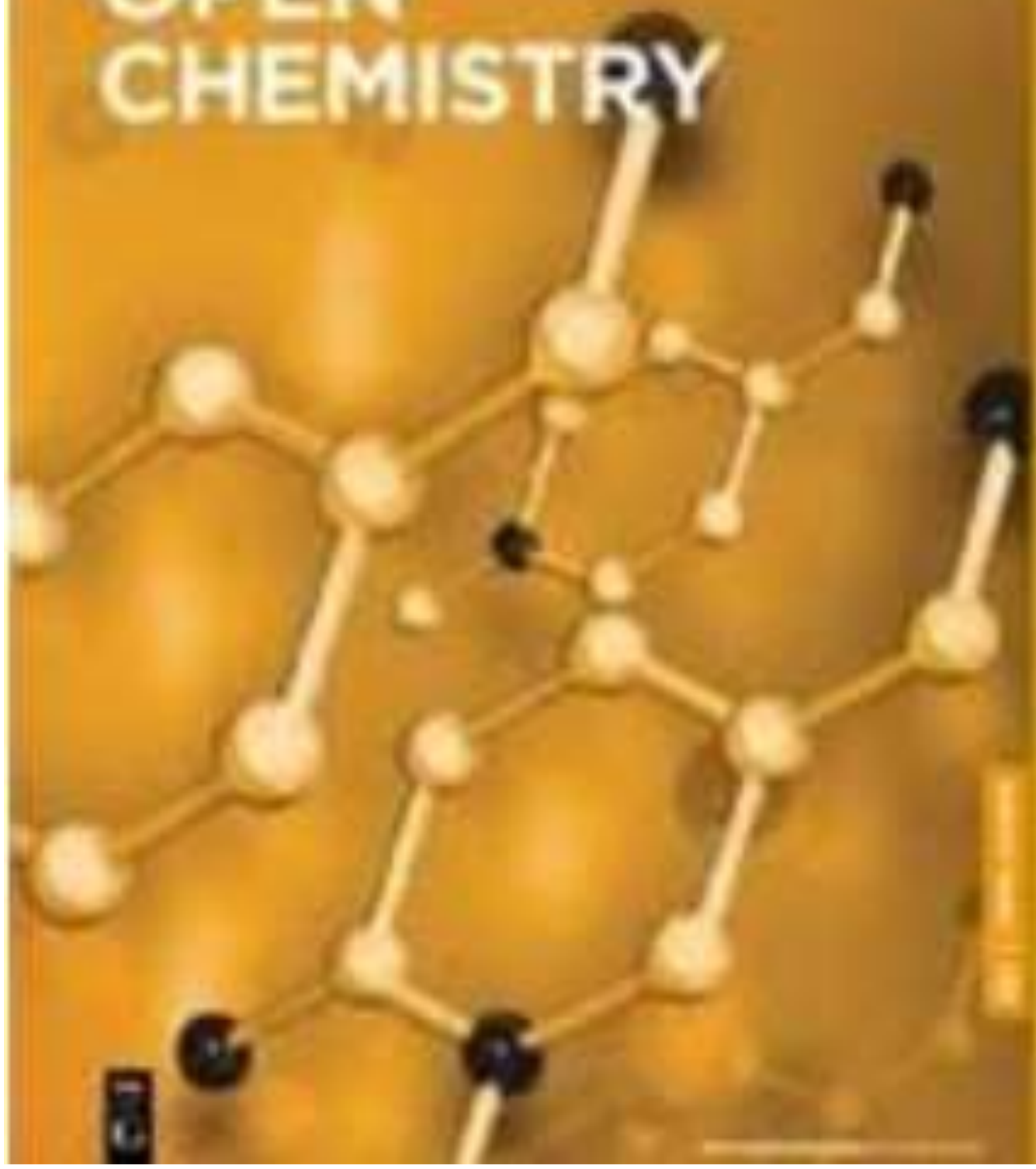


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Research Article

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Antidiabetes and Antioxidant agents from *Clausena excavata* root as medicinal plant of Myanmar

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Abstract: All around the world, patients with diabetes and the prevalence of its disease are currently growing. Due to these side effects of oral hypoglycemic agents and oxidative stress in complicating diabetes, there is growing interest in drugs, which possess dual function as both type II diabetes mellitus treatment and oxidative stress treatment. The objective of this research is to search effective antidiabetes and antioxidant bioactive compounds from the Myanmar medicinal plant *Clausena excavata*. The root part of *C. excavata* was successfully extracted with 95% ethanol and followed by column chromatographic separation technique. The structure of isolated pure compounds was elucidated by using methods of spectroscopic such as UV-Vis, IR, NMR and HRFABMS spectrometry. The α -glucosidase inhibition assay was performed against baker's yeast and rat intestine (sucrose and maltase) α -glucosidases. The activity of isolated compounds' antioxidant was measured by using DPPH assay. Among the tested enzymes, the two isolated compounds, which were dentatin (**1**) and heptaphylline (**2**), exhibited highest inhibitory on maltase enzymes with IC_{50} values 6.75 and 11.46 μ M; as positive control, acarbose (IC_{50} , 2.35 μ M) was utilized. Moreover, scavenging activity was found to be present upon seeing

the result of antioxidant activity investigation of (**1**) and (**2**) (IC_{50} values 2.66 and 1.55 mM), where ascorbic acid (IC_{50} 0.012 mM) was used as standard. Both compounds showed their antidiabetic and antioxidant activity with different fashion, especially exhibited strongest activity against on maltase α -glucosidase.

