EXPERIMENTAL SECTION

Commented [W1]: add more references in secondary metabolites parts that have been found in these plant.

Author : the references has been added

.

Materials

Rhodomyrtus tomentosa (fruits, leaves, and stem) were collected from Inderalaya, Ogan Ilir, South Sumatera. The plant was identified at Herbarium Anda, Department of Biology, University of Andalas. The solvents (methanol, *n*-hexane and ethyl acetate) were a technical quality that is distilled while *n*-butanol and dimethylsulfoxide (DMSO) were pro analysis (p.a) from Merck. α-glucosidase (from *Saccharomyces cerevisiae*) and *p*-nitro-phenyl-α-D-glucopyranoside were purchased from Sigma-Aldrich. Bovine serum albumin (BSA) was purchased from Merck. Silica gel 60G (Merck) was used for vacuum liquid chromatography and silica gel 60 PF_{254} (Merck) was used for radial chromatography. TLC analysis was performed on Kieselgel 60 GF_{254}, 0.25 mm aluminum plate (Merck) and visualized with cerium sulfate.

Instrumentation

Incubator Biosan PST-60HL was used for sample incubation process. The absorbance of *p*-nitrophenol was measured by a Tecan Infinite F50 Microplate reader. UV spectrum was recorded with Shimadzu UV-1240 spectrophotometer. IR spectrum was determined using KBr pellets on a Perkin Elmer FTIR Spectrum One spectrophotometer. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (500 MHz) spectra were recorded with Agilent DD2 spectrometer, using residual and deuterated solvent peaks as reference standards.

Procedure

Extraction of sample for assay

100 gr of the dried powdered sample (fruits, leaves, and stem) of *R.tomentosa* were extracted by maceration method using methanol (400 mL) as the solvent at the room temperature. The maceration process was carried out three times (@ 24 hours). The methanol solvents were evaporated in under reduce pressure to give a crude extract of methanol of fruit, leaves, and stem (4.6, 4.2 and 3.9 g respectively). The crude of methanol extract of fruit was partitioned successively with *n*-hexane, ethyl acetate, and *n*-butanol and produce of each fraction after the solvent was evaporated.

Commented [W2]: For how many hours each times of maceration process?

Author : has been added

In-vitro α-glucosidase inhibition assay

The α-glucosidase assay has been performed using the spectrophotometric method as previously described [2, 12, 13] with slight modification. 10 μL of the sample at various concentrations was added with 55 μL of 50 mM phosphate buffer (pH 6.8) and 10 μL of 10 mM *p*-nitrophenyl-α-D-glucopyranoside as the substrate. After preincubated for 5 min at 37 °C, 25 μl of

0.1 U/mL α-glucosidase (in the phosphate buffer pH 6.8 containing 0.1 mg/mL bovine serum albumin) was added. The mixture was then incubated for 30 min at 37 °C. After that, the stopped solution (100 µl of 200 mM Na_2CO_3) was added to the mixture. The absorbance of the *p*-nitrophenol released due to hydrolysis of the substrate by the α- glucosidase was measured by microplate reader at 405 nm. The blank solution was prepared by replaced sample solution by DMSO. Acarbose (Glucobay®) is used as a positive control. The percentage inhibition of α-glucosidase was calculated using the following equation: Inhibition % = [1-(A_{sample} / A_{blank})] x 100. The IC_{50} was calculated by linear regression equation analysis between concentration and percentage inhibition.

Extraction and Isolation of *R. tomentosa* fruits

The dried fruits (2 kg) were extracted with methanol (3 x 7 L, 24 h each) by maceration method. The methanol extract was concentrated under reduced pressure to give 1.1 L syrup which was suspended in distilled water. This suspension was partitioned successively with *n*-hexane, ethyl acetate, and *n*-butanol to afford *n*-hexane, ethyl acetate and *n*-butanol fraction. The ethyl acetate fraction (15 g) was fractionated by vacuum liquid chromatography on silica gel 60 G, eluting with *n*-hexane-ethyl acetate system with increment ethyl acetate gradually (9:1→8:2→7:3→6:4→4:6 →2:8→1:9→0:10, each 150 mL) to give 8 fractions (A-H). Fraction C (374 mg) was further separated using ~~by~~ radial chromatography over silica gel 60 PF_{254} (1 mm), eluted with n-hexane-ethyl acetate gradually (85:15→80:20→75:25→70:30→60:40→50:50) to yield 7 subfractions. Subfraction C2 yielded a leptospermone derivative **1** (8.9 mg).

Commented [W3]: write down the objectives of the extraction method in the previous and in this section

Author : has been added the objective of this section and previous

Commented [W4]: using

Author : has been changed

RESULTS AND DISCUSSION

α-Glucosidase inhibition of extracts and fractions

The extraction of three parts of *R. tomentosa* (fruit, stem, and leaves) produced methanol extract (4.6, 3.9, and 4.2 g respectively). All of these extracts were tested for α-glucosidase inhibitory using *p*-nitrophenyl-α-D-glucopyranoside as the substrate and acarbose as the reference or positive control. The methanol extract from the stem and fruit have a similar ability to inhibit α-glucosidase activity (IC_{50} 20.36 and 20.57 µg/mL). Both of these extracts demonstrated two times more potent than the methanol extract of the leaves (IC_{50} 43.99 µg/mL) (Figure 1). All three methanol extracts possessed high potency in inhibiting α-glucosidase compare to the reference drug, acarbose (IC_{50} 383.68 µg/mL) (Table 1). Previously, it has been reported that *R. tomentosa* fruit contains stilbenoid compound, such as resveratrol, and piceatannol [7]. These stilbenoids showed the more potent inhibition of α-glucosidase activity with IC_{50} 91 and 60 µg/mL respectively than acarbose (IC_{50} 247 µg/mL) [4]. d . In addtition, another phenolic compounds such as flavonoid isolated from *Morus alba* and anthocyanins isolated from noble muscadine grapes have

Commented [W5]: better displayed in mass units

Author: has been changed to mass units

Commented [W6]: is there any evidence that the extract of karamunting fruit containing stilbenoids? (e.a the TLC data between fruit extracts with standard resveratrol) or please delete this sentence.

Author : we did't have any evidence, only base on the literature [7]. So, the sentence has been deleted

been reported as potential α-glucosidase inhibitory [11,14]. Meanwhile, the triterpenoid saponins from *Gypsophila oldhamiana* also showed significant α-glucosidase inhibitory comparing to acarbose [15].

Base on its inhibition of α-glucosidase, the methanol extract of fruits was partitioned into *n*-hexane, ethyl acetate, and butanol. Ethyl acetate fraction had the highest α-glucosidase inhibitory (IC_{50} 13.49 μg/mL than butanol fraction (IC_{50} 19.29 μg/mL) due to its phenolic content, meanwhile, the *n*-hexane fraction was not as potent as α-glucosidase inhibitory (IC_{50} 1175.16 μg/mL) (Table 1 and Figure 1). Compounds typically found in hexane fractions are non-polar triterpenoid and steroid, while triterpenoid saponins and highly oxygenated triterpenoid found in the polar fraction such as ethyl acetate and *n*-butanol [1, 15].

Table 1. Inhibitory effect of the extract, fraction and compound on α-glucosidase activity

Extract/compound	Inhibitor concentration (IC_{50}, μg/mL)
MeOH extract of the leaves	43.99
MeOH extract of the stem	20.36
MeOH extract of the fruit	20.57
n-hexane fraction of the fruit	1175.16
Ethyl acetate fraction of the fruit	13.49
Butanol fraction of the fruit	19.29
Compound 1	110.45
Acarbose*	383.68

*positive control

Commented [W7]: Please mark this compound as a positive control of this test

Author : has been marked

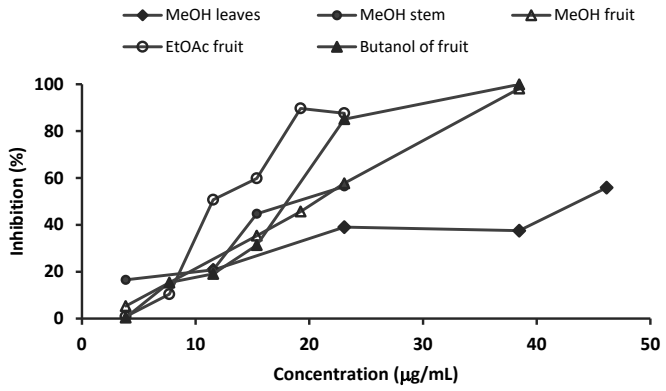


Figure 1. Effect of extracts and fractions on the inhibition of α-glucosidase

Isolation and structural elucidation

The sequential partition to the methanol crude extract of *R. tomentosa* fruits (87 g) yielded *n*-hexane, ethyl acetate and butanol fraction (1.54, 17.81 and 0.44 g respectively). Ethyl acetate fraction with the highest α-glucosidase inhibition was chromatographed over silica gel with some chromatographic technique to afford compound **1.**

Compound **1** was isolated as a white powder with m.p. 120-121 °C. The UV spectrum in methanol showed the maximum absorption at 242 nm which indicated the presence of α,β carbonyl unsaturated. The IR spectrum displayed absorption for the isolated carbonyl group at 1715 cm^{-1} as well as conjugated carbonyl group at 1678 and 1663 cm^{-1} which consisted to UV spectrum. In addition, there is absorption for C-H aliphatic group at 2976 and 2941 cm^{-1}. The ^{13}C-NMR (125 MHz, $CDCl_3$) was showed the presence of 14 signal. Two of the signal confirmed the existence of the isolated and conjugated carbonyl at δ_C 212.2 ppm and δ_C 192.2 ppm respectively. In addition, ^{13}C-NMR displayed the presence of five other quarternary carbon signal (δ_C 175.5 (oxy-carbon), 128.3, 113.2, 56.6, and 45.3 ppm), two signal for methine carbon (δ_C 46.6 and 34.5 ppm), and five signal for methyl carbon (δ_C 25.9, 24.5, 24.0, 22.4 and 15.6 ppm). Considering the intensity of quarternary carbon signal at 128.3 ppm with the six other quarternary carbon (included the carbonyl) which has a ratio of 1:2, indicating that the six quarternary carbon is equivalent to twelve carbon. Furthermore, the five methyl carbon signals have an intensity ratio of 2: 1 with a carbon methine signal at δ_C 34.5 ppm, consequently each of these methyl signals is identical for 2 methyl carbon (there are a total of 10 methyls). Based on this, compound **1** actually has 25 carbon atoms. The ^{1}H-NMR (500MHz, $CDCl_3$) spectrum exhibited the presence of a singlet signal of methine proton at δ_H 4.67 ppm. The spectrum also indicated the presence of an isopropyl unit with the appearance of a doublet signal at δ_H 1.00 ppm (6H, *d*, *J* = 6.9 Hz, 2xCH_3) which is adjacent to the methine proton at δ_H 2.35 ppm (1H, *sept*, *J* = 6.9 Hz). In addition, there are three singlet signals at δH 1.41 (12H), 1.32 (6H) and 1.25 ppm (6H) which indicate the presence of 8 methyl groups. The HMBC correlation revealed a correlation of both methyl on a geminal dimethyl group (δ_H 1.25 and 1.32 ppm) to the isolated and conjugated carbonyl group (δ_C 212.2 and 192.2 ppm) as well as correlation of both methyl on another geminal dimethyl group to the isolated carbonyl (δ_C 212.2 ppm) and oxy-carbon (δ_C 175.5 ppm). These explained that both of geminal dimethyl are α position in β-tri-ketone unit. Based on the previous NMR data, there is actually two symmetrical unit of β-tri-ketone. Furthermore, the correlation between of proton δ_H 4.67 ppm to isopropyl unit (δ_C 34.5 ppm) and oxy-carbon (δC 175.5 and 128.3 ppm) indicating that the isopropyl group was an adjacent bis-furan ring and the bis-furan ring was integrated with the β-tri-ketone unit. According to these spectroscopic evidence and comparing to the those of reported literature [11], the structure of compound **1** was established as rhodomyrtosone D.

Commented [W8]: better displayed in mass units

Author: has been changed to mass units

Commented [W9]: Please use dot instead of comma

Author : has been revised

Commented [W10]: Specthroscopic evidence

Author : has been changed to "spectroscopic evidence"

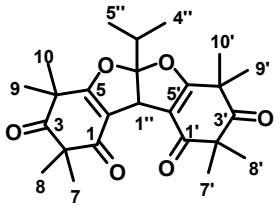


Figure 2. Structure of compound **1** (rhodomyrtosone D)

Table 2. NMR data of compound **1** in $CDCl_3$ and rhodomyrtosone D

No	Compound 1			Rhodomyrtosone D [11]	
	δC	δ_H (ΣH, *mult*, J_{Hz})	HMBC (H→C)	δ_C	δ_H (*mult*, J_{Hz})
1 (1')	192.2	-	-	192.4	-
2(2')	56.6	-	-	56.4	-
3(3')	212.2	-	-	212.1	-
4(4')	45.3	-	-	45.2	-
5(5')	175.5	-	-	175.7	-
6(6')	113.2	-	-	113.0	-
7(7')	25.8	1.25 (6H, *s*)	C-3(3'), C-1 (1'), C-2(2'), C- 8(8')	25.7	1.27 (*s*)
8(8')	22.4	1.32 (6H, *s*)	C-3(3'), C- 1 (1'), C- 2(2'), C- 7(7')	22.3	1.34 (*s*)
9(9')	24.0	1.41 (6H, *s*)	C-3(3'), C-5(5'), C-4(4'), C- 9(9'), C-10(10')	23.9	1.44 (*s*)
10(10')	24.5	1.41 (6H, *s*)	C-3(3'), C-5(5'), C-4(4'), C- 9(9'), C-10(10')	24.4	1.44 (*s*)
1''	46.6	4.67 (1H, *s*)	C-5(5'), C- 2'', C- 6(6'), C- 3''	46.5	4.69 (*s*)
2''	128.3	-	-	128.2	-
3''	34.5	2.35 (1H, s*ept,* 6.9)	C-2'', C- 1'', C- 4'', C- 5"	34.4	2.37 (*sept*, 6.9)
4'', 5"	15.6	1.00 (6H, *d*, 6.9)	C-2'', C- 3'', C- 4', C- 5"	15.5	1.02 (*d*, 6.9)

Commented [W11]: Where is the comparing/literature data of rhodomyrtosone D?

