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Coumarins from Myanmar edible fruit tree (*Casimiroa edulis*)

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This research expresses the phytochemical study from the Myanmar edible fruit tree, *Casimiroa edulis* (Rutaceae). The result revealed that the isolation and identification of two furanocoumarins (bergapten **1** and isopimpinellin **2**) from the stem bark of this plant. Their molecular structures were elucidated and identified by using NMR spectroscopy in combination with IR, UV and HRMS spectra data, respectively. Furthermore, these two compounds were investigated for their anti-diabetic activity. According to the result, bergapten **1** and isopimpinellin **2** are not essentially good for anti-diabetic activity. This is the first report of two furanocoumarins from the Myanmar edible fruit tree.

Keywords: *Casimiroa edulis*, furanocoumarins, NMR spectroscopy, anti-diabetic activity.

Introduction

Casimiroa edulis La Llave (Rutaceae) is a genus of comprising only 10 species which is distributed in tropical and subtropical regions, including Myanmar. Among then, *Casimiroa edulis* is the well-known species^{1,2}. The traditional used and the pharmacological studies of the leaves and seeds of *Casimiroa edulis* displayed various biological activities. In Myanmar, this plant is named Thar-kyar-thee.

Phytochemical studied from genus *Casimiroa* assist to the separation of various chemical constituents such as 18 flavonoids (isolated from leaves, fruits, and seeds), 24 alkaloids (isolated from leaves, fruits, bark, and seeds), and 16 coumarins (isolated from leaves, fruits, and seeds). Pharmacological investigations of isolated flavonoids are antioxidant, anti-mutagenic, solid tumor selective cytotoxicity, vasodilation and radical-scavenging activities, the investigations of isolated alkaloids are anti-mutagenic, solid tumor selective cytotoxicity, anti-hypertensive, cardiovascular activities, and the investigations of isolated coumarins are anti-coagulant, vasodilation and radical-scavenging, adipogenesis, solid tumor selective cytotoxicity activities, respectively³⁻¹⁴. This

paper deals with the structure elucidation of two furanocoumarins from the stem bark of *Casimiroa edulis*.

Experimental

General:

The infrared spectra (IR) were obtained on FTIR-8400S (Shimadzu) using KBr. UV spectra were examined in MeOH by using UV-Vis Shimadzu spectrometer. Column chromatography (CC) was achieved on silica gel (BW-820H). Analytical thin-layer-chromatography (TLC) was completed at room temperature on pre-coated Kieselgel silica gel 60 F₂₅₄ (Merck) aluminium plates. Melting points were determined by fisher john melting point apparatus. All NMR spectra were checked in CDCl₃ by using a Bruker Avance 3 (¹H: 600 MHz and ¹³C: 151 MHz) with TMS as an internal standard. Chemical shifts are described in part per million (δ , ppm) based on their solvent signal (chloroform-*d*: δ_{H} 7.26, δ_{C} 77.16) coupling constants *J* value in Hertz (Hz). HR-FAB-MS was attained with a JEOL JMS HX-110 mass spectrometer.

Plant material:

Casimiroa edulis La Llave was collected from Namp-see

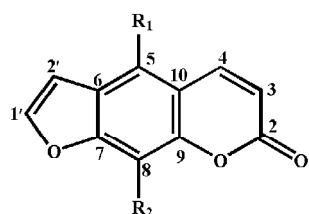
Village, Taunggyi (Shan State), Myanmar, in August 2016, and was identified by the Professor of Botany Department, University of Taunggyi.

Extraction and isolation:

The dry sample of *Casimiroa edulis* (1 kg) was exhaustively extracted with MeOH (3 L) at room temperature for four weeks. The methanol extract was concentrated under pressure using rotator evaporator for dryness. Then, the methanolic extract was partition between *n*-hexane and MeOH (300 ml×3, v/v). The concentrated MeOH extract (50 g) was subjected to vacuum liquid chromatography (VLC) and eluted with *n*-hexane-EtOAc in a stepwise gradient solvent system (10:0, 6:4, 0:10, v/v) to afford several fractions. Column eluate was collected in 150 ml glass bottles to yield 28 fractions and examined under UV. Based on analytical TLC, the fractions were grouped and evaporated on a rotary evaporator to afford six fractions (J-1 to J-6). Fractions J-5 (2 g) were additionally purified by silica gel column chromatography with a gradient solvent system of *n*-hexane in EtOAc (10:0, 0:10) to give colourless needles of compound **1**, and pale-yellow crystalline solids of compound **2**.