Keywords: *Clausena excavata*; dentatin; heptaphylline; α -glucosidase; antidiabetes; antioxidant; DPPH.

1 Introduction

In general, diabetes is a chronic metabolic disorder that could be serious and lethal. It is characterized by relative or absolute insufficiencies which occurs either when the body cannot effectively use the insulin (a hormone that regulates blood glucose) it produces, or when the pancreas does not produce adequate insulin [1]. World Health Organization (WHO) recognized three types of diabetes mellitus such as, (i) type 1 diabetes (insulin-dependent) (ii) type 2 diabetes (non-insulin-dependent) and (iii) gestational diabetes. One of the most frequent effects of uncontrolled diabetes is raised blood glucose. It could lead to fatal damages to the blood vessels, nerves, eyes, kidneys and eventually the heart [1, 2]. Diabetes is not only lethal but it is also the major cause of blindness, heart attacks, kidney failure, strokes, gangrene, and neuropathy especially for adults [3]. The patients of diabetes who also live with the prevalence of the disease are growing in the whole world. There were 422 million diabetic adults (or about 8.5% of the world's population) in 2014. The number increased highly compared to the year of 1980 with 108 million of diabetic adults (4.7% of the world's population) [4].

One inevitable consequence of Type 2 Diabetes Mellitus (T2DM) is Hyperglycemia. The symptom is marked by high level of blood sugar and it is said to be the most destructive effect associated with T2DM. The high level of blood sugar stimulates glucose auto-oxidation so that free radicals could be formed. The presence of free

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radicals lead to macro and microvascular dysfunction and polyneuropathy since it is exceeding the scavenging abilities of endogenous antioxidant defenses [5]. There are many harms that could be caused by free radicals such as damaging DNA, proteins, cellular molecules, and lipids that further could lead to altered cellular functions. In fact, recently there are numerous studies that reveal the capability of antioxidants in neutralizing free radicals effectively. For instance, animals induced with diabetes through experiments are able to prevent themselves in developing the disease. Moreover, it is also effective in reducing the severity of diabetic complications [6].

Even though there are several drugs to tightly regulate blood glucose, to reduce microvascular and macrovascular complications, the main undesirable effects of this antidiabetic drug that are currently available are brain damage, swelling, erythema, abdominal pain, weight gain, metallic taste, vitamin B₁₂ deficiency, heart failure and gastro intestinal disturbances. Due to these side effects of oral hypoglycemic agents and oxidative stress in complicating diabetes, there is growing interest in herbal medicament as one of the methods to cure T2DM and oxidative stress [7].

Besides insulin, there are other already-developed drugs that help the body lower blood glucose levels, such as insulin secretagogues, insulin sensitizers, α -glucosidase inhibitors, peptide analogues, dipeptidylpeptidase-4 inhibitors and glucagon like peptide-1. However, these synthetic medications possess dangerous side effects, which include hypoglycemia, nausea, diarrhea, gastrointestinal discomfort, weight gain, liver disease, heart failure, etc. [2]. Natural products emerge as one promising area in the activity of drug developments given that the secondary metabolite derived from plants and animals are proven effective to be healing agents for various diseases. The secondary metabolites of the plants, grant the natural defense mechanisms against pathogens, predators, as well as self-protection against herbivores and microbes [8].

The currently available modern medicines have numerous harmful effects. Therefore, to develop safe and effective medications for patients of diabetes is undeniably needed. Plants are endowed with safe and effective natural ability as medications, proven since the ancient times where plants have been an exemplary source of medicine. After preclinical and clinical evaluation, various plants are proven to possess significant characteristics of antidiabetic property. Moreover, many phytoconstituents (compounds that are responsible for antidiabetic effects) have been isolated from plants in the past few years [9].

A shrub named *C. excavata* Burm. f. (Rutaceae) is mainly distributed in southern and southeastern Asia, including Myanmar. It is usually known as 'SatPu Khar Yar' by Myanmar people while the roots are commonly used as medications for colds, fever, headache, cough, malaria colic, sores, wounds, rhinitis, and detoxification. The plant possesses bioactive constituents which are mainly reported to be carbazole alkaloids [10, 11, 12], coumarins [13, 14, 15] along with a little amount of triterpenoids [16], limonoids [17] and pyrano coumarin [18]. The bioactivities, such as antiplatelet, antiplasmodial, antimicrobial, antitumor, anti-HIV, antimycobacterial and antifungal activities, of coumarins and carbazoles, have been reported from a different plant part and location [19], but there was not yet reported secondary metabolites from Myanmar *C. excavata* and their antidiabetic and antioxidant activity. In this research, we isolated constituents from *C. excavata* and evaluated their antidiabetic activity that was measured by an α -glucosidase inhibition assay. Meanwhile, the antioxidant activity was done by DPPH assay. The highest inhibition activity against maltase α -glucosidases was shown from the two isolated compounds especially against on maltase α -glucosidases.