Author : the literature data has been added

The isolated compound **1** (rhodomyrtosone D) was examined for α-glucosidase inhibitory activity with concentration range about 30.77 to 0.24 μg/mL. The α- glucosidase inhibitory effect of rhodomyrtosone D (17.7% at 30.77 μg/mL) seems higher than the acarbose (8.54 % at 30.77 μg/mL). Using the extrapolation method to linear regression, the IC_{50} of rhodomyrtosone D on inhibiting α-glucosidase was 110.45 μg/mL.

CONCLUSION

All parts of *R. tomentosa* plant were potential as a source of a natural antidiabetic, especially from the ethyl acetate fraction. A bioactive compound, rhodomyrtosone D was isolated from the fruit of *Rhodomyrtus tomentosa* and showed higher α-glucosidase inhibition than acarbose.

ACKNOWLEDGMENTS

The author would like thanks to Dr. Nurainas, M.Si from Herbarium Anda, Andalas University for identification of plant specimen. We are grateful to the Ministry of Research, Technology and Higher Education for research grants through PD-UPT scheme (No: 093/SP2H/LT/DPRM/IV/2018).

REFERENCES

1. Yin, Z., Zhang, W., Feng, F., Zhang, Y., and Kang, W., 2014, α-Glucosidase Inhibitors Isolated from Medicinal Plants, *Food Science, and Human Wellness*, 3, 136-174.
2. Choudhary, M.I., Adhikari, A., Rasheed, S., Marasini, B.P., Hussain, N., Kaleem, W.A., and Rahman, A., 2011, Cyclopeptyde Alkaloid of *Ziziphus oxyphylla* Edgw as Novel Inhibitors of α-Glucosidase Enzyme and Protein Glycation, *Phytochemistry Letters*, 4, 404-406.
3. Lam. S.H., Cheng, J.M., Kang, C.J., Chen, C.H., and Lee, S.S., 2008, α-Glucosidase Inhibitors from the Seed of *Syagrus romanzoffiana*, *Phytochemistry*, 1173-1178.
4. Zhang, A.J., Rimando, A.M., Mizuno, C.S., and Mathews, S.T., 2017, α-Glucosidase Inhibitory Effect of Resveratrol and Piceatannol, *The Journal of Nutritional Biochemistry*, 47, 86-93.
5. Lavanya, G., Voravuthikunchai, S.P, and Towatana, N.H., 2012, Acetone Extract from *Rhodomyrtus tomentosa*: A Potent Natural Antioxidant, *Evidence-Based Complementary and Alternative Medicine,* 2012, Article ID 535479, 1-8.
6. Limsuwan, S., Kayser, O., and Voravuthikunchai, S.P., 2012, Antibacterial Activity of Rhodomyrtus tomentosa (Aiton) Hassk. Leaf Extract against Clinical Isolates of *Streptococcus pyogenes*, *Evidence-Based Complementary and Alternative Medicine,* 2012, Article ID 697183, 1-6.
7. Lai, T.N.H., Herent, M.F., Quetin-Leclercq, J., Nguyen, T.B.T., Larondelle, Y., Andre, C.M., and Rogez, H., 2013, Piceatannol, a Potent Bioactive Stilbene, as Major Phenolic Component in *Rhodomyrtus tomentosa*, *Food Chemistry*, 138: 1421-1430
8. Wu, P., Ma, G., Li, N., Deng, Q., Yin, Y., and Huang, R., 2015, Investigation of In Vitro and in Vivo Antioxidant Activities of Flavonoids Rich Extract from the Berries of *Rhodomyrtus tomentosa* (Ait.) Hassk., *Food Chemistry*, 173, 194-202.
9. Hiranrat, A., Mahabusakaram, W., Carrol, A.R., Duffy, S., and Avery, V.M., 2012, Tomentosones A and B, Hexacyclic Phloroglucinol Derivatives from the Thai Shrub *Rhodomyrtus tomentosa*, *J. Org. Chem.*, 77, 680-683.

10. Limsuwan, S., Trip, E.N., Kouwen, T.R.H.M., Piersma, S.,Hiranrat, A., Mahabusakaram, W., Voravuthikunchai, S.P., Dijl, JM., and Kayse, O., 2009, Rhodomyrtone, A new Candidate as Natural Antibacterial Drug from *Rhodomyrtus tomentosa*, *Phytomedicine*, 16, 645-651.
11. Hiranrat, A., and Mahabusakaram, W., 2008, New Acylphloroglucinols from the Leaves of *Rhodomyrtus tomentosa*, *Tetrahedron*, 64, 11193-11197
12. Yang, Z., Wang, Y., Wang, Y., and Zhang, Y., 2012, Bioassay-Guided Screening and Isolation of α-glucosidase and Tyrosinase Inhibitors from Leaves of *Morus alba*, *Food Chemistry*, 617-625.
13. Anisah, L.N., Syafii, W., Pari, G., and Sari, R.K., 2018, Antidiabetic Activities and Identification of Chemical Compound from Samama (*Anthocephalus macrophyllus* (Roxb) Havil), *Indones. J. Chem*, 18(1), 66-74.

α-Glucosidase Inhibitory and A LeptospermoneDerivative from *Rhodomyrtustomentosa* Extract

ABSTRACT

One of the treatmentsfor diabetes mellitus disease is to control blood sugar level using an inhibitor of α-glucosidase enzyme. The methanol extracts of the fruit, stem,and leaves of *R. tomentosa*were found significant in inhibiting α-glucosidase (IC_{50} 20.57, 20.36 and 43.99 μg/mL respectively). The ethyl acetate and *n*-butanol fraction from the methanol extract of *R. tomentosa* fruit exhibited the potent inhibition (IC_{50} 13.49 and 19.29 μg/mL) compare to acarbose and *n*-hexane fraction (IC_{50} 383.68 and 1175.16 μg/mL). A leptospermone derivative, rhodomyrtosone D was isolated from the ethyl acetate fraction of *R. tomentosa* fruit. The structure was identified base on spectroscopic analysis, as well as comparing with literature data. The α-glucosidase inhibition of rhodomyrtosone D (IC_{50} 110.45 μg/mL) was 3.5 fold more potent than acarbose. Thus, the plant could be potential as a natural resourceof α-glucosidase inhibitor

Keywords:α-glucosidase, *Rhodomyrtustomentosa*, antidiabetic, rhodomyrtosone D, ethyl acetate fraction

ABSTRAK

Salah satupenanganan diabetes mellitus adalahdenganmengontrolkadarguladarahmenggunakanpenghambatkerjaenzimα-glukosidase. Ekstrakmetanolbuah, batangdandaun*R. tomentosa*menunjukkanpenghambatanα-glukosidase yang signifikan (IC_{50} 20,57; 20,36 dan 43,99 μg/mL). Fraksietilasetatdan butanol yang diperolehdariekstrakmetanolbuah R. tomentosamenunjukkanpenghambatan yang potensial (IC_{50} 13,49dan 19,29 μg/mL) dibandingkandenganakarbosadanfraksi*n*-hexane (IC_{50} 383,68 and 1175,16 μg/mL). SuatuturunanleptospermonyaiturhodomyrtosonD telahdiisolasidarifraksietilasetatbuah*R. tomentosa*. Strukturtersebutdiidentifikasiberdasarkananalisisspektroskopidanmembandingkandenganliteratur. Penghambatanα-glukosidasedarirhodomyrtoson D 3,5 kali lebihkuatdibandingkandenganakarbosa. Dengandemikian, tumbuhaniniberpotensisebagaisumberalamipenghambatenzimα-glukosidase.

Kata kunci:α-glukosidase, *Rhodomyrtustomentosa*, antidiabetes, rhodomyrtosone D, fraksietilasetat.

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorder, in which there are high blood sugar levels (hyperglycemia) over a prolonged period [1]. It will happen if the pancreas does not produce enough insulin that is able to convert sugar into energy, or the body's cells do not respond well to the insulin produced.Some serious complication of hyperglycemia such as cardiovascular disease, damage to the eyes, atherosclerosis, and chronic kidney disease (nephropathy) can also occur [2]. The control of blood sugar level by inhibition of carbohydrate-hydrolyzing enzymes in the digestive organ is believed to be important in hyperglycemia treatment [1]. α-glucosidase, an enzyme in the small intestine is responsible for the degradation of carbohydrate. The α-glucosidase inhibitor will interfere withthe digestion of carbohydrate and thereby reduce the postprandial glucose level and insulin responses in a diabetic patient [2-3]. Acarbose, miglitol,andvoglibose have been found as anα-glucosidase inhibitor and currently clinically used to control blood glucose of diabetic patients [4]. However, they have been caused serious gastrointestinal side effects. Nowadays, natural resources have received tremendous attention as a therapeutic agent in the inhibition of α-glucosidase and have shown very promising biological activity.

Karamuntingis locally named (Sumatera island) for *Rhodomyrtustomentosa*and belonging to Myrtaceae family. This plant is an evergreen shrub which is native to Southern Asia and Southeast Asia and is widely distributed in Indonesia. *R. tomentosa*is widely used as traditional medicines to treat a variety of disease caused by bacteriasuch asdiarrhea, dysenteryand urinary tract infections [5-6]. In addition, its ripe fruits are usedtoboost the immune system [7]. Biologically, ethanolic extract of *R. tomentosa* fruits possesses potent antioxidant activities on DPPH radical scavenging activity, reducing power as well as inhibition of lipid peroxidation activity [8] Furthermore, some extract of this plants were reported as antibacterial and anti-hepatitis properties [9]. Chemically, various secondary metabolites have been reported such as polyketide, flavonoids, anthocyanins, stilbenoids,and triterpenoids. Rhodomyrtone, a phloroglucinolpolyketide from *R.tomentosa* have displayed significant antibacterial activities against Gram-positive bacteria and suggested as a new candidate as a natural antibacterial drug [10]. Meanwhile, tomentosone A, a hexacyclicphloroglucinolwas reported as antimalaria against chloroquine-resistant and sensitive strains of *Plasmodium falciparum*. Resveratrol and piceatannol, a stilbenoid compound has been characterized from this plant [7]. A stilbenoid compound from *Syagrusromanzoffiana*was reported as a potentialhypoglycemic agent. In a search for potentialα-glucosidase inhibitor from natural resources, we have been investigated the ability of *R. tomentosa* plant to inhibit the activity of the α–glucosidase enzyme as well as to isolate the bioactive compound. One active compound, rhodomyrtosone (**1**) was isolated and its α-glucosidase inhibition was determined. The following describes the outcomes of these efforts.

EXPERIMENTAL SECTION

.

Materials

Rhodomyrtustomentosa(fruits, leaves,and stem) were collected from Inderalaya, OganIlir, South Sumatera. The plant was identified at Herbarium Anda, Department of Biology, University of Andalas. The solvents (methanol, *n*-hexane and ethyl acetate) were a technical quality that is distilled while *n*-butanol and dimethylsulfoxide (DMSO) were pro analysis (p.a) from Merck.α-glucosidase (from *Saccharomyces cerevisiae*) and *p*-nitro-phenyl-α-D-glucopyranoside were purchased from Sigma-Aldrich. Bovine serum albumin (BSA) was purchased from Merck. Silica gel 60G (Merck) was used for vacuum liquid chromatography and silica gel 60 PF_{254}(Merck) was used for radial chromatography. TLC analysis was performed on Kieselgel 60 GF_{254}, 0.25 mmaluminum plate (Merck) and visualized with cerium sulfate.

Instrumentation

IncubatorBiosan PST-60HL was used for sample incubation process. The absorbance of *p*-nitrophenol was measured by a Tecan Infinite F50 Microplate reader.UV spectrumwas recorded with Shimadzu UV-1240 spectrophotometer.IR spectrumwas determined using KBr pellets on a Perkin Elmer FTIR Spectrum One spectrophotometer. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (500 MHz) spectra were recorded with Agilent DD2spectrometer, using residual and deuterated solvent peaks as reference standards.

Procedure

Extraction of Sample

100 gr of the dried powdered sample (fruits, leaves,and stem) of *R.tomentosa* were extracted by maceration method using methanol (400 mL) as the solvent at theroom temperature. The maceration process was carried out three times. The methanol solvents were evaporated in under reduce pressure to give a crude extract of methanol of fruit, leaves,and stem (4.6, 4.2 and 3.9 g respectively). The crude of methanol extract of fruit was partitioned successively with *n*-hexane, ethyl acetate,and*n*-butanol and produce of each fraction after the solvent was evaporated.