Results and discussion

Compound **1** was obtained as colorless needle crystals. Its melting point was 186–206°C. Based on the molecular ion peak at m/z 217.0501 $[M + H]^+$ (Calcd. 216.0423) in the HR-FAB-MS, the molecular formula was determined as $C_{12}H_8O_4$. IR spectrum informed the characteristic of sharp bands at 3143, 3115, 3088, 3010, 2924, 2852, 1737, 1720, 1625 and 1581 cm^{-1} which could be assigned to $-OCH_3$, sp^2-CH , sp^3-CH , lactone $C=O$, $C=C$ ring skeletal stretching vibration, respectively. The UV spectrum showed the bands



Compds.	R ₁	R ₂
1	OCH ₃	H
2	OCH ₃	OCH ₃

Fig. 1. Chemical structure of compound **1** and **2**.

at λ_{max} 221, 249, 259, 268, and 311 nm, which were typical of a furanocoumarin skeleton. The 1H NMR spectrum was exhibited as an easily distinguishable pair of doublets at δ_H 6.28 (d, 1H, 9.8 Hz, H-3) and δ_H 8.16 (d, 1H, 9.8 Hz, H-4). It also displayed two furan doublets at δ_H 7.60 and 7.02 (each, d, 1H, 2.4 Hz, H-2' and H-3'), and an aromatic singlet at δ_H 7.14 (s, 1H, H-8). Combining analysis of ^{13}C NMR and DEPT spectra data, compound **1** displayed 12 carbons resonance, including five methine, one oxygenated and six quaternary carbons, respectively. In the 1H - 1H COSY experiment, the cross-peak correlations of H-3 with H-4, and the cross-peak correlations of H-2' through H-3' were observed. The long-range HMBC attachment from H-3 to C-2 and C-10, H-4 to C-2 and C-9, H-8 to C-6, C-7, C-9 and C-10, H-2'' to C-3'', C-6 and C-7, H-3'' to C-2', C-6 and C-7 were also observed. The remaining one singlet proton at δ 4.27 (integrating for three protons) showed the presence of methoxy group on the aromatic ring. This confirmation was deduced by HMBC spectrum, which confirms that methoxy proton showed the long-range correlation with the C-5 carbon at δ_C 149.6. Analysis of UV, IR, 1D, 2D NMR and HRMS spectra as well as on comparison with Literature, were allowed to determine the structure of compound **1** as bergapten^{15–17}.

Compound **2** was obtained as pale-yellow crystalline solids with a melting point at 178–180°C. Its molecular formula was assigned as $C_{13}H_{10}O_5$ based on HR-FAB-MS (m/z 247.0606 $[M + H]^+$, Calcd. 246.0528). The UV spectrum ex-

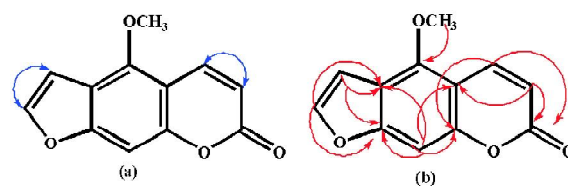


Fig. 2. 1H - 1H COSY correlations (a) and major 1H - ^{13}C long-range HMBC (b) correlation in compound **1**.

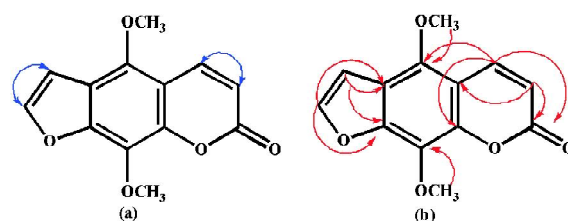


Fig. 3. 1H - 1H COSY correlations (a) and major 1H - ^{13}C long-range HMBC (b) correlation in compound **2**.