2 Experimental Section

2.1 Plant material

In October 2016, the researchers collected the roots of *C. excavata* from Pyin Ma Nar Township, Mandalay Division, Myanmar [18]. Afterwards, the plant materials were sent to Mandalay University, Myanmar, to be identified by Prof. Soe Myint Aye, a botanist from Department of Botany of Mandalay University.

2.2 Extraction and isolation

Over a period of two weeks, as much as 3.6 kg of the dried roots were consecutively extracted with 95% EtOH (12.0 L) at room temperature. Once the solvent was removed by evaporation, as much as 156g of dark gummy extracts was obtained. As much as 100g of the extracts was effectively partitioned three times using *n*-hex and methanol solvents with ratio 1:1(v/v). Afterward, as much as 80.4g of methanol portion was subjected to vacuum liquid chromatography over silica gel that has been eluted with different mixtures of *n*-hexane: ethylacetate by gradually increasing gradient polarity. Ultimately, there were 7 combined fractions

(MF-1 to -7) obtained from the process. One of the sub fractions namely sub fraction MF-1 was fractionated by silica gel column eluted with gradient polarity *n*-hexane : ethylacetate, (0-10% EtOAc). From it, three sub fractions (MF1.1 to MF-1.3) including pure compound (**1**, 368 mg) were obtained. Finally, fraction MF-1.1 (1.3 g) was purified on silica gel column eluted with gradient polarity *n*-hexane : ethylacetate, (0-5% EtOAc) and thereafter produced pure compound (**2**, 98 mg).

2.3 General procedure

1D and 2D NMR tests were performed on a Bruker 600 MHz (¹H) and 151 MHz (¹³C) in solvent CDCl₃. The report of chemical shifts was shown in ppm as referenced to solvent residues (CDCl₃, δ_H 7.26 ppm and δ_C 77.0 ppm). Fisher-Johns Melting Apparatus was utilized to determine melting points. Infrared spectra were recorded on KBr disks on IRTracer-100, ν in cm⁻¹. UV spectra and antioxidant assay (DPPH) were measured with UV-1800 spectrophotometer. Vacuum liquid chromatography and analytical preparative thin layer chromatography were performed by using Kieselgel 60 (F₂₅₄, Merck). Merck Kieselgel 60 (40-63 μm) was employed for column chromatography. Sigma Aldrich supplied rat intestinal acetone powder and baker's yeast. Inhibition of α-glucosidase was measured with a TECAN Infinite 50 microplate reader spectrophotometer, whereas the spectrophotometric measurement of yeast was conducted using iMark™ microplate reader.

2.4 α-Glucosidase inhibitory activities

The inhibition of α-glucosidase was examined using a method explained by Ramadhan et al. [20]. The aforementioned protocol was used to determine the inhibitory reaction of these isolated compounds against maltase from rat intestine and sucrase. 20 μL of raw enzyme solution, 80 μL of glucose kit, and buffer of phosphate with 0.1 M of molarity that consists of 10 μL of buffer (pH 6.9, 30 μL) and 20 μL of the substrate solution (10mM maltose and 100 mM sucrose). The released glucose concentration was identified by the method of glucose oxidase with a glu-kit. After that, this reaction mixture was incubated at 37°C. Maltase was incubated for 10 minutes, whereas sucrose was incubated for 40 minutes. The glucose oxidase method was employed using a glu-kit to detect the glucose concentration released from the action mixture (Human, Germany). Enzyme activity was calculated through absorbance measurement at 503 nm. The reaction

inhibition percentage was determined using $[(A_0 - A_1)/A_0] \times 100$, in which A₀ refers to sample-less absorbance, while A₁ refers to absorbance with sample. A graph of inhibition percentage against concentration of inhibition was used to determine the value of IC₅₀. The standard control was Acarbose® and the test was conducted in duplicate. Moreover, to determine the inhibition of all isolated compounds against yeast, the method used by Damsud et al. [21] was applied with a slight modification. 10 μL of sample and yeast (0.4 U/ml) was mixed in 1 mM phosphate buffer (pH 6.9) and shook with microplate shaker for about 2 min and pre-incubated for 10 minutes at 37°C. 50 μL of *p*-nitrophenyl-α-D-glucopyranoside (PNPG) was added to the reaction mixture. Then, the mixture was put in the incubator for 20 minutes at 37°C. After the incubation, Na₂CO₃ (100 μL) was added to the reaction in order to quench it. The *p*-nitrophenoxide that was released from PNPG was identified by iMark microplate reader at 405 nm. The equation mentioned above was employed to calculate the percentage of inhibition.