In-vitro α-glucosidase inhibition assay

The α-glucosidase assay has been performed using the spectrophotometric method as previously described [2, 11, 12] with slight modification. 10 μL of the sample at various concentrations was added with 55 μL of 50 mM phosphate buffer (pH 6.8) and 10 μL of 10 mM*p*-

nitrophenyl-α-D-glucopyranoside as the substrate. After preincubated for 5 min at 37 °C, 25 µl of 0.1 U/mL α-glucosidase (in the phosphate buffer pH 6.8 containing 0.1 mg/mL bovine serum albumin) was added. The mixture was then incubated for 30 min at 37 °C. After that, the stopped solution (100 µl of 200 mM Na_2CO_3) was added to the mixture. The absorbance of the *p*-nitrophenolreleased due to hydrolysis of the substrateby the α- glucosidase was measured by microplate reader at 405 nm. The blank solution was prepared by replaced sample solution by DMSO. Acarbose (Glucobay®) is used as a positive control. The percentage inhibition of α-glucosidase was calculated using the following equation: Inhibition% = [1-(A_{sample}/A_{blank})] x 100. The IC_{50} was calculated by linear regression equation analysis between concentration and percentage inhibition.

Extractionand Isolation

The dried fruits (2 kg) were extracted with methanol (3 x 7 L, 24 h each) by maceration method. The methanol extract was concentrated under reduced pressure to give 1.1 L syrup which was suspended in distilled water. This suspension was partitioned successively with *n*-hexane, ethyl acetate,and *n*-butanol to afford *n*-hexane, ethyl acetate and *n*-butanol fraction. The ethyl acetate fraction (15 g) was fractionated by vacuum liquid chromatographyon silica gel 60 G, eluting with *n*-hexane-ethyl acetate system with increment ethyl acetate gradually (9:1→8:2→7:3→6:4→4:6 →2:8→1:9→0:10, each 150 mL) to give 8 fractions (A-H). Fraction C (374 mg) was further separated by radial chromatography over silica gel 60 PF_{254} (1 mm), eluted with n-hexane-ethyl acetate gradually (85:15→80:20→75:25→70:30→60:40→50:50) to yield 7 subfractions. Subfraction C2 yielded a leptospermone derivative **1** (8.9 mg).

RESULTS AND DISCUSSION

α-Glucosidaseinhibition of extracts and fractions

The extraction of three parts of karamunting (*R. tomentosa*) plants namely fruit, stem,and leaves) produced methanol extract of 4.6%, 3.9%,and 4.2% respectively. All of these extractswere tested for α-glucosidase inhibitory using *p*-nitrophenyl-α-D-glucopyranoside as the substrateand acarbose as the reference or positive control. The methanol extract from the stem and fruit have the similar ability to inhibit α-glucosidase activity (IC_{50} 20.36 and 20.57µg/mL). Both of these extracts demonstratedtwo times more potent than the leaves methanol extract (IC_{50}43.99µg/mL) (Figure 1). All three methanol extracts possessed high potency in inhibiting α-glucosidase compare to the reference drug, acarbose (IC_{50} 383.68 µg/mL) (Table 1).Previously reported by Lai et al, *R. tomentosa* fruit contains stilbenoid compound, such as resveratrol,andpiceatannol. These stilbenoidsshowed the more potent inhibition of α-glucosidase activity with IC_{50} 91 and 60 mg/mL

respectively than acarbose (IC_{50} 247 μg/mL) [4]. Therefore, it is assumed that the inhibition of α-glucosidase in this plant is due to the content of the stilbenoid compound.

Base on its inhibition of α-glucosidase, the methanol extract of fruits waspartitioned into *n*-hexane, ethyl acetate, and butanol. Ethyl acetate fraction had the highest α-glucosidase inhibitory (IC_{50} 13.49 μg/mL than butanol fraction (IC_{50} 19.29 μg/mL) due to its phenolic content,meanwhile, the *n*-hexane fraction was not as potent as α-glucosidase inhibitory (IC_{50}1175.16 μg/mL) (Table 1 and Figure 1).

Table 1. Inhibitory effect of the extract, fraction and compound on α-glucosidase activity

Extract/compound	**Inhibitor concentration (IC_{50}, μg/mL)**
MeOH extract of the leaves	43.99
MeOH extract of the stem	20.36
MeOH extract of the fruit	20.57
n-hexane fraction of the fruit	1175.16
Ethyl acetate fraction of the fruit	13.49
Butanol fraction of the fruit	19.29
Compound 1	110.45
Acarbose	383.68

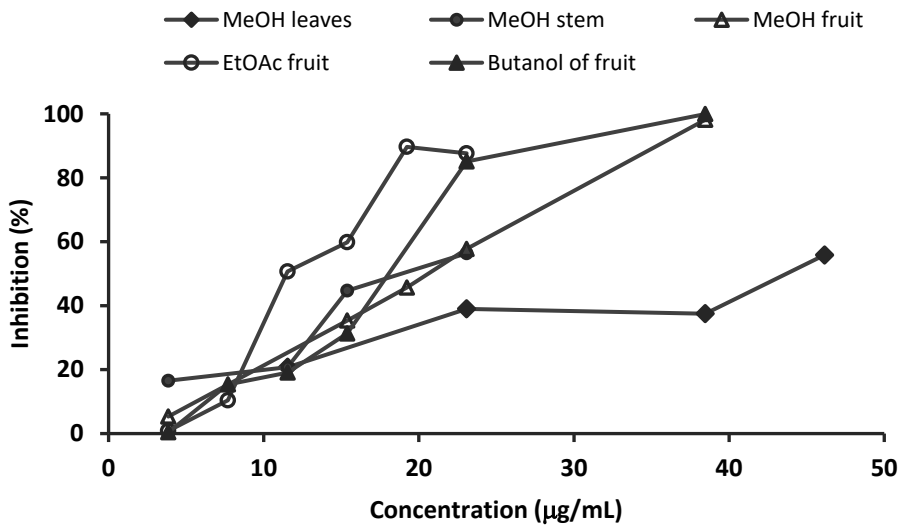


Figure 1. Effect of extracts and fractions on the inhibition of α-glucosidase

Isolation and structural elucidation

The sequentialpartition tothe methanol crude extract of *R. tomentosa*fruits (87 g) yielded *n*-hexane, ethyl acetate and butanol fraction of1.7%, 20.5%,and0.5% respectively. Ethyl acetate fraction with the highest α-glucosidase inhibition was chromatographed over silica gel with some chromatographic technique to afford a leptospermone derivative **1.**

Compound **1** was isolated as a white powder with m.p. 120-121 ^{o}C.The UV spectrum in methanol showed the maximum absorption at 242 nm which indicated the presence of α,β carbonyl unsaturated. The IR spectrum displayed absorption for the isolated carbonyl group at 1715 cm^{-1} as well as conjugated carbonyl group at 1678 and 1663 cm^{-1}whichconsisted to UV spectrum. In addition, there is absorption for C-H aliphatic group at 2976 and 2941 cm^{-1}. The ^{13}C-NMR (125 MHz, $CDCl_3$) was showed the presence of 14 signal. Two of the signal confirmed the existence of the isolated and conjugated carbonyl at pada δ_C 212,2 ppm and δ_C 192,2 ppm respectively. In addition, ^{13}C-NMR displayed the presence of five other quarternary carbon signal (δ_C175.5 (oxy-carbon), 128.3, 113.2, 56.6, and 45.3 ppm), two signal for methine carbon (δ_C 46.6 and 34.5 ppm), and five signal for methyl carbon (δ_C 25.9, 24.5, 24.0, 22.4 and 15.6 ppm). Considering the intensity of quarternary carbon signal at 128.3 ppm with the six other quarternary carbon (included the carbonyl) which has a ratio of 1:2, indicating that the six quarternary carbon is equivalent to twelve carbon. Furthermore, the five methyl carbon signals have an intensity ratio of 2: 1 with a carbon methinesignal at δ_C 34.5 ppm, consequently each of these methyl signals is identical for 2 methyl carbon (there are a total of 10 methyls). Based on this, compound **1** actually has 25 carbon atoms. The ^{1}H-NMR (500MHz, $CDCl_3$) spectrum exhibited the presence of a singlet signal of methine proton at δ_H 4.67 ppm.The spectrum also indicated the presence of an isopropyl unit with the appearance of a doublet signal at δ_H1.00 ppm (6H, *d*, *J* = 6.9 Hz, 2xCH_3) which is adjacent to the methineproton at δ_H 2.35 ppm (1H, *sept*, *J* = 6.9 Hz). In addition, there are three singlet signals atδH 1.41 (12H), 1.32 (6H) and 1.25 ppm (6H) which indicate the presence of 8 methyl groups. The HMBC correlation revealed a correlation of both methyl on a geminaldimethyl group(δ_H 1.25 and 1.32 ppm) to the isolated and conjugated carbonyl group (δ_C 212.2 and 192.2 ppm) as well as correlation of both methyl on another geminal dimethyl group to the isolated carbonyl (δ_C 212.2 ppm) and oxy-carbon (δ_C175.5 ppm). These explained that both of geminal dimethyl are α position in β-triketone unit. Based on the previous NMR data, there is actually two symmetrical unit of β-triketone. Furthermore, the correlation between of proton δ_H4.67 ppm to isopropyl unit (δ_C 34.5 ppm) and oxy-carbon (δC 175.5 and 128.3 ppm) indicating that the isopropyl group was an adjacentbis-furan ring and the bis-furan ring was integrated with the β-triketone unit. According to these spectral studies and comparing to the those of reported literature [13], the structure of compound **1** was established as rhodomyrtosone D.

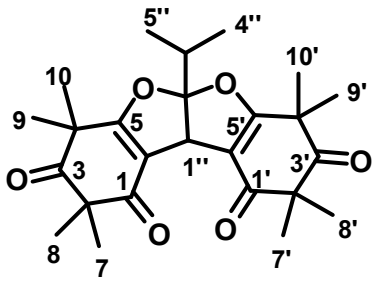


Figure 2. Structure of compound **1** (rhodomyrtosone D)

Table 2. NMR data of compound **1**in $CDCl_3$

No	Compound 1		
	δC[a]	δ_H (ΣH, *mult*,J_{Hz})[b]	HMBC (H→C)
1 (1')	192.2	-	-
2(2')	56.6	-	-
3(3')	212.2	-	-
4(4')	45.3	-	-
5(5')	175.5	-	-
6(6')	113.2	-	-
7(7')	25.8	1.25 (6H, *s*)	C-3(3'), C-1 (1'), C-2(2'), C- 8(8')
8(8')	22.4	1.32 (6H, *s*)	C-3(3'), C- 1 (1'), C- 2(2'), C- 7(7')
9(9')	24.0	1.41 (6H, *s*)	C-3(3'),C-5(5'),C-4(4'), C- 9(9'), C-10(10')
10(10')	24.5	1.41 (6H, *s*)	C-3(3'),C-5(5'),C-4(4'), C- 9(9'), C-10(10')
1''	46.6	4.67 (1H, *s*)	C-5(5'), C- 2'', C- 6(6'), C- 3''
2''	128.3	-	-
3''	34.5	2.35 (1H, s*ept*)	C-2'', C- 1'', C- 4'', C- 5"
4'', 5"	15.6	1.00 (6H, *d*, 6.9)	C-2'', C- 3'', C- 4', C- 5"

[a] 125 MHz
[b] 500 MHz

The isolated compound **1** (rhodomyrtosone D) was examined for α-glucosidase inhibitory activity with concentration range about 30.77 to 0.24 μg/mL.The α- glucosidase inhibitory effect of rhodomyrtosone D (17.7% at 30.77 μg/mL) seems higher than the acarbose (8.54 % at 30.77 μg/mL). Using the extrapolation method to linear regression, the IC_{50} of rhodomyrtosone D on inhibiting α-glucosidase was 110.45 μg/mL.

CONCLUSION

In summary, all parts of *R. tomentosa* plant were potential as a source of a natural antidiabetic, especially from the ethyl acetate fraction. A bioactive compound, rhodomyrtosone D was isolated from the fruit of *Rhodomyrtustomentosa* and showed higher α-glucosidase inhibition than acarbose.

ACKNOWLEDGMENTS

The author would like thanks to Dr.Nurainas, M.Si from Herbarium Anda, Andalas University for identification of plant specimen. We are grateful to the Ministry of Research, Technology and Higher Education for research grants through PD-UPT scheme (No: 093/SP2H/LT/DPRM/IV/2018).