hibited the absorption maxima at 241, 248, 268 and 311 nm, which is consistent with the presence of a furocoumarin. The IR spectrum revealed the presence of an α,β -unsaturated lactone (ν_{\max} 1751 and 1718 cm^{-1}), methoxy group (3132 and 3159 cm^{-1}), C-H stretching vibration of sp^2 hydrocarbon (3084 and 3005 cm^{-1}), asymmetrical and symmetrical C-H stretching vibration of sp^3 hydrocarbon (2843 and 2953 cm^{-1}), and C=C ring skeleton stretching vibration (ν_{\max} 1604 and 1593 cm^{-1}) groups. ^{13}C NMR of compound **2** included 13 carbon signals, comprising 2 methoxy carbon signals, 4 methine carbon signals, and 7 quaternary carbon signals, which were classified by DEPT and HSQC experiment. Its ^1H NMR spectrum was exhibited a distinct pair of doublets at δ_{H} 6.27 and 8.11 (each, 1H, 9.8 Hz) assignable to C-3, C-4 protons, a furan doublet at δ_{H} 7.62 and 6.99 (each, 1H, 2.2 Hz) assignable to C-2', C-3' protons. The ^1H - ^1H COSY spectrum showed a correlation between H-3 and H-4 protons while H-2' proton had a correlation to the H-4' proton. In HMBC spectrum, the following correlations were observed; the proton signal at δ_{H} 6.27 was connected with C-2 and C-10, the proton signal at δ_{H} 8.11 was connected with C-2 and C-9, the proton signal at δ_{H} 7.62 was correlated with C-2', C-6 and C-7, the proton signal at δ_{H} 6.99 was correlated with C-3', C-6 and C-7. The remaining methoxy protons at δ 4.16 (integrating for 6 protons) were placed at C-5 and C-8 on the

Table 1. ^1H NMR and ^{13}C NMR data of compound **1** in CDCl_3 and bergapten ^1H in CDCl_3

Position	Compd. 1		Lit. [Ref. 16]	
	^1H NMR ^a	^{13}C NMR ^b	^1H NMR ^c	^{13}C NMR ^d
2	–	161.2	–	161.4
3	6.28 (d, 9.8 Hz)	112.6	6.27 (d, 9.8 Hz)	112.6
4	8.16 (d, 9.8 Hz)	139.2	8.15 (d, 9.8 Hz)	139.4
5	–	149.6	–	149.7
6	–	112.7	–	112.9
7	–	158.4	–	158.5
8	7.14 (s)	93.9	7.13 (s)	94.0
9	–	152.7	–	151.9
10	–	106.5	–	106.6
2'	7.60 (d, 2.4 Hz)	144.8	7.59 (d, 2.4 Hz)	144.9
3'	7.02 (d, 2.4 Hz)	105.0	7.02 (d, 2.4 Hz)	105.2
5-OMe	4.27 (s)	60.1	4.27 (s)	60.3
8-OMe	–	–	–	–

^aSpectra recorded in 600 MHz, ^bspectra recorded in 151 MHz, ^cspectra recorded in 400 MHz and ^dspectra recorded in 100 MHz.

Table 2. ^1H NMR and ^{13}C NMR data of compound **2** in CDCl_3 and isopimpinellin in CDCl_3

Position	Compd. 2		Lit. [Ref. 16]	
	^1H NMR ^a	^{13}C NMR ^b	^1H NMR ^c	^{13}C NMR ^d
2	–	160.4	–	160.4
3	6.27 (d, 9.8 Hz)	112.8	6.26 (d, 9.8 Hz)	112.8
4	8.11 (d, 9.8 Hz)	139.4	8.10 (d, 9.8 Hz)	139.3
5	–	144.3	–	144.3
6	–	114.8	–	114.8
7	–	150.0	–	150.0
8	–	128.2	–	128.2
9	–	143.7	–	143.7
10	–	107.6	–	107.6
2'	7.62 (d, 2.2 Hz)	145.1	7.61 (d, 2.3 Hz)	145.1
3'	6.99 (d, 2.2 Hz)	105.1	6.99 (d, 2.3 Hz)	105.1
5-OMe	4.16 (s)	61.7	4.16 (s)	61.7
8-OMe	4.16 (s)	60.8	4.14 (s)	60.8

^aSpectra recorded in 600 MHz, ^bspectra recorded in 151 MHz, ^cspectra recorded in 400 MHz and ^dspectra recorded in 100 MHz.

basic of long range HMBC correlations. With the aid of above data and previous report, compound **2** was identified as isopimpinellin^{16–18}.