2.5 DPPH radical scavenging assay

DPPH radical scavenging activity of isolated pure compounds was performed using the method employed by Aminah et al. [22]. In this method, DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent was utilized as free radicals. The reducing ability of antioxidants towards DPPH was evaluated by monitoring the decrease of its absorbance at 523 nm by UV spectrometer. Firstly, isolated compound was dissolved with methanol. It was then added with buffer acetate solution with the molarity of 0.1 M and a pH of 5.5 as well as 5.10⁻⁴ M DPPH radical solution. The dissolution was done to determine antioxidant activity. With a spectrometer at 523 nm, isolated compounds inhibition against DPPH radical was observed following 30 minute incubation at 25°C. The percentage of inhibition activity was quantified using this equation: $[(A_{control} - A_{sample}) / A_{control} \times 100]$. In this equation, A_{control} refers to the stable DPPH radical's initial concentration in the absence of the test compound. Meanwhile, A_{sample} shows the absorbance of the residual DPPH concentration with methanol. The values of IC₅₀ (mM) were calculated using a plotted graph of extract concentration versus scavenging; IC₅₀ refers to the complete sum of antioxidant needed to lower the initial concentration of DPPH radical by fifty percent.

Ethical approval: The conducted research is not related to either human or animal use.

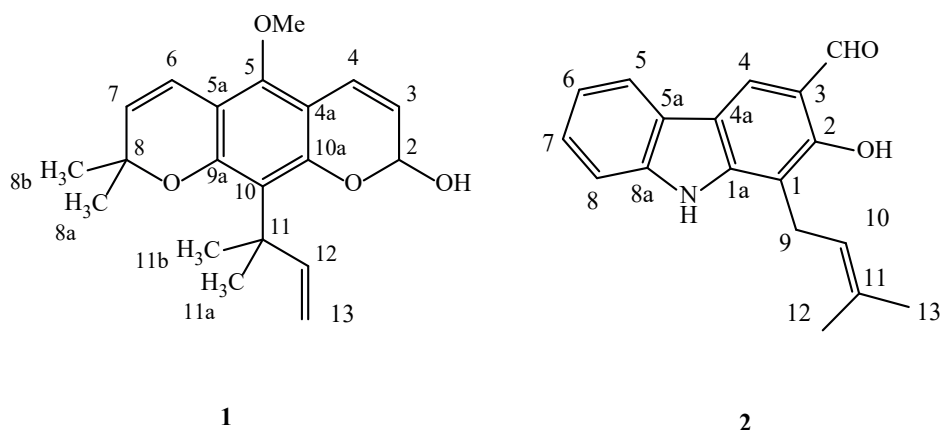


Figure 1: The structure of isolated compounds **1** and **2** from *C. excavata*.

3 Results and Discussion

Compound (1) yielded as white prism like crystal with melting points 93–94 °C. According from HR-FABMS spectrum it show molecular formula $C_{20}H_{22}O_4$ (m/z 327.1597 $[M + H]^+$ (calcd. for $C_{20}H_{22}O_4$, 327.1596). The presence of aromatic (1641, 1590, 1469 cm^{-1}) groups and conjugated lactone (1686 cm^{-1}) was denoted by IR spectrum. Due to 7-oxygenated coumarin, absorption maxima were exhibited by the spectrum of UV at 330 and 272 nm. The 1H -NMR spectrum of compound (**1**) showed 9 signals and representing 27 protons it pattern is similar to nordentatin only one methoxy signal at δ 3.83 (s, 3H). As a typical pyrano coumarin, compound (**1**) showed the presence of two pyrone protons δ 6.19 (d, $J = 9.6$ Hz, 1H, H-3) and 7.87 (d, $J = 9.6$ Hz, 1H, H-4). The presence of chromene ring was showed by two olefinic proton pair δ 5.69 (d, $J = 9.9$ Hz, 1H, H-7) and 6.56 (d, $J = 9.9$ Hz, 1H, H-6). The prenyl group exomethylene protons was showed by δ 6.30 (dd, $J = 17.4$, 10.6 Hz, 1H, H-12), δ 4.94 (dd, $J = 17.4$, 1.1 Hz, 1H, H-13), δ 4.88 (dd, $J = 10.6$, 1.1 Hz, 1H, H-13). Moreover the existence of 1,1-dimethyl groups was revealed by two singlet peaks δ 1.45 (s, 6H) and δ 1.66 (s, 6H) (Table S1). The ^{13}C NMR data revealed the presence of lactone carbonyl with δ (160.7), together with 8 quaternary carbons, 5 methine carbons, one sp^2 methylene, one methoxy carbon and 4 methyl carbons (Figure S2). After carefully checked all NMR data and matched with literature values compound (**1**) was elucidated as dentatin (Figure 1) [11].