REFERENCES

1. Yin, Z., Zhang, W., Feng, F., Zhang, Y., and Kang, W., 2014, α-Glucosidase InhibitorsIsolated from Medicinal Plants, *Food Science,and Human Wellness*, 3, 136-174.
2. Choudhary, M.I., Adhikari, A., Rasheed, S., Marasini, B.P., Hussain, N.,Kaleem, W.A., and Rahman, A., 2011, Cyclopeptyde Alkaloid of *Ziziphusoxyphylla*Edgw as Novel Inhibitors of α-Glucosidase Enzyme and Protein Glycation, *Phytochemistry Letters*, 4, 404-406.
3. Lam. S.H., Cheng, J.M., Kang, C.J., Chen, C.H., and Lee, S.S., 2008, α-Glucosidase Inhibitors from the Seed of *Syagrusromanzoffiana*, *Phytochemistry*, 1173-1178.
4. Zhang, A.J., Rimando, A.M., Mizuno, C.S., and Mathews, S.T., 2017, α-Glucosidase Inhibitory Effect of Resveratrol and Piceatannol, *The Journal of Nutritional Biochemistry*, 47, 86-93.
5. Lavanya, G., Voravuthikunchai, S.P, and Towatana, N.H., 2012,Acetone Extract from *Rhodomyrtustomentosa*: A Potent Natural Antioxidant, *Evidence-Based Complementary and Alternative Medicine,*2012, Article ID 535479, 1-8.
6. Limsuwan, S., Kayser, O., and Voravuthikunchai, S.P., 2012, Antibacterial Activity ofRhodomyrtustomentosa (Aiton) Hassk. Leaf Extract against Clinical Isolates of *Streptococcus pyogenes*, *Evidence-Based Complementary and Alternative Medicine,*2012, Article ID 697183, 1-6.
7. Lai, T.N.H., Herent, M.F., Quetin-Leclercq, J.,Nguyen, T.B.T.,Larondelle, Y., Andre, C.M., and Rogez, H., 2013, Piceatannol, a Potent Bioactive Stilbene, as Major Phenolic Component in *Rhodomyrtustomentosa*, *Food Chemistry*, 138: 1421-1430
8. Wu,P.,Ma,G.,Li,N.,Deng,Q.,Yin,Y.,andHuang,R., 2015, Investigationof In Vitro and in VivoAntioxidant Activities of Flavonoids Rich Extract from theBerriesof*Rhodomyrtustomentosa*(Ait.)Hassk., *FoodChemistry*,173, 194-202.
9. Hiranrat, A., Mahabusakaram, W., Carrol, A.R., Duffy, S., and Avery, V.M., 2012, Tomentosones A and B, HexacyclicPhloroglucinol Derivatives from the Thai Shrub *Rhodomyrtustomentosa*, *J. Org. Chem*., 77, 680-683.
10. Limsuwan, S., Trip, E.N., Kouwen, T.R.H.M., Piersma, S.,Hiranrat, A., Mahabusakaram, W., Voravuthikunchai, S.P., Dijl, JM., and Kayse, O., 2009, Rhodomyrtone, A new Candidate as Natural Antibacterial Drug from *Rhodomyrtustomentosa*, *Phytomedicine*, 16, 645-651.
11. Yang, Z., Wang, Y., Wang, Y., and Zhang, Y., 2012, Bioassay-Guided Screening and Isolation of α-glucosidase and Tyrosinase Inhibitors from Leaves of *Morus alba*, *Food Chemistry*, 617-625.
12. Anisah, L.N., Syafii, W., Pari, G., and Sari, R.K., 2018, Antidiabetic Activities and Identification of Chemical Compound from Samama (*Anthocephalusmacrophyllus* (Roxb) Havil), *Indones. J. Chem*, 18(1), 66-74.
13. Hiranrat, A., and Mahabusakaram, W., 2008, New Acylphloroglucinols from the Leaves of *Rhodomyrtustomentosa*, *Tetrahedron*, 64, 11193-11197.

α-Glucosidase Inhibitory and A Leptospermone Derivative from *Rhodomyrtus tomentosa*

Ferlinahayati[1]*, Daniel Alfarado[1], Eliza[1,] and Budi Untari[2]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Sriwijaya
Jalan Raya Palembang Prabumulih Km 32, Ogan Ilir, South Sumatera, Indonesia 30622

[2]Department of Pharmacy, Faculty of Mathematics and Natural Sciences, University of Sriwijaya
Jalan Raya Palembang Prabumulih Km 32, Ogan Ilir, South Sumatera, Indonesia 30622

* Corresponding author, tel/: 081394741890, email: etihayati74@yahoo.com

ABSTRACT

One of the treatments for diabetes mellitus disease is to control blood sugar level using an inhibitor of α-glucosidase enzyme. The methanol extracts of the fruit, stem, and leaves of *Rhodomyrtus tomentosa* were found significant in inhibiting α-glucosidase (IC_{50} 20.57, 20.36 and 43.99 μg/mL respectively). The ethyl acetate and *n*-butanol fractions from the methanol extract of *R. tomentosa* fruit exhibited the potent inhibition (IC_{50} 13.49 and 19.29 μg/mL) compare to acarbose and *n*-hexane fraction (IC_{50} 383.68 and 1175.16 μg/mL). A leptospermone derivative, rhodomyrtosone D was isolated from the ethyl acetate fraction of *R. tomentosa* fruit. The structure of rhodomyrtosone D was identified base on spectroscopic analysis, as well as comparing with literature data. The α-glucosidase inhibition of rhodomyrtosone D (IC_{50} 110.45 μg/mL) was 3.5 fold more potent than acarbose. Thus, *R. tomentosa* plant could be potential as a natural resource of α-glucosidase inhibitor.

Keywords: α-glucosidase, *Rhodomyrtus tomentosa*, antidiabetic, rhodomyrtosone D, ethyl acetate fraction

ABSTRAK

Salah satu penanganan diabetes mellitus adalah dengan mengontrol kadar gula darah menggunakan penghambat kerja enzim α-glukosidase. Ekstrak metanol buah, batang dan daun *R. tomentosa* menunjukkan penghambatan α-glukosidase yang signifikan (IC_{50} 20,57; 20,36 dan 43,99 μg/mL). Fraksi etil asetat dan *n*-butanol yang diperoleh dari ekstrak metanol buah R. tomentosa menunjukkan penghambatan yang potensial (IC_{50} 13,49 dan 19,29 μg/mL) dibandingkan dengan akarbosa dan fraksi *n*-hexana (IC_{50} 383,68 and 1175,16 μg/mL). Suatu

turunan leptospermon yaitu rhodomyrtoson D telah diisolasi dari fraksi etil asetat buah *R. tomentosa*. Struktur senyawa rhodomyrtosone D ditetapkan berdasarkan analisis spektroskopi dan membandingkan dengan literatur. Penghambatan α-glukosidase dari rhodomyrtoson D menunjukkan 3,5 kali lebih kuat dibandingkan dengan akarbosa. Dengan demikian, tumbuhan *R. tomentos*a berpotensi sebagai sumber alami penghambat enzim α-glukosidase.

Kata kunci: α-glukosidase, *Rhodomyrtus tomentosa*, antidiabetes, rhodomyrtosone D, fraksi etil asetat.

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorder, in which there are high blood sugar levels (hyperglycemia) over a prolonged period [1]. It will happen if the pancreas does not produce enough insulin that is able to convert sugar into energy, or the body's cells do not respond well to the insulin produced. Some serious complication of hyperglycemia such as cardiovascular disease, damage to the eyes, atherosclerosis, and chronic kidney disease (nephropathy) can also occur [2]. The control of blood sugar level by inhibition of carbohydrate-hydrolyzing enzymes in the digestive organ is believed to be important in hyperglycemia treatment [1]. The α-glucosidase, an enzyme in the small intestine is responsible for the degradation of carbohydrate. The α-glucosidase inhibitor will interfere with the digestion of carbohydrate and thereby reduce the postprandial glucose level and insulin responses in a diabetic patient [2-3]. Acarbose, miglitol, and voglibose have been found as an α-glucosidase inhibitor and currently clinically used to control blood glucose of diabetic patients [4]. However, they have been caused serious gastrointestinal side effects. Nowadays, natural resources have received tremendous attention as a therapeutic agent in the inhibition of α-glucosidase and have shown very promising biological activity.

Karamunting is locally named (Sumatera island) for *Rhodomyrtus tomentosa* and belonging to Myrtaceae family. This plant is an evergreen shrub which is native to Southern Asia and Southeast Asia and is widely distributed in Indonesia. *R. tomentosa* is widely used as traditional medicines to treat a variety of disease caused by bacteria such as diarrhea, dysentery and urinary tract infections [5-6]. In addition, its ripe fruits are used to boost the immune system [7]. Biologically, ethanolic extract of *R. tomentosa* fruits possesses potent antioxidant activities on DPPH radical scavenging activity, reducing power as well as inhibition of lipid peroxidation activity [8] Furthermore, some extract of this plants were reported as antibacterial and anti-hepatitis properties [9]. Chemically, various secondary metabolites have been reported such as polyketide, flavonoids, anthocyanins, stilbenoids, and triterpenoids [7-11]. Rhodomyrtone, a phloroglucinol polyketide from *R. tomentosa* have displayed significant antibacterial activities against Gram-positive bacteria and suggested as a new candidate as a natural antibacterial drug [10]. Meanwhile, tomentosone A, a hexacyclic phloroglucinol was reported as antimalarial against

chloroquine-resistant and sensitive strains of *Plasmodium falciparum*. Resveratrol and piceatannol, a stilbenoid compound has been characterized from this plant [7]. A stilbenoid compound from *Syagrus romanzoffiana* was reported as a potential hypoglycemic agent. However, there is no literature on the α-glucosidase inhibitory of *R. tomentosa* and its bioactive chemical compound. In a search for potential α-glucosidase inhibitor from natural resources, we have been investigated the ability of *R. tomentosa* plant to inhibit the activity of the α–glucosidase enzyme as well as to isolate the bioactive compound. One active compound, rhodomyrtosone D (**1**) was isolated and its α-glucosidase inhibition was determined. The following describes the outcomes of these efforts.

EXPERIMENTAL SECTION

.

Materials

Rhodomyrtus tomentosa (fruits, leaves, and stem) were collected from Inderalaya, Ogan Ilir, South Sumatera. The plant was identified at Herbarium Anda, Department of Biology, University of Andalas. The solvents (methanol, *n*-hexane and ethyl acetate) were a technical quality that is distilled while *n*-butanol and dimethylsulfoxide (DMSO) were pro analysis (p.a) from Merck. The α-glucosidase (from *Saccharomyces cerevisiae*) and *p*-nitro-phenyl-α-D-glucopyranoside were purchased from Sigma-Aldrich. Bovine serum albumin (BSA) was purchased from Merck. Silica gel 60G (Merck) was used for vacuum liquid chromatography and silica gel 60 PF_{254} (Merck) was used for radial chromatography. TLC analysis was performed on Kieselgel 60 GF_{254}, 0.25 mm aluminum plate (Merck) and visualized with cerium sulfate.

Instrumentation

Incubator Biosan PST-60HL was used for sample incubation process. The absorbance of *p*-nitrophenol was measured by a Tecan Infinite F50 Microplate reader. UV spectrum was recorded with Shimadzu UV-1240 spectrophotometer. IR spectrum was determined using KBr pellets on a Perkin Elmer FTIR Spectrum One spectrophotometer. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded with Agilent DD2 spectrometer, using residual and deuterated solvent peaks as reference standards.

Procedure

Extraction of sample for assay

As much as 100 gr of each the dried powdered sample (fruits, leaves, and stem) of *R.tomentosa* were extracted by maceration method using methanol (400 mL) as the solvent at the

room temperature. The maceration process was carried out three times (@ 24 hours). The methanol solvents were evaporated in under reduce pressure to give a crude extracts of methanol of fruit, leaves, and stem (4.6, 4.2 and 3.9 g respectively). The crude of methanol extract of fruit was partitioned successively with *n*-hexane, ethyl acetate, and *n*-butanol and produce of each fraction after the solvent was evaporated.

In-vitro α-glucosidase inhibition assay

The α-glucosidase assay has been performed using the spectrophotometric method as previously described [2, 12, 13] with slight modification. As much as 10 μL of the sample at various concentrations was added with 55 μL of 50 mM phosphate buffer (pH 6.8) and 10 μL of 10 mM *p*-nitrophenyl-α-D-glucopyranoside as the substrate. After preincubated for 5 min at 37 °C, 25 μL of 0.1 U/mL α-glucosidase (in the phosphate buffer pH 6.8 containing 0.1 mg/mL bovine serum albumin) was added. The mixture was then incubated for 30 min at 37 °C. After that, the stopped solution (100 μL of 200 mM Na_2CO_3) was added to the mixture. The absorbance of the *p*-nitrophenol released due to hydrolysis of the substrate by the α- glucosidase was measured by microplate reader at 405 nm. The blank solution was prepared by replaced sample solution by DMSO. Acarbose (Glucobay®) is used as a positive control. The percentage inhibition of α-glucosidase was calculated using the following equation: Inhibition % = [1-(A_{sample} / A_{blank})] x 100. The IC_{50} was calculated by linear regression equation analysis between concentration and percentage inhibition.

Extraction and Isolation of *R. tomentosa* fruits

The dried fruits (2 kg) was extracted with methanol (3 x 7 L, 24 h each) by maceration method. The methanol extract was concentrated under reduced pressure to give 1.1 L syrup which was suspended in distilled water. This suspension was partitioned successively with *n*-hexane, ethyl acetate, and *n*-butanol to afford *n*-hexane, ethyl acetate and *n*-butanol fractions. The ethyl acetate fraction (15 g) was fractionated by vacuum liquid chromatography on silica gel 60 G, eluting with *n*-hexane-ethyl acetate system with increment ethyl acetate gradually (9:1, 8:2, 7:3, 6:4, 4:6, 2:8, 1:9, and 0:10, each 150 mL) to give 8 fractions (A-H). Fraction C (374 mg) was further separated using radial chromatography over silica gel 60 PF_{254} (1 mm), eluted with n-hexane-ethyl acetate gradually (85:15, 80:20, 75:25, 70:30, 60:40, 50:50) to yield a leptospermone derivative 1 (8.9 mg)

RESULTS AND DISCUSSION

The α-Glucosidase inhibition of extracts and fractions

The extraction of three parts of *R. tomentosa* (fruit, stem, and leaves) produced methanol extract 4.6, 3.9, and 4.2 g respectively. All of these extracts were tested for α-glucosidase inhibitory using *p*-nitrophenyl-α-D-glucopyranoside as the substrate and acarbose as the reference or positive control. The methanol extract from the stem and fruit have a similar ability to inhibit α-glucosidase activity (IC_{50} 20.36 and 20.57 μg/mL). Both of these extracts demonstrated two times more potent than the leaves methanol extract (IC_{50} 43.99 μg/mL) (Figure 1). All three methanol extracts possessed high potency in inhibiting α-glucosidase compare to the reference drug, acarbose (IC_{50} 383.68 μg/mL) (Table 1). Previously, it has been reported that *R. tomentosa* fruit contains stilbenoid compound, such as resveratrol, and piceatannol [7]. These stilbenoids showed the more potent inhibition of α-glucosidase activity with IC_{50} 91 and 60 μg/mL respectively than acarbose (IC_{50} 247 μg/mL) [4]. In addtition, another phenolic compounds such as flavonoid isolated from *Morus alba* and anthocyanins isolated from noble muscadine grapes have been reported as potential α-glucosidase inhibitory [11,14]. Meanwhile, the triterpenoid saponins from *Gypsophila oldhamiana* and highly oxigenated triterpenoid from *Fagara tessmannii* and *Luculia pinceana* also showed significant α-glucosidase inhibitory comparing to acarbose [1,15].