Conclusions

In this study, two known compounds were obtained from the stem bark of *Casimiroa edulis*. The structure of isolated compounds identified as bergapten **1** and isopimpinellin **2** based on their spectroscopic data, such as UV-Vis, FTIR, 1D NMR, 2D NMR, and HRMS. The isolated compounds are not important for their anti-diabetic activity. A lot of researchers were reported these two compounds from many sources of natural product. This is the first report on the occurrence of bergapten and isopimpinellin from Myanmar edible fruit tree *Casimiroa edulis*.

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Supporting Information

1D and 2D NMR spectrum are described.

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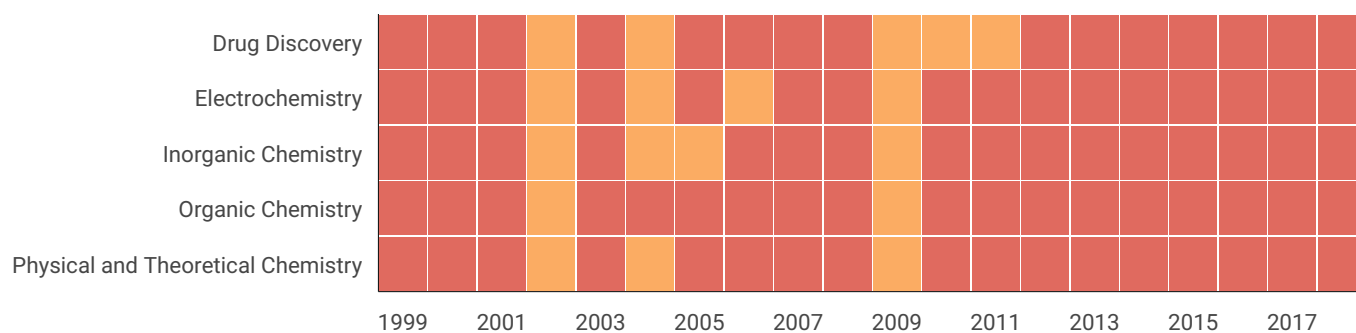
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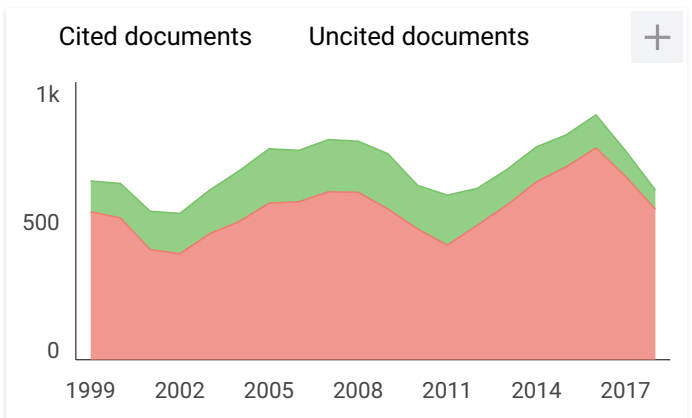
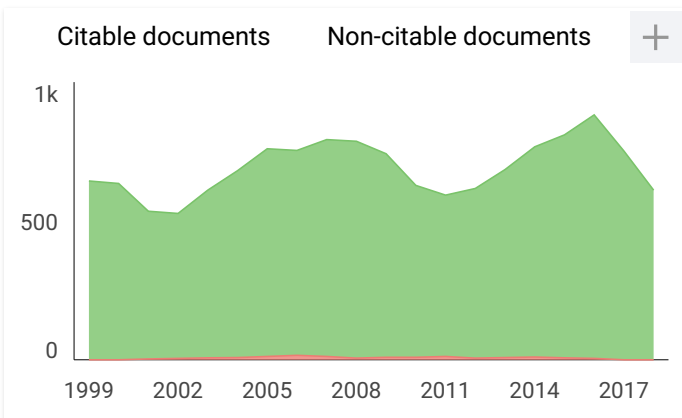