Compound (2) afforded as yellow green crystal UV (MeOH), λ_{max} (log ϵ) 342 (0.97), 298(3.32), 278(3.02), 249(1.64), 235(2.26). Compound (**2**)'s IR spectrum pointed out that -OH and -NH functional groups at (3437 cm^{-1}) and carbonyl and aromatic benzene groups at (1728, 1612 and 1588 cm^{-1}) were present. In the spectrum of 1H NMR,

compound (**2**) revealed a total of 11 signals representing 14 protons. In the down field region three singlets; intramolecular bonding OH-(δ 11.66) with -CHO (δ 9.91) and one broad signal due to -NH (δ 8.21) and another singlet proton H-4 (δ 8.03). In the aromatic region four signals with δ values, 7.97 (dd, $J = 7.7$ Hz, 1H, H-5) 7.26 (td, $J = 3.8$, 7.7 Hz, 1H, H-6), 7.40 (td, $J = 3.8$, 7.7 Hz, 1H, H-7) and 7.41 (dd, $J = 7.7$, 3.8 Hz, 1H, H-8) showed the absence of substituent in ring A. the presence of prenyl group was revealed by one methylene protons at δ 3.64 (d, $J = 6.9$ Hz, 2H, H-9), δ 5.33 (t, $J = 6.9$ Hz, 1H, H-10) and one dimethyl group (δ 1.91 (s, 3H, H-12), δ 1.78 (s, 3H, H-13). According to the spectra of ^{13}C NMR and DEPT, it was revealed that there were 18 signals and 18 carbons. Furthermore, the DEPT spectrum showed the presence of one aldehyde carbon, 8 quaternary carbons, 6 methine carbons, one methylene carbon, and two methyl carbon as shown in (Table 2). The 2D NMR spectra such as DQF-COSY data revealed the correlation of two adjacent protons (Figure S9). The HSQC spectrum gave the direct correlation protons and carbon. Finally, the combination of fragments and attachments of substituents were confirmed by HMBC data (Table S2) (Figure S1) and compound (**2**) was elucidated as heptaphylline (Figure 1). It was also reliable with the data which reported earlier [11].

Every isolated compound was utilized by α -glucosidase assay to detect antidiabetes activity and also by DPPH assay to detect antioxidant activity. Based on the tested compounds, dentatin (**1**) and heptaphylline (**2**) displayed inhibition against on maltase and yeast α -glucosidase that have IC_{50} values of (6.75, 11.46 μM) and (0.482, 24.96 mM) respectively. However, compound (**1**) showed no inhibition whereas compound (**2**) IC_{50} value 223.9 (μM) against on sucrase enzymes (Table 1). According to this data, compound (**1**) exhibited 2-fold more potent

Table 1: α -Glucosidase inhibitory and antioxidant activities of isolated compounds (1-2).

Compound	α -Glucosidase			DPPH IC ₅₀ (mM)
	Baker's yeast IC ₅₀ (mM)	Maltase IC ₅₀ (μ M)*	Sucrase IC ₅₀ (μ M)*	
1	0.481843	6.75	NI**	2.66
2	24.9641	11.46	223.91	1.55
Acarbose	0.1030	2.35	15.48	-
Ascorbic acid	-	-	-	0.0118

* Nonlinear regression analyzes were evaluated by SigmaPlot 12.5

than compound (2) but 3-fold less potent than standard control, acarbose (IC₅₀ 2.35 μ M) against on maltase α -glucosidase. Each isolated compound demonstrated antioxidant activity with IC₅₀ values of 2.66, and 1.55 mM respectively. The results showed that the compound (1) is higher inhibitory than compound (2) against on maltase and yeast enzymes.

4 Conclusion

In searching antidiabetic and antioxidant agents from Myanmar medicinal plant *C. excavata* root ethanolic extract lead to the isolation of two known bioactive compounds namely dentatin (1) and heptaphylline (2). Each isolated compound was evaluated on its antidiabetic activity and antioxidant activity by employing α -glucosidase inhibition and DPPH assays, respectively. Both compound (1) and compound (2) demonstrated the highest activity of inhibition against maltase (IC₅₀ 6.75 μ M), (IC₅₀ 11.46 μ M) respectively. Nevertheless, compound (1) and compound (2) showed that there was inhibition on yeast α -glucosidase in the presence of IC₅₀ values of 0.482 and 24.96 mM. Furthermore, each isolated compound displayed antioxidant activity; the IC₅₀ values were 2.66 and 1.55 mM (ascorbic acid, IC₅₀ 0.012 mM). The study showed both compounds demonstrated inhibition against α -glucosidases and free radical scavenging activity. Compound (1) showed a stronger activity. Hence, isolated compound (1) from *C. excavata* root can be a prospective candidate for natural antidiabetes and antioxidant agent.

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Conflict of interest: Authors state no conflict of interest.