Base on its inhibition of α-glucosidase, the methanol extract of fruits was partitioned into *n*-hexane, ethyl acetate, and *n*-butanol. Ethyl acetate fraction had the highest α-glucosidase inhibitory (IC_{50} 13.49 μg/mL than n-butanol fraction (IC_{50} 19.29 μg/mL) due to its phenolic content, meanwhile, the *n*-hexane fraction was not as potent as α-glucosidase inhibitory (IC_{50} 1175.16 μg/mL) (Table 1 and Figure 1). Compounds typically found in hexane fractions are non-polar triterpenoid and steroid, while triterpenoid saponins and highly oxygenated triterpenoid found in the polar fraction such as ethyl acetate and *n*-butanol [1, 15].

Table 1. Inhibitory effect of the extract, fraction and compound on α-glucosidase activity

Extract/compound	**Inhibitor concentration (IC_{50}, μg/mL)**
MeOH extract of the leaves	43.99
MeOH extract of the stem	20.36
MeOH extract of the fruit	20.57
n-hexane fraction of the fruit	1175.16
Ethyl acetate fraction of the fruit	13.49
n-butanol fraction of the fruit	19.29
Compound **1**	110.45
Acarbose*	383.68

*positive control

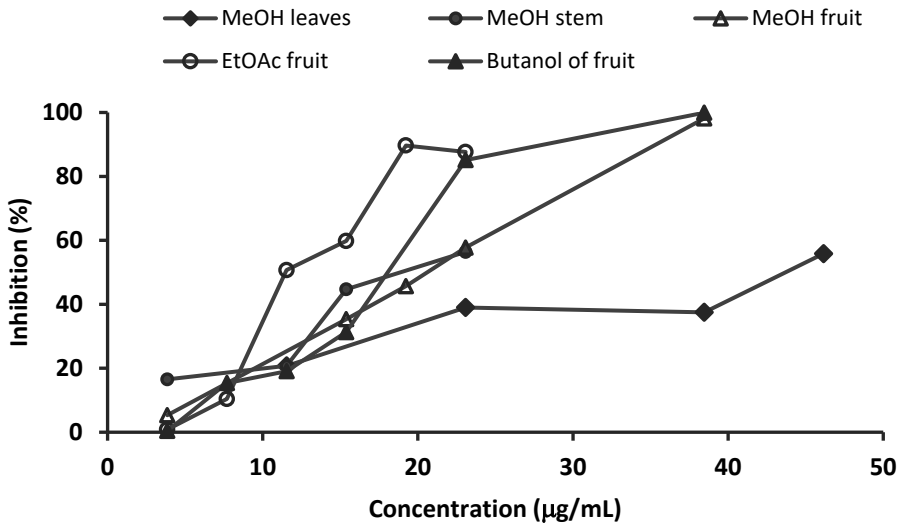


Figure 1. Effect of extracts and fractions on the inhibition of α-glucosidase

Isolation and structural elucidation

The sequential partition to the methanol crude extract of *R. tomentosa* fruits (87 g) yielded *n*-hexane, ethyl acetate and *n*-butanol fraction (1.54, 17.81 and 0.44 g respectively). Ethyl acetate fraction with the highest α-glucosidase inhibition was chromatographed over silica gel with some chromatographic technique to afford compound **1.**

Compound **1** was isolated as a white powder with m.p. 120-121 °C. The UV spectrum in methanol showed the maximum absorption at 242 nm which indicated the presence of α,β carbonyl unsaturated. The IR spectrum displayed absorption for the isolated carbonyl group at 1715 cm^{-1} as well as conjugated carbonyl group at 1678 and 1663 cm^{-1} which consisted to UV spectrum. In addition, there is absorption for C-H aliphatic group at 2976 and 2941 cm^{-1}. The ^{13}C-NMR (125 MHz, $CDCl_3$) was showed the presence of 14 signal. Two of the signal confirmed the existence of the isolated and conjugated carbonyl at δ_C 212.2 ppm and δ_C 192.2 ppm respectively. In addition, ^{13}C-NMR displayed the presence of five other quarternary carbon signal (δ_C 175.5 (oxy-carbon), 128.3, 113.2, 56.6, and 45.3 ppm), two signal for methine carbon (δ_C 46.6 and 34.5 ppm), and five signal for methyl carbon (δ_C 25.9, 24.5, 24.0, 22.4 and 15.6 ppm). Considering the intensity of quarternary carbon signal at 128.3 ppm with the six other quarternary carbon (included the carbonyl) which has a ratio of 1:2, indicating that the six quarternary carbon is equivalent to twelve carbon. Furthermore, the five methyl carbon signals have an intensity ratio of 2: 1 with a carbon methine signal at δ_C 34.5 ppm, consequently each of these methyl signals is identical for 2 methyl carbon (there are a total of 10 methyls). Based on this, compound **1** actually has 25 carbon atoms. The ^{1}H-NMR (500MHz, $CDCl_3$) spectrum exhibited the presence of a singlet signal of methine proton at δ_H 4.67 ppm. The spectrum also indicated the presence of an isopropyl unit with the appearance of a doublet signal at δ_H 1.00 ppm (6H, *d*, *J* = 6.9 Hz, 2xCH_3) which is adjacent to

the methine proton at δ_H 2.35 ppm (1H, *sept*, *J* = 6.9 Hz). These constant coupling value indicates that the both signals are correlated to each other as vicinal aliphatic protons. In addition, there are three singlet signals at δH 1.41 (12H), 1.32 (6H) and 1.25 ppm (6H) which indicate the presence of 8 methyl groups. The HMBC correlation revealed a correlation of both methyl on a geminal dimethyl group (δ_H 1.25 and 1.32 ppm) to the isolated and conjugated carbonyl group (δ_C 212.2 and 192.2 ppm) as well as correlation of both methyl on another geminal dimethyl group to the isolated carbonyl (δ_C 212.2 ppm) and oxy-carbon (δ_C 175.5 ppm). These explained that both of geminal dimethyl are α position in β-triketone unit. Based on the previous NMR data, there is actually two symmetrical unit of β-triketone. Furthermore, the correlation between of proton δ_H 4.67 ppm to isopropyl unit (δ_C 34.5 ppm) and oxy-carbon (δC 175.5 and 128.3 ppm) indicating that the isopropyl group was an adjacent bis-furan ring and the bis-furan ring was integrated with the β-triketone unit. According to these spectroscopic evidence and comparing to the those of reported literature [11], the structure of compound **1** was established as rhodomyrtosone D. This compound has been previously reported from *R. tomentosa* leaves [11].

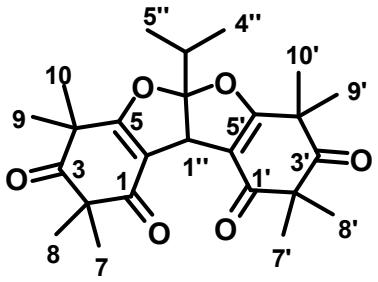


Figure 2. Structure of compound **1** (rhodomyrtosone D)

Table 2. NMR data of compound **1** in $CDCl_3$ and rhodomyrtosone D

No	Compound 1			Rhodomyrtosone D [11]	
	δC	δ_H (ΣH, *mult*, J_{Hz})[b]	HMBC (H→C)	δ_C	δ_H (*mult*, J_{Hz})
1 (1')	192.2	-	-	192.4	-
2(2')	56.6	-	-	56.4	-
3(3')	212.2	-	-	212.1	-
4(4')	45.3	-	-	45.2	-
5(5')	175.5	-	-	175.7	-
6(6')	113.2	-	-	113.0	-
7(7')	25.8	1.25 (6H, *s*)	C-3(3'), C-1 (1'), C-2(2'), C- 8(8')	25.7	1.27 (*s*)
8(8')	22.4	1.32 (6H, *s*)	C-3(3'), C- 1 (1'), C- 2(2'), C- 7(7')	22.3	1.34 (*s*)
9(9')	24.0	1.41 (6H, *s*)	C-3(3'), C-5(5'), C-4(4'), C- 9(9'), C-10(10')	23.9	1.44 (*s*)
10(10')	24.5	1.41 (6H, *s*)	C-3(3'), C-5(5'), C-4(4'), C- 9(9'), C-10(10')	24.4	1.44 (*s*)

1''	46.6	4.67 (1H, *s*)	C-5(5'), C- 2'', C- 6(6'), C- 3''	46.5	4.69 (*s*)
2''	128.3	-	-	128.2	-
3''	34.5	2.35 (1H, s*ept,* 6.9)	C-2'', C- 1'', C- 4'', C- 5"	34.4	2.37 (*sept*, 6.9)
4'', 5"	15.6	1.00 (6H, *d*, 6.9)	C-2'', C- 3'', C- 4', C- 5"	15.5	1.02 (*d*, 6.9)

The isolated compound **1** (rhodomyrtosone D) was examined for α-glucosidase inhibitory activity with concentration range about 30.77 to 0.24 μg/mL. The α- glucosidase inhibitory effect of rhodomyrtosone D (17.7% at 30.77 μg/mL) seems higher than the acarbose (8.54 % at 30.77 μg/mL). Using the extrapolation method to linear regression, the IC_{50} of rhodomyrtosone D on inhibiting α-glucosidase was 110.45 μg/mL.

CONCLUSION

The leaves, the stem, and the fruit of *R. tomentosa* plant were potential as a source of a natural antidiabetic, especially from the ethyl acetate fraction of the fruit. A bioactive compound, rhodomyrtosone D was isolated from the fruit of *Rhodomyrtus tomentosa* and showed higher α-glucosidase inhibition than acarbose.

ACKNOWLEDGMENTS

The author would like thanks to Dr. Nurainas, M.Si from Herbarium Anda, Andalas University for identification of plant specimen. We are grateful to the Ministry of Research, Technology and Higher Education for research grants through PD-UPT scheme (No: 093/SP2H/LT/DPRM/IV/2018).

REFERENCES

1. Yin, Z., Zhang, W., Feng, F., Zhang, Y., and Kang, W., 2014, α-Glucosidase Inhibitors Isolated from Medicinal Plants, *Food Science, and Human Wellness*, 3, 136-174.
2. Choudhary, M.I., Adhikari, A., Rasheed, S., Marasini, B.P., Hussain, N., Kaleem, W.A., and Rahman, A., 2011, Cyclopeptyde Alkaloid of *Ziziphus oxyphylla* Edgw as Novel Inhibitors of α-Glucosidase Enzyme and Protein Glycation, *Phytochemistry Letters*, 4, 404-406.
3. Lam. S.H., Cheng, J.M., Kang, C.J., Chen, C.H., and Lee, S.S., 2008, α-Glucosidase Inhibitors from the Seed of *Syagrus romanzoffiana*, *Phytochemistry*, 1173-1178.
4. Zhang, A.J., Rimando, A.M., Mizuno, C.S., and Mathews, S.T., 2017, α-Glucosidase Inhibitory Effect of Resveratrol and Piceatannol, *The Journal of Nutritional Biochemistry*, 47, 86-93.
5. Lavanya, G., Voravuthikunchai, S.P, and Towatana, N.H., 2012, Acetone Extract from *Rhodomyrtus tomentosa*: A Potent Natural Antioxidant, *Evidence-Based Complementary and Alternative Medicine,* 2012, Article ID 535479, 1-8.

6. Limsuwan, S., Kayser, O., and Voravuthikunchai, S.P., 2012, Antibacterial Activity of Rhodomyrtus tomentosa (Aiton) Hassk. Leaf Extract against Clinical Isolates of *Streptococcus pyogenes*, *Evidence-Based Complementary and Alternative Medicine,* 2012, Article ID 697183, 1-6.
7. Lai, T.N.H., Herent, M.F., Quetin-Leclercq, J., Nguyen, T.B.T., Larondelle, Y., Andre, C.M., and Rogez, H., 2013, Piceatannol, a Potent Bioactive Stilbene, as Major Phenolic Component in *Rhodomyrtus tomentosa*, *Food Chemistry*, 138: 1421-1430
8. Wu, P., Ma, G., Li, N., Deng, Q., Yin, Y., and Huang, R., 2015, Investigation of In Vitro and in Vivo Antioxidant Activities of Flavonoids Rich Extract from the Berries of *Rhodomyrtus tomentosa* (Ait.) Hassk., *Food Chemistry*, 173, 194-202.
9. Hiranrat, A., Mahabusakaram, W., Carrol, A.R., Duffy, S., and Avery, V.M., 2012, Tomentosones A and B, Hexacyclic Phloroglucinol Derivatives from the Thai Shrub *Rhodomyrtus tomentosa*, *J. Org. Chem*., 77, 680-683.
10. Limsuwan, S., Trip, E.N., Kouwen, T.R.H.M., Piersma, S.,Hiranrat, A., Mahabusakaram, W., Voravuthikunchai, S.P., Dijl, JM., and Kayse, O., 2009, Rhodomyrtone, A new Candidate as Natural Antibacterial Drug from *Rhodomyrtus tomentosa*, *Phytomedicine*, 16, 645-651.
11. Yang, Z., Wang, Y., Wang, Y., and Zhang, Y., 2012, Bioassay-Guided Screening and Isolation of α-glucosidase and Tyrosinase Inhibitors from Leaves of *Morus alba*, *Food Chemistry*, 617-625.
12. Anisah, L.N., Syafii, W., Pari, G., and Sari, R.K., 2018, Antidiabetic Activities and Identification of Chemical Compound from Samama (*Anthocephalus macrophyllus* (Roxb) Havil), *Indones. J. Chem*, 18(1), 66-74.
13. Hiranrat, A., and Mahabusakaram, W., 2008, New Acylphloroglucinols from the Leaves of *Rhodomyrtus tomentosa*, *Tetrahedron*, 64, 11193-11197.
14. You, Q., Chen, F., Wang, X., Luo, P.G., and jiang, Y., 2011, Inhibitory Effect of Muscadine Anthocyanins on α-Glucosidase and Pancreatic Lipase Activities, *J. Agric. Food. Chem*., 59, 9506-9511.
15. Luo, J.G., Ma, L., and Kong, L.Y., 2008, New Triterpenoid Saponins with Strong α-Glucosidase Inhibitory Activity from the Roots of *Gypsophila oldhamiana*, *Bioorg. Med. Chem.*,16, 2912–2920.