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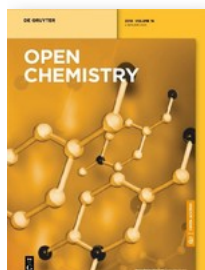
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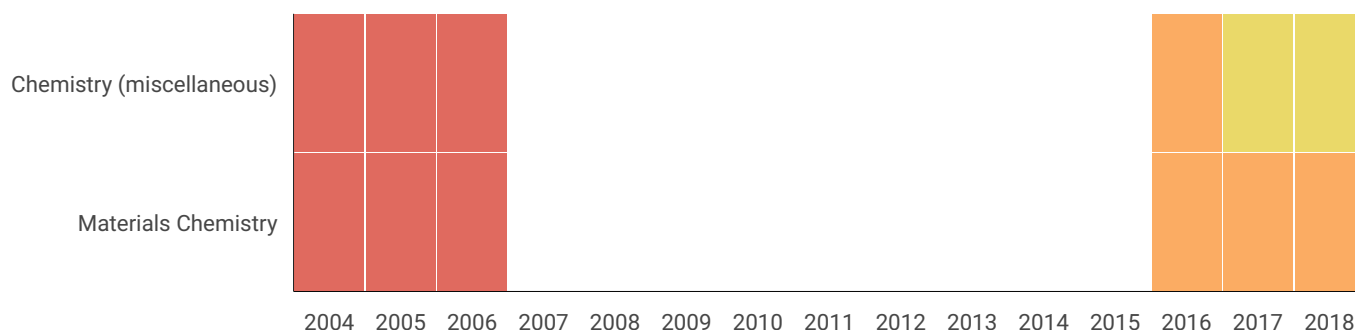
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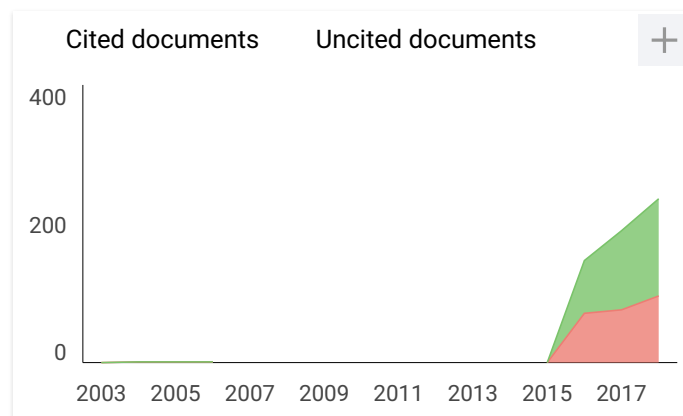
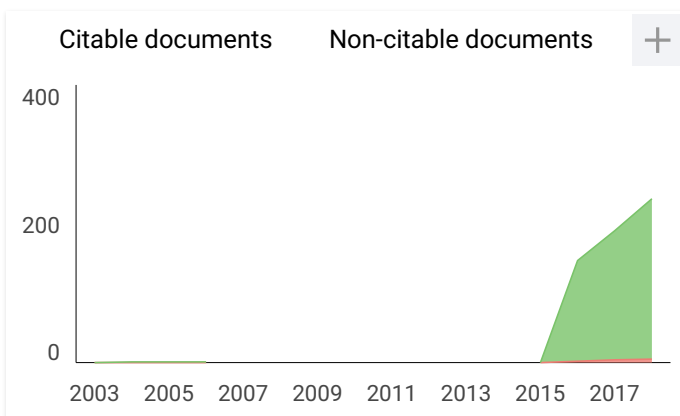
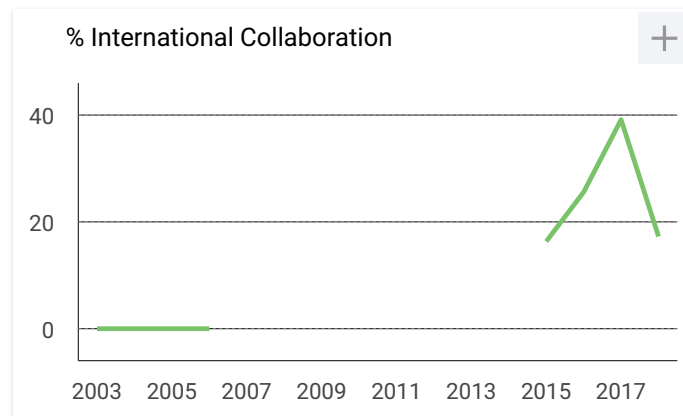
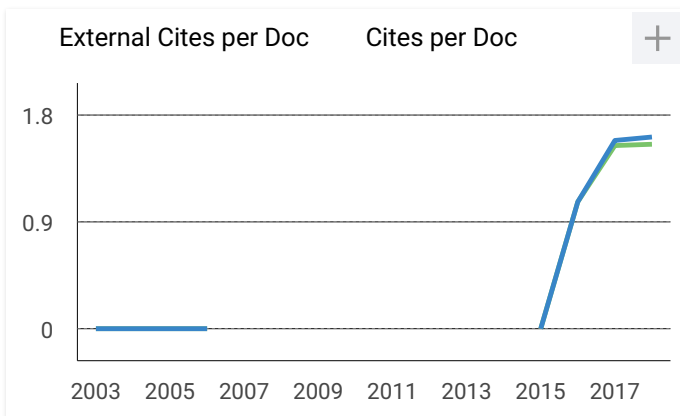
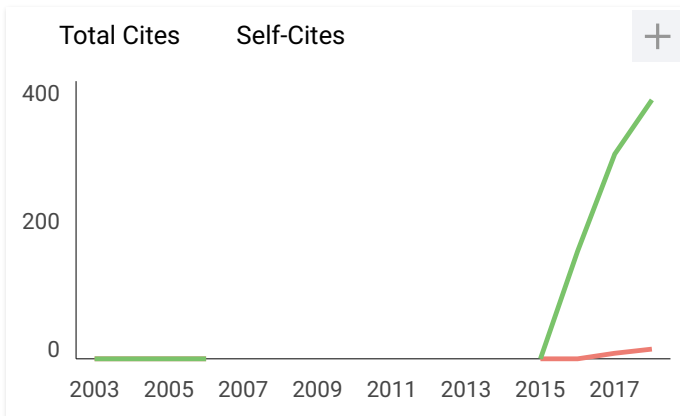
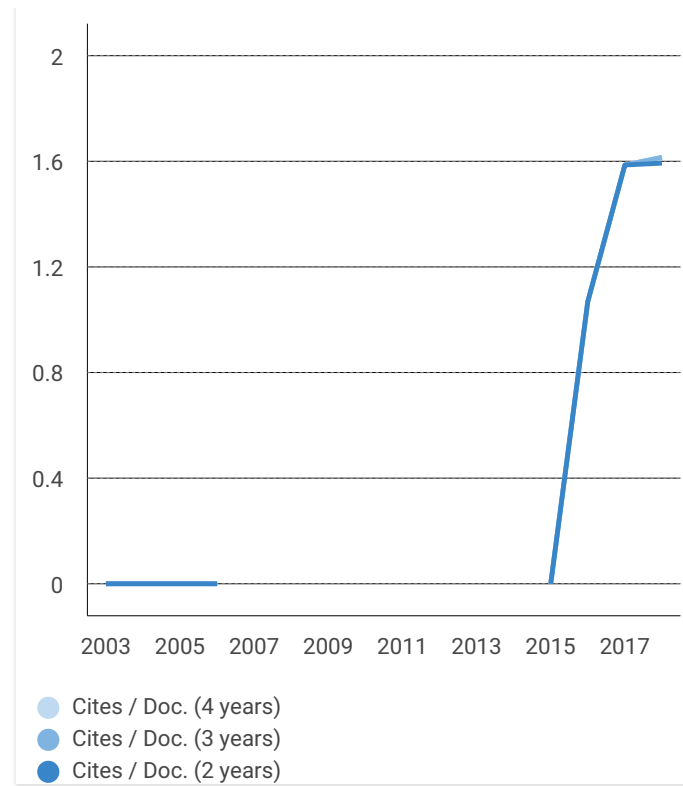
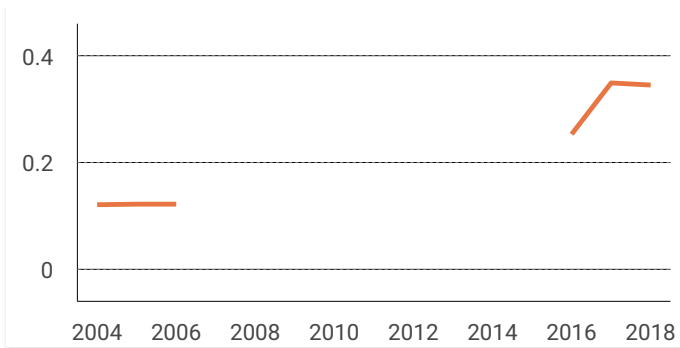


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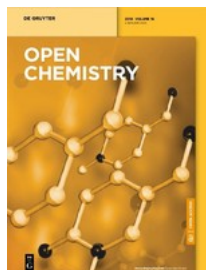
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Thant, T. M. / **Aminah, N. S.** / Kristanti, A. N. / Ramadhan, R. / Aung, H. T. / Takaya, Y.

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Article: **OPENCHEM-D-18-00445R1**

Title: "Antidiabetes and Antioxidant agents from *Clausena excavata* root as medicinal plant of Myanmar"

date:	Jan 30, 2019, 7:20 PM
subject:	Your Submission

Dear Dr Aminah,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be happy to receive your revised paper.

For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you submit the revised manuscript.

Your revision is due by 2019/03/03.

To submit a revision, go to <https://www.editorialmanager.com/openchem/> and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

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Yours sincerely,
Agnieszka Topolska
Managing Editor
Open Chemistry

Reviewers' comments:

Reviewer #1: The overall manuscript might have some scientific worth, but the manuscript is extremely weak grammatically, lacks appropriate references in multiple occasions, arbitrary hyphens have been used to make simple words immensely complicated, unacceptable use of copying-pasting of computer screenshots for Table.1 and Table.3 which are also seems to be the same screenshot.

Some of the key points which also needs to be addressed are mentioned herewith:

-In the extraction and isolation section, (i) what was the reduced pressure, (ii) what is meant by "gave 7 combined fractions (MF-1 to -7)".

- Please provide an explanation for 'Prison crystal'

-It is very difficult to follow the results section, with all data put in the sentences.