Dear reviewer,

Thank you to for reviewing my paper entittled "**α-Glucosidase Inhibitory and A Leptospermone Derivative from *Rhodomyrtus tomentosa"*** This is my response to your comments :

Reviewer A

The revised part of **reviewer A** indicated with red color

No	Revised/Comment of author
A1	It was deleted
A2	It has been revised to "*Rhodomyrtus tomentosa"*
A3	It has been revised to "fractions"
A4	It has been added "The structure of rhodomyrtosone D was"
A5	It has been added "rhodomyrtosone D was"
A6	It has been added with "."
A7	It has been added with "rhodomyrtosone D"
A8	It has been added with " menunjukkan"
A9	It Has been revised to "*R. tomentosa*"
A10	It has been added with "the"
A11	It has been revised to " antimalarial"
A12	It has been added with "The"
A13	It has been revised to "^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz)
A14	It has been revised to "As much as 100 gr of each"
A15	It has been added with "@ 24 hours"
A16	It has been revised to " extracs"
A17	It has been added with :As much as"
A18	It has been revised
A19	It has been revised
A20	has been revised to "was"
A21	It has been revised
A22	It has been revised and state clearly.
A23	It has been added with "the"
A24	It has been added with ","
A25	The sentence has been revised and the reference has been changed to

	numbering
A26	Yes, that is correct
A27	Yes, that is correct
A28 & A29	It has been explained.
A30	The reference has been added
A31	It has been revised
Respond to the additional comments:	
• Reference of the comparison compounds have been written. • The value of the coupling constant (J) on the H-NMR have been explained in the text	

Reviewer B

The revised part of **reviewer B** indicated with blue color

No	Revised/Comment of author
W1	The references has been added
W2	It has been added
W3	It has been added the objective of this section and previous
W4	It has been changed
W5	It has been changed to mass units
W6	we did't have any evidence, only base on the literature [7]. So, the sentence has been deleted
W7	It has been marked
W8	It has been changed to mass units
W9	It has been revised
W10	It has been changed to "spectroscopic evidence"
W11	the literature data has been added
Respond to the additional comments:	
• The originality of this study has been added in introduction part. • Discussion on inhibitory activity has been added	

Reviewer C

The revised part of **reviewer B** indicated with green color

- Butanol has been changed to "*n*-butanol).
- The sign of '$\rightarrow$" has been changed to " comma"

Sincerely yours,

Ferlinahayati

Chemistry Department, FMIPA, University of Sriwijaya

Re: IJC article information

From: ferlina hayati (etihayati74@yahoo.com)

To: nuryono_mipa@ugm.ac.id; ijc@ugm.ac.id; ijcugm@yahoo.com

Date: Monday, April 22, 2019, 5:06 PM GMT+7

Yth : Editor IJC

Mohon maaf atas kesalahpahaman dari saya.
Sebelumnya pada tanggal 9 Januari saya telah mengupload kembali revisi artikel sesuai saran reviewer (40990-113820-4-ED.docx), beserta summary perbaikan dan jawaban atas pertanyaan reviwer pada file terpisah (40990-113820-3-ED.docx).
Karena isi email pada tanggal 11 Januari tersebut persis sama dengan email yang saya terima sebelumnya, maka saya kira email tersebut terkirim ulang by system saja.

Namun saya akan jawab kembali pertanyaan dari reviewer lebih rinci sebagai berikut :

Response to Reviewer A:
1. The isolated compound is not a new compound. On table 2, we have added the NMR data of the comparison compound.
2. We have added an explanation about the value of coupling constant with this sentence "These constant coupling value indicates that the both signals are correlated to each other as vicinal aliphatic protons".
3. We just checked using Grammarly

Response to Reviewer B:
1. The orinality have been added in the introduction with this sentence "However, there is no literature on the a-glucosidase inhibitory of *R. tomentosa* and its bioactive chemical compound"
2. The discussion of inhibitory activity have been added with this sentence
"In addtition, another phenolic compounds such as flavonoid isolated from *Morus alba* and anthocyanins isolated from noble muscadine grapes have been reported as potential a-glucosidase inhibitory [11,14]. Meanwhile, the triterpenoid saponins from *Gypsophila oldhamiana* and highly oxigenated triterpenoid from *Fagara tessmannii* and *Luculia pinceana* also showed significant a-glucosidase inhibitory comparing to acarbose [1,15]."

and also with this sentence "
Compounds typically found in hexane fractions are non-polar triterpenoid and steroid, while triterpenoid saponins and highly oxygenated triterpenoid found in the polar fraction such as ethyl acetate and *n*-butanol [1, 15]."

Response to reviewer C:
1. We have revised as reviewer suggestion.

Demikianlah yang dapat saya sampaikan, dan saya sangat berharap artikel tersebut dapat diproses lebih lanjut dan bisa terbit di IJC.

Wassalam

Ferlinahayati
Jurusan Kimia FMIPA UNSRI

.

> On Sunday, April 21, 2019, 9:59:51 PM GMT+7, Nuryono Nuryono <nuryono_mipa@ugm.ac.id> wrote:
>
> Sdr Ferlinahayati
> Tanggal 11 Januari editor kami telah mengirim decision sebagai berikut. Namun, samai sekarang tdk ada respon revisi dari author.
>
> Ferlinahayati Ferlinahayati:

We have reached a decision regarding your submission to Indonesian Journal of Chemistry, "-Glucosidase Inhibitory and A Leptospermone Derivative from Rhodomyrtus tomentosa".

Our decision is: Revisions Required

Please answer the
1. This compound is not new, it should be written reference of the comparison compounds.
2. The value of the coupling constant (J) on the H-NMR to be explained
3. It would be better, when using proof reader

1. The significance and objective of this study have been explained clearly in the introduction. However, there is no originality stated in introduction. It would be better if the authors could confirm the originality of this study on introduction part.
2. At discussion section, discussion of inhibitory activity must be added more, compare with other papers.
3. Others, please check the manuscript.

Best regards,

Tri Joko Raharjo
Laboratory of Organic Chemistry, Department of Chemistry, Universitas Gadjah Mada
trijr_mipa@ugm.ac.id

Indonesian Journal of Chemistry
https://jurnal.ugm.ac.id/ijc
Indexed by SCOPUS since 2012

Pada tanggal Jum, 19 Apr 2019 pukul 13.13 ferlina hayati <etihayati74@yahoo.com> menulis:

> Dear Editor of IJC
>
> Relating to our article ID 40990 with the tittle " a-Glucosidase Inhbitory and A Leptospermone Derivative from Rhodomyrtus tomentosa", we need the information about the progress and status of the article.
>
> The revised article has been submitted throught the system on 9th Jan 2019.
>
> We look forward to hear from you
>
> Best Regards,
>
> Ferlinahayati
> Department of Chemistry,
> FMIPA UNSRI

--

Prof. Dr.rer.nat. Nuryono, MS
Editor in Chief
Indonesian Journal of Chemistry
Accredited by DIKTI; Indexed in Scopus since 2012

Re: [IJC] -Glucosidase Inhibitory and A Leptospermone Derivative from Rhodomyrtus tomentosa

From: ferlina hayati (etihayati74@yahoo.com)

To: trijr_mipa@ugm.ac.id

Date: Saturday, May 4, 2019, 1:41 PM GMT+7

Dear Editor,

Thank you for the information. We really hope to get good news soon.

Best Regards,
Ferlinahayati

> On Thursday, May 2, 2019, 9:10:25 PM GMT+7, Tri Joko Raharjo <trijr_mipa@ugm.ac.id> wrote:
>
> Dear Authors
> Regarding your submission to IJC #40990, I could inform you that at this moment the manuscript is under review to see if the reviewer's comment from previous round have been addressed properly.
> Soon after the reviewer give the feedback I am ready to make decision and I will let you know at the first occasion.
> Best regards.
> Editor
> _____
> Indonesian Journal of Chemistry
> https://jurnal.ugm.ac.id/ijc
> Indexed by SCOPUS since 2012

α-Glucosidase Inhibitory and A Leptospermone Derivative from *Rhodomyrtus tomentosa*

Ferlinahayati[1]*, Daniel Alfarado[1], Eliza[1,] and Budi Untari[2]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Sriwijaya
Jalan Raya Palembang Prabumulih Km 32, Ogan Ilir, South Sumatera, Indonesia 30622

[2]Department of Pharmacy, Faculty of Mathematics and Natural Sciences, University of Sriwijaya
Jalan Raya Palembang Prabumulih Km 32, Ogan Ilir, South Sumatera, Indonesia 30622

* Corresponding author, tel/: 081394741890, email: etihayati74@yahoo.com

ABSTRACT

One of the treatments for diabetes mellitus disease is to control blood sugar level using an inhibitor of α-glucosidase enzyme. The methanol extracts of the fruit, stem, and leaves of *Rhodomyrtus tomentosa* were found significant in inhibiting α-glucosidase with an IC_{50} value of 20.57, 20.36 and 43.99 μg/mL respectively. The ethyl acetate and *n*-butanol fractions from the methanol extract of *R. tomentosa* fruit exhibited the potent inhibition (IC_{50} 13.49 and 19.29 μg/mL) compare to acarbose and *n*-hexane fraction (IC_{50} 383.68 and 1175.16 μg/mL). A leptospermone derivative, rhodomyrtosone D, was isolated from the ethyl acetate fraction of *R. tomentosa* fruit. The structure of rhodomyrtosone D was identified based on spectroscopic analysis, as well as comparing with literature data. The α-glucosidase inhibition of rhodomyrtosone D (IC_{50} 110.45 μg/mL) was 3.5 fold more potent than acarbose. Thus, *R. tomentosa* plant could be potential as a natural resource of α-glucosidase inhibitor.

Keywords: α-glucosidase, *Rhodomyrtus tomentosa*, antidiabetic, rhodomyrtosone D, ethyl acetate fraction

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorder, in which there are high blood sugar levels (hyperglycemia) over a prolonged period [1]. It will happen if the pancreas does not produce enough insulin that is able to convert sugar into energy, or the body's cells do not respond well to the insulin produced. Some serious complication of hyperglycemia such as cardiovascular disease, damage to the eyes, atherosclerosis, and chronic kidney disease (nephropathy) can also occur [2]. The control of blood sugar level by inhibition of carbohydrate-hydrolyzing enzymes in the digestive organ is believed to be important in hyperglycemia treatment [1]. The α-glucosidase, an enzyme in the small intestine, is responsible for the degradation of carbohydrate. The α-glucosidase inhibitor

will interfere with the digestion of carbohydrate and thereby reduce the postprandial glucose level and insulin responses in a diabetic patient [2-3]. Acarbose, miglitol, and voglibose have been found as an α-glucosidase inhibitor and currently clinically used to control blood glucose of diabetic patients [4]. However, they have caused severe gastrointestinal side effects. Nowadays, natural resources have received tremendous attention as a therapeutic agent in the inhibition of α-glucosidase and have shown very promising biological activity.

Karamunting is locally named (Sumatera island) for *Rhodomyrtus tomentosa* and belonging to the Myrtaceae family. This plant is an evergreen shrub which is native to Southern Asia and Southeast Asia and is widely distributed in Indonesia. *R. tomentosa* is widely used as traditional medicines to treat a variety of disease caused by bacteria such as diarrhea, dysentery, and urinary tract infections [5-6]. In addition, its ripe fruits are used to boost the immune system [7]. Biologically, ethanolic extract of *R. tomentosa* fruits possesses potent antioxidant activities on DPPH radical scavenging activity, reducing power as well as inhibition of lipid peroxidation activity [8] Furthermore, some extract of this plants were reported to have antibacterial and anti-hepatitis properties [9]. Chemically, various secondary metabolites have been reported, such as polyketide, flavonoids, anthocyanins, stilbenoids, and triterpenoids [7-11]. Rhodomyrtone, a phloroglucinol polyketide from *R. tomentosa* have displayed significant antibacterial activities against Gram-positive bacteria and suggested as a new candidate as a natural antibacterial drug [10].

Meanwhile, tomentosone A, hexacyclic phloroglucinol was reported as antimalarial against chloroquine-resistant and sensitive strains of *Plasmodium falciparum*. Resveratrol and piceatannol, a stilbenoid compound has been characterized by this plant [7]. A stilbenoid compound from *Syagrus romanzoffiana* was reported as a potential hypoglycemic agent. However, there is no literature on the α-glucosidase inhibitory of *R. tomentosa* and its bioactive chemical compound. In a search for potential α-glucosidase inhibitor from natural resources, we have been investigated the ability of *R. tomentosa* plant to inhibit the activity of the α–glucosidase enzyme as well as to isolate the bioactive compound. One active compound, rhodomyrtosone (**1**) was isolated, and its α-glucosidase inhibition was determined. The following describes the outcomes of these efforts.