Please provide appropriate tables, and try to summarize the main outcomes in the result section

-Please explain in the conclusion section, if the anti-diabetic effect and anti-oxidant

activity of *C. excavata* have been studied recently, where is the novelty of the present manuscript

Reviewer #3: The paper presented by T.M. Thant et al describes an interesting research on one natural extract with antidiabetes and antioxidant properties from local plants. The paper is logically and clear written. However there are some errors which have to be corrected before publication. I will not present here these errors but I ask the authors to carefully check the whole manuscript. At the same time I think the conclusions have to be rewritten in order to avoid to be similar with the abstract. The paper proposes to extract two different agents from a local medicinal plant and shows their antidiabetes and antioxidant properties. Tacking into accont that the methodology is quite expensive to separe the compounds did the authors tried to preppare an alcoholic extract with both compounds and test it for antidiabetes and antioxidant properties ?

Reviewer #5: Dear Authors,
the reviewed manuscript entitled: "Antidiabetes and Antioxidant agents from *Clausena excavata* root as medicinal plant of Myanmar" shows the invastgation of antidiabetes and antioxidant activity isolated compounds: dentatin and heptaphylline. To study the activity of isolated metabolites, proposed α -glucosidase inhibition assay and DPPH assay. The undertaken subject is interesting, and presenting up-to-date information, but there are some issues which should be clarified prior to the publication:

- please, add a chromatogram recorded for the extract and for the plasma samples, indicating the analyzed peaks
- The authors need to correct references and remove a little mistake in the text.
- Maybe, apply the test with Folina-Cliocalteu solution

date:	Feb 27, 2019, 3:18 PM
subject:	Submission Confirmation for OPENCHEM-D-18-00445R1

Dear Dr Aminah,

Open Chemistry has received your revised submission.

You may check the status of your manuscript by logging onto Editorial Manager at (<https://www.editorialmanager.com/openchem/>).

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Kind regards,

Editorial Office
Open Chemistry

date:	Feb 27, 2019, 2:58 PM
subject:	Your PDF Antidiabetes and Antioxidant agents from Clausena excavata root as medicinal plant of Myanmar has been built and requires approval

Dear Dr Aminah,

The PDF for your submission, "Antidiabetes and Antioxidant agents from Clausena excavata root as medicinal plant of Myanmar" is ready for viewing.

This is an automatic email sent when your PDF is built. You may have already viewed and approved your PDF while on-line, in which case you do not need to return to view and approve the submission

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Your submission must be approved in order to complete the submission process and send the manuscript to the Open Chemistry editorial office.

Please view the submission before approving it to be certain that your submission remains free of any errors.

Thank you for your time and patience.

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subject:	Your Submission OPENCHEM-D-18-00445R1 - Open Chemistry

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It was accepted on 2019/02/27

In order to proceed with your publication I would like to remind you that we charge a publication fee - the APC is 300 EUR plus VAT (if applicable), plus money transfer charges. The discount code will be sent to you asap.

Thank you for submitting your work to this journal.

With kind regards

Agnieszka Topolska
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date:	Nov 25, 2019, 11:37 PM
subject:	OPENCHEM-D-18-00445R1

Dear Authors,

please find attached your article after language editing. Note to check the references list carefully (especially year of publication and the journal title - both are essential for a proper indexing). For any corrections use the attached version of your manuscript. Thank you!

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Agnieszka

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Hopefully, this is the last process of our manuscript to be published.

Thank for your patient to teach me for all of the process,

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Nanik

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Dear Nanik,
thank you very much for the last corrections. I will proceed to the technical editing in coming days.

Best regards,
Agnieszka

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Nanik

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Thank you very much for your kind help and cooperation.

With best regard,
 Nanik

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Best regards,
 Agnieszka

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From: em.openchem.3d34.68159e.c839b08f@editorialmanager.com <em.openchem.3d34.68159e.c839b08f@editorialmanager.com> On Behalf Of Nanik Siti Aminah

Sent: Saturday, December 21, 2019 3:28 PM

To: Topolska, Agnieszka <Agnieszka.Topolska@degruyter.com>

Subject: OPENCHEM-D-18-00445R1 - Proofreading after article composition has been done

Article: OPENCHEM-D-18-00445R1

Title: "Antidiabetes and Antioxidant agents from Clausena excavata root as medicinal plant of Myanmar"

Author: N.S. Aminah

Dear Dr. Agnieszka Topolska,

The proofreading after composition of article titled "Antidiabetes and Antioxidant agents from Clausena excavata root as medicinal plant of Myanmar" has been finished. Please find below my comments:

Here I send you small revise with a yellow block in lines 40 and 46 of the manuscript. For the Supplementary Information, perfect.

Best regards,
 N.S. Aminah

date:	Dec 27, 2019, 11:03 AM
subject:	Re: OPENCHEM-D-18-00445R1 - Proofreading after article composition has been done

Dear Agnieszka Topolska

We agree with the latest changes to the manuscript you have made.

Thank you for your kind help and cooperation.

With best regard,
Nanik

from: **Topolska, Agnieszka** Agnieszka.Topolska@degruyter.com
to: nanik siti aminah <nanik-s-a@fst.unair.ac.id>
date: Dec 28, 2019, 7:40 PM
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Agnieszka

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I'd like to thank you very much for your contribution to our journal! It was a pleasure to work with you.

Best wishes,
Agnieszka