EXPERIMENTAL SECTION

Materials

Rhodomyrtus tomentosa (fruits, leaves, and stem) were collected from Inderalaya, Ogan Ilir, South Sumatera. The plant was identified at Herbarium Anda, Department of Biology, University of Andalas. The solvents (methanol, *n*-hexane and ethyl acetate) were the technical grade that is distilled, while *n*-butanol and dimethylsulfoxide (DMSO) were pro analysis grade (p.a) from Merck. The α-glucosidase (from *Saccharomyces cerevisiae*) and *p*-nitro-phenyl-α-D-glucopyranoside were purchased from Sigma-Aldrich. Bovine serum albumin (BSA) was purchased from Merck. Silica gel

60G (Merck) was used for vacuum liquid chromatography, and silica gel 60 PF_{254} (Merck) was used for radial chromatography. TLC analysis was performed on Kieselgel 60 GF_{254}, 0.25 mm aluminum plate (Merck) and visualized with cerium sulfate.

Instrumentation

Incubator Biosan PST-60HL was used for the sample incubation process. The absorbance of *p*-nitrophenol was measured by a Tecan Infinite F50 Microplate reader. The UV spectrum was recorded with Shimadzu UV-1240 spectrophotometer. IR spectrum was determined using KBr pellets on a Perkin Elmer FTIR Spectrum One spectrophotometer. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded with Agilent DD2 spectrometer, using residual and deuterated solvent peaks as reference standards.

Procedure

Extraction of sample for assay

As much as 100 g of each the dried powdered sample (fruits, leaves, and stem) of *R.tomentosa* were extracted by maceration method using methanol (400 mL) as the solvent at the room temperature. The maceration process was carried out three times (@ 24 hours). The methanol solvents were evaporated in under reduce pressure to give crude extracts of methanol of fruit, leaves, and stem (4.6, 4.2 and 3.9 g respectively). The crude of methanol extract of fruit was partitioned successively with *n*-hexane, ethyl acetate, and *n*-butanol and produce of each fraction after the solvent was evaporated.

In-vitro α-glucosidase inhibition assay

The α-glucosidase assay has been performed using the spectrophotometric method as previously described [2,-12,-13] with slight modification. As much as 10 μL of the sample at various concentrations was added with 55 μL of 50 mM phosphate buffer (pH 6.8) and 10 μL of 10 mM *p*-nitrophenyl-α-D-glucopyranoside as the substrate. After preincubated for 5 min at 37 °C, 25 μL of 0.1 U/mL α-glucosidase (in the phosphate buffer pH 6.8 containing 0.1 mg/mL bovine serum albumin) was added. The mixture was then incubated for 30 min at 37 °C. After that, the stopped solution (100 μL of 200 mM Na_2CO_3) was added to the mixture. The absorbance of the *p*-nitrophenol released due to hydrolysis of the substrate by the α-glucosidase was measured by a microplate reader at 405 nm. The blank solution was prepared by replaced sample solution by DMSO. Acarbose (Glucobay®) is used as a positive control. The percentage inhibition of α-glucosidase was calculated using the following equation: Inhibition % = [1-(A_{sample} / A_{blank})] ~~×~~ 100. The IC_{50} was calculated by linear regression equation analysis between concentration and percentage inhibition.

Extraction and Isolation of *R. tomentosa* fruits

The dried fruits (2 kg) was extracted with methanol (3 ~~x~~ 7 L, 24 h each) by maceration method. The methanol extract was concentrated under reduced pressure to give 1.1 L syrup, which was suspended in distilled water. This suspension was partitioned successively with *n*-hexane, ethyl acetate, and *n*-butanol to afford *n*-hexane, ethyl acetate, and *n*-butanol fractions. The ethyl acetate fraction (15 g) was fractionated by vacuum liquid chromatography on silica gel 60 G, eluting with *n*-hexane-ethyl acetate system with increment ethyl acetate gradually (9:1, 8:2, 7:3, 6:4, 4:6, 2:8, 1:9, and 0:10, each 150 mL) to give 8 fractions (A-H). Fraction C (374 mg) was further separated using radial chromatography over silica gel 60 PF_{254} (1 mm), eluted with n-hexane-ethyl acetate gradually (85:15, 80:20, 75:25, 70:30, 60:40, 50:50) to yield a leptospermone derivative 1 (8.9 mg)

RESULTS AND DISCUSSION

The α-Glucosidase inhibition of extracts and fractions

The extraction of three parts of *R. tomentosa*, namely, ~~(~~fruit, stem, and leaves~~)~~ produced methanol extract of 4.6, 3.9, and 4.2 g, respectively. All of these extracts were tested for the α-glucosidase inhibitory using *p*-nitrophenyl-α-D-glucopyranoside as the substrate and acarbose as the reference or positive control. The methanol extract from the stem and fruit have a similar ability to inhibit α-glucosidase activity with the IC_{50} value were ~~(IC_{50}~~ 20.36 and 20.57 μg/mL respectively~~),~~ Both of these extracts demonstrated two times more potent than the leaves methanol extract with the ~~(~~IC_{50} was 43.99 μg/mL~~)~~ (Figure 1). Base on the IC_{50} value, ~~A~~all three methanol extracts possessed high potency in inhibiting α-glucosidase compare to the reference drug, acarbose (IC_{50} 383.68 μg/mL) (Table 1).

~~-~~Previously, it has been reported that *R. tomentosa* fruit contains stilbenoid compound, such as resveratrol, and piceatannol [7]. These stilbenoids showed the more potent inhibition of α-glucosidase activity with IC_{50} 91 and 60 μg/mL respectively than acarbose (IC_{50} 247 μg/mL) [4]. In addition, other phenolic compounds such as flavonoid isolated from *Morus alba* and anthocyanins isolated from noble muscadine grapes have been reported as a potential α-glucosidase inhibitory [11,14]. Meanwhile, the triterpenoid saponins from *Gypsophila oldhamiana* and highly oxygenated triterpenoid from *Fagara tessmannii* and *Luculia pinceana* also showed significant α-glucosidase inhibitory comparing to acarbose [1,15].

Base on its inhibition of α-glucosidase, the methanol extract of fruits was partitioned into *n*-hexane, ethyl acetate, and *n*-butanol. Ethyl acetate fraction had the highest α-glucosidase inhibitory (IC_{50} 13.49 μg/mL) than an ~~an~~ n-butanol fraction (IC_{50} 19.29 μg/mL) due to its phenolic content, meanwhile, the *n*-hexane fraction was not as potent as α-glucosidase inhibitory (IC_{50} 1175.16 μg/mL) (Table 1 and Figure 1). ~~-~~Compounds typically found in hexane fractions are non-polar

Commented [a1]: Try to rewrite the sentence, remove the brackets and describe them

Commented [f2R1]: Have been rewrite n remove the bracket

Formatted: Subscript

Commented [a3]: What is IC50?

Formatted: Subscript

Commented [a4]: Based on what value?

Commented [f5R4]: Base on IC50 value

Formatted: Highlight

Formatted: Highlight

triterpenoid and steroid, while triterpenoid saponins and highly oxygenated triterpenoid found in the polar fraction such as ethyl acetate and *n*-butanol [1,-15].

Table 1. Inhibitory effect of the extract, fraction and compound on α-glucosidase activity

Extract/compound	Inhibitor concentration (IC_{50}, µg/mL)
MeOH extract of the leaves	43.99
MeOH extract of the stem	20.36
MeOH extract of the fruit	20.57
n-hexane fraction of the fruit	1175.16
Ethyl acetate fraction of the fruit	13.49
n-butanol fraction of the fruit	19.29
Compound **1**	110.45
Acarbose*	383.68

*positive control

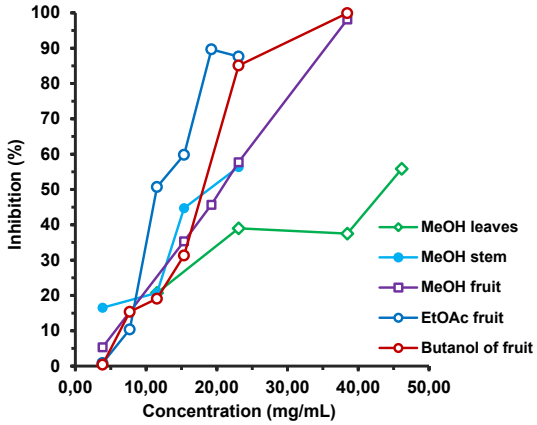


Figure 1. Effect of extracts and fractions on the inhibition of α-glucosidase

Isolation and structural elucidation

The sequential partition to the methanol crude extract of *R. tomentosa* fruits (87 g) yielded *n*-hexane, ethyl acetate and *n*-butanol fraction (1.54, 17.81 and 0.44 g respectively). Ethyl acetate fraction with the highest α-glucosidase inhibition was chromatographed over silica gel with some chromatographic technique to afford compound **1.**

Compound **1** was isolated as a white powder with m.p. 120-121 °C. The UV spectrum in methanol showed the maximum absorption at 242 nm, which indicated the presence of α,β carbonyl unsaturated. The IR spectrum displayed absorption for the isolated carbonyl group at 1715 cm^{-1} as well as conjugated carbonyl group at 1678 and 1663 cm^{-1}, which consisted of the UV spectrum. In

Commented [a6]: Then? What is the correlation with the previous sentences?

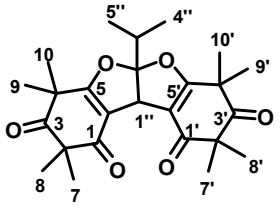
Formatted: Font: Not Bold
Formatted: Centered
Formatted Table
Formatted: Indent: Left: -0,02 cm, Tab stops: 3,48 cm, Decimal aligned
Formatted: Indent: Left: -0,02 cm, Tab stops: 3,48 cm, Decimal aligned
Formatted: Indent: Left: -0,02 cm, Tab stops: 3,48 cm, Decimal aligned
Formatted: Indent: Left: -0,02 cm, Tab stops: 3,48 cm, Decimal aligned
Formatted: Indent: Left: -0,02 cm, Tab stops: 3,48 cm, Decimal aligned
Formatted: Indent: Left: -0,02 cm, Tab stops: 3,48 cm, Decimal aligned
Formatted: Indent: Left: -0,02 cm, Tab stops: 3,48 cm, Decimal aligned
Formatted: Indent: Left: -0,02 cm, Tab stops: 3,48 cm, Decimal aligned

Commented [a7]: What is the correct unit of concetration? µg/mL or mg/mL)?

Commented [a8]: Try to rewrite the data/result not in the brackets
Formatted: Highlight

addition, there is absorption for C-H aliphatic group in 2976 and 2941 cm^{-1}. The ^{13}C-NMR (125 MHz, $CDCl_3$) was showed the presence of 14 signal. The two ~~of the~~ signals confirmed the existence of the isolated and conjugated carbonyl at δ_C 212.2 ppm and δ_C 192.2 ppm respectively. In addition, ^{13}C-NMR displayed the presence of five other quarternary carbon signal (δ_C 175.5 (oxy-carbon), 128.3, 113.2, 56.6, and 45.3 ppm), two signal for methine carbon (δ_C 46.6 and 34.5 ppm), and five signal for methyl carbon (δ_C 25.9, 24.5, 24.0, 22.4 and 15.6 ppm). ~~Considering~~ The intensity of quarternary carbon signal at 128.3 ppm with the six other quarternary carbon (included the carbonyl) which has a ratio of 1:2, indicating that the six quarternary carbon is equivalent to twelve carbon. Furthermore, the five methyl carbon signals have an intensity ratio of 2:-1 with a carbon methine signal at δ_C 34.5 ppm, consequently each of these methyl signals is identical for 2 methyl carbon (there are a total of 10 methyls). Based on this, compound **1** ~~actually~~ has 25 carbon atoms. The ^{1}H-NMR (500MHz, $CDCl_3$) spectrum exhibited the presence of a singlet signal of methine proton at δ_H 4.67 ppm. The spectrum also indicated the presence of an isopropyl unit with the appearance of a doublet signal at δ_H 1.00 ppm (6H, *d*, *J* = 6.9 Hz, 2xCH_3) which is adjacent to the methine proton at δ_H 2.35 ppm (1H, ***sept***, *J* = 6.9 Hz). This constant coupling value indicates that both signals are correlated to each other as vicinal aliphatic protons.

____-In addition, there are three singlet signals at δH 1.41 (12H), 1.32 (6H), and 1.25 ppm (6H) which indicate the presence of 8 methyl groups. The HMBC correlation revealed a correlation of both methyl on a geminal dimethyl group (δ_H 1.25 and 1.32 ppm) to the isolated and conjugated carbonyl group (δ_C 212.2 and 192.2 ppm) as well as correlation of both methyl on another geminal dimethyl group to the isolated carbonyl (δ_C 212.2 ppm) and oxy-carbon (δ_C 175.5 ppm). These explained that both of geminal dimethyl is α position in β-triketone unit. Based on the previous NMR data, there is ~~actually~~ two symmetrical units of β-triketone. Furthermore, the correlation between of proton δ_H 4.67 ppm to isopropyl unit (δ_C 34.5 ppm) and oxy-carbon (δC 175.5 and 128.3 ppm) indicating that the isopropyl group was an adjacent bis-furan ring and the bis-furan ring was integrated with the β-triketone unit. According to this spectroscopic evidence and comparing to the those of reported literature [11], the structure of compound **1** was established as rhodomyrtosone D. This compound has been previously reported from *R. tomentosa* leaves [11].



Fig~~ure~~ 2. Structure of compound **1** (rhodomyrtosone D)

Table 2. NMR data of compound **1** in $CDCl_3$ and rhodomyrtosone D

No	Compound 1			Rhodomyrtosone D [11]	
	δC	δ_H (ΣH, *mult*, J_{Hz})[b]	HMBC (H→C)	δ_C	δ_H (*mult*, J_{Hz})
1 (1')	192.2	-	-	192.4	-
2(2')	56.6	-	-	56.4	-
3(3')	212.2	-	-	212.1	-
4(4')	45.3	-	-	45.2	-
5(5')	175.5	-	-	175.7	-
6(6')	113.2	-	-	113.0	-
7(7')	25.8	1.25 (6H, *s*)	C-3(3'), C-1 (1'), C-2(2'), C- 8(8')	25.7	1.27 (*s*)
8(8')	22.4	1.32 (6H, *s*)	C-3(3'), C- 1 (1'), C- 2(2'), C- 7(7')	22.3	1.34 (*s*)
9(9')	24.0	1.41 (6H, *s*)	C-3(3'), C-5(5'), C-4(4'), C- 9(9'), C-10(10')	23.9	1.44 (*s*)
10(10')	24.5	1.41 (6H, *s*)	C-3(3'), C-5(5'), C-4(4'), C- 9(9'), C-10(10')	24.4	1.44 (*s*)
1''	46.6	4.67 (1H, *s*)	C-5(5'), C- 2'', C- 6(6'), C- 3''	46.5	4.69 (*s*)
2''	128.3	-	-	128.2	-
3''	34.5	2.35 (1H, s*ept,* 6.9)	C-2'', C- 1'', C- 4'', C- 5"	34.4	2.37 (*sept*, 6.9)
4'', 5"	15.6	1.00 (6H, *d*, 6.9)	C-2'', C- 3'', C- 4', C- 5"	15.5	1.02 (*d*, 6.9)

The isolated compound **1** (rhodomyrtosone D) was examined for α-glucosidase inhibitory activity with concentration range about 30.77 to 0.24 μg/mL. The α- glucosidase inhibitory effect of rhodomyrtosone D (17.7% at 30.77 μg/mL) ~~seems~~ was higher than the acarbose (8.54 % at 30.77 μg/mL). Using the extrapolation method to linear regression, the IC_{50} of rhodomyrtosone D on inhibiting α-glucosidase was 110.45 μg/mL.

CONCLUSION

The leaves, ~~the~~ stem, and ~~the~~ fruit of *R. tomentosa* plant were potential as a source of a natural antidiabetic, especially from the ethyl acetate fraction of the fruit. A bioactive compound, rhodomyrtosone D, was isolated from the fruit of *Rhodomyrtus tomentosa* and showed higher α-glucosidase inhibition than acarbose.

ACKNOWLEDGMENTS

The author would like thanks to Dr. Nurainas, M.Si. from Herbarium Anda, Andalas University for identification of plant specimen. We are grateful to the Ministry of Research, Technology, and Higher Education for research grants through PD-UPT scheme (No: 093/SP2H/LT/DPRM/IV/2018).

REFERENCES

Formatted Table

Commented [a9]: The α- glucosidase inhibitory is indicated by what? The percentage or the IC50 value.
If the both of them can be used, try to rewrite it.
Consider describing the percentage first then the IC50 later.

Please pay attention to the previous sub-heading for inhibitory explanation also, thanks.

Commented [a10]: How high?

Commented [a11]: Write the name of journal in abbreviated form.

Please add references from the latest appropriate journal articles to a minimum reference total of 20, then cite them carefully and correctly.

1. Yin, Z., Zhang, W., Feng, F., Zhang, Y., and Kang, W., 2014, α-Glucosidase Inhibitors Isolated from Medicinal Plants, *Food Science, and Human Wellness*, 3, 136-174.
2. Choudhary, M.I., Adhikari, A., Rasheed, S., Marasini, B.P., Hussain, N., Kaleem, W.A., and Rahman, A., 2011, Cyclopeptyde Alkaloid of *Ziziphus oxyphylla* Edgw as Novel Inhibitors of α-Glucosidase Enzyme and Protein Glycation, *Phytochemistry Letters*, 4, 404-406.
3. Lam. S.H., Cheng, J.M., Kang, C.J., Chen, C.H., and Lee, S.S., 2008, α-Glucosidase Inhibitors from the Seed of *Syagrus romanzoffiana*, *Phytochemistry*, 1173-1178.
4. Zhang, A.J., Rimando, A.M., Mizuno, C.S., and Mathews, S.T., 2017, α-Glucosidase Inhibitory Effect of Resveratrol and Piceatannol, *The Journal of Nutritional Biochemistry*, 47, 86-93.
5. Lavanya, G., Voravuthikunchai, S.P, and Towatana, N.H., 2012, Acetone Extract from *Rhodomyrtus tomentosa*: A Potent Natural Antioxidant, *Evidence-Based Complementary and Alternative Medicine,* 2012, Article ID 535479, 1-8.
6. Limsuwan, S., Kayser, O., and Voravuthikunchai, S.P., 2012, Antibacterial Activity of Rhodomyrtus tomentosa (Aiton) Hassk. Leaf Extract against Clinical Isolates of *Streptococcus pyogenes*, *Evidence-Based Complementary and Alternative Medicine,* 2012, Article ID 697183, 1-6.
7. Lai, T.N.H., Herent, M.F., Quetin-Leclercq, J., Nguyen, T.B.T., Larondelle, Y., Andre, C.M., and Rogez, H., 2013, Piceatannol, a Potent Bioactive Stilbene, as Major Phenolic Component in *Rhodomyrtus tomentosa*, *Food Chemistry*, 138: 1421-1430
8. Wu, P., Ma, G., Li, N., Deng, Q., Yin, Y., and Huang, R., 2015, Investigation of In Vitro and in Vivo Antioxidant Activities of Flavonoids Rich Extract from the Berries of *Rhodomyrtus tomentosa* (Ait.) Hassk., *Food Chemistry*, 173, 194-202.
9. Hiranrat, A., Mahabusakaram, W., Carrol, A.R., Duffy, S., and Avery, V.M., 2012, Tomentosones A and B, Hexacyclic Phloroglucinol Derivatives from the Thai Shrub *Rhodomyrtus tomentosa*, *J. Org. Chem*., 77, 680-683.
10. Limsuwan, S., Trip, E.N., Kouwen, T.R.H.M., Piersma, S.,Hiranrat, A., Mahabusakaram, W., Voravuthikunchai, S.P., Dijl, JM., and Kayse, O., 2009, Rhodomyrtone, A new Candidate as Natural Antibacterial Drug from *Rhodomyrtus tomentosa*, *Phytomedicine*, 16, 645-651.
11. Yang, Z., Wang, Y., Wang, Y., and Zhang, Y., 2012, Bioassay-Guided Screening and Isolation of α-glucosidase and Tyrosinase Inhibitors from Leaves of *Morus alba*, *Food Chemistry*, 617-625.
12. Anisah, L.N., Syafii, W., Pari, G., and Sari, R.K., 2018, Antidiabetic Activities and Identification of Chemical Compound from Samama (*Anthocephalus macrophyllus* (Roxb) Havil), *Indones. J. Chem*, 18(1), 66-74.
13. Hiranrat, A., and Mahabusakaram, W., 2008, New Acylphloroglucinols from the Leaves of *Rhodomyrtus tomentosa*, *Tetrahedron*, 64, 11193-11197.

14. You, Q., Chen, F., Wang, X., Luo, P.G., and jiang, Y., 2011, Inhibitory Effect of Muscadine Anthocyanins on α-Glucosidase and Pancreatic Lipase Activities, *J. Agric. Food. Chem*., 59, 9506-9511.
15. Luo, J.G., Ma, L., and Kong, L.Y., 2008, New Triterpenoid Saponins with Strong α-Glucosidase Inhibitory Activity from the Roots of *Gypsophila oldhamiana*, *Bioorg. Med. Chem*.,16, 2912–2920.

[IJC] Copyediting Completed

From: Aulia Ratri Hapsari (aulia.ratri.h@ugm.ac.id)

To: etihayati74@yahoo.com

Cc: trijr_mipa@ugm.ac.id

Date: Thursday, May 23, 2019, 2:27 PM GMT+7

Dear Ferlinahayati Ferlinahayati,

We have now copyedited your submission "-Glucosidase Inhibitory and A Leptospermone Derivative from Rhodomyrtus tomentosa" for Indonesian Journal of Chemistry. To review the proposed changes and respond to Author Queries, please follow these steps:

1. Log into the journal using URL below with your username and password (use Forgot link if needed).
2. Click on the file at 1. Initial Copyedit File to download and open copyedited version.
3. Review the copyediting, making changes using Track Changes in Word, and answer queries.
4. Save file to desktop and upload it in 2. Author Copyedit.
5. Click the email icon under COMPLETE and send an email to the editor.

This is the last opportunity that you have to make substantial changes. You will be asked at a later stage to proofread the galleys, but at that point, only minor typographical and layout errors can be corrected.

Manuscript URL: https://jurnal.ugm.ac.id/ijc/author/submissionEditing/40990
Username: ferlinahayati

If you are unable to undertake this work at this time or have any questions, please contact me. Thank you for your contribution to this journal.

Best regards,
Aulia Ratri Hapsari

Indonesian Journal of Chemistry
https://jurnal.ugm.ac.id/ijc
Indexed by SCOPUS since 2012

40990-129453-1-CE.docx
76.3kB

[IJC] Copyediting Review Acknowledgement

From: Dwi Siswanta (dsiswanta@ugm.ac.id)

To: etihayati74@yahoo.com

Date: Wednesday, May 29, 2019, 12:52 PM GMT+7

Dear Ferlinahayati Ferlinahayati:

Thank you for reviewing the copyediting of your manuscript, "-Glucosidase Inhibitory and A Leptospermone Derivative from Rhodomyrtus tomentosa," for Indonesian Journal of Chemistry. We look forward to publishing this work.

Kind Regards,
Dwi Siswanta
Laboratory of Analytical Chemistry,
Department of Chemistry,
Universitas Gadjah Mada
Phone +628157951198
Fax +62545188
dsiswanta@ugm.ac.id

Indonesian Journal of Chemistry
https://jurnal.ugm.ac.id/ijc
Indexed by SCOPUS since 2012

Re: [IJC] Proofreading Request (Author)

From: ferlina hayati (etihayati74@yahoo.com)

To: dsiswanta@ugm.ac.id

Date: Friday, June 14, 2019 at 09:10 PM GMT+7

Dear editor,

we have done proofreading and made some improvement as follows:

1. in abstract line 8, we have change *R. tometosa* to R. tomentosa
2. In introduction line 14, we have change The -glucosidase inhibitor to the α-glucosidase inhibitor.
3. on table 2 in the 2nd line, we have deleted **"b" (superscribe)**
4. **we have refer fig 2 & table 2 in the text.**

Best regard

Ferlinahayati

> On Monday, June 10, 2019, 3:57:12 PM GMT+7, Dwi Siswanta <dsiswanta@ugm.ac.id> wrote:
>
> Dear Ferlinahayati Ferlinahayati,
>
> Your submission "α-Glucosidase Inhibitory and A Leptospermone Derivative from Rhodomyrtus tomentosa" to Indonesian Journal of Chemistry now needs to be proofread by following these steps.
> 1. Click on the Submission URL below.
> 2. Log into the journal and view PROOFING INSTRUCTIONS
> 3. Click on VIEW PROOF in Layout and proof the galley in the one or more formats used.
> 4. Enter corrections (typographical and format) in Proofreading Corrections.
> 5. Save and email corrections to Layout Editor and Proofreader.
> 6. Send the COMPLETE email to the editor.
>
> ==========
> Author is required to complete the proofreading stage in no more than ONE WEEK.
> ==========
>
> Submission URL: https://journal.ugm.ac.id/ijc/author/submissionEditing/40990
> Username: ferlinahayati
>
> Best regards,
> Dwi Siswanta
> Laboratory of Analytical Chemistry,
> Department of Chemistry,
> Universitas Gadjah Mada
> Phone +628157951198
> Fax +62545188
> dsiswanta@ugm.ac.id
> _____
> Indonesian Journal of Chemistry
> https://jurnal.ugm.ac.id/ijc
> Indexed by SCOPUS since 2012

40990-131829-1-PB - author.docx
89kB

[IJC] Proofreading Acknowledgement (Author)

From: Dwi Siswanta (dsiswanta@ugm.ac.id)

To: etihayati74@yahoo.com

Date: Saturday, June 15, 2019, 11:48 AM GMT+7

Dear Ferlinahayati Ferlinahayati,

Thank you for proofreading the galleys for your manuscript, "α-Glucosidase Inhibitory and A Leptospermone Derivative from Rhodomyrtus tomentosa," in Indonesian Journal of Chemistry. We are looking forward to publishing your work shortly.

If you subscribe to our notification service, you will receive an email of the Table of Contents as soon as it is published. If you have any questions, please contact me.

Best regards,
Dwi Siswanta
Laboratory of Analytical Chemistry,
Department of Chemistry,
Universitas Gadjah Mada
Phone +628157951198
Fax +62545188
dsiswanta@ugm.ac.id

Indonesian Journal of Chemistry
https://jurnal.ugm.ac.id/ijc
Indexed by SCOPUS since 2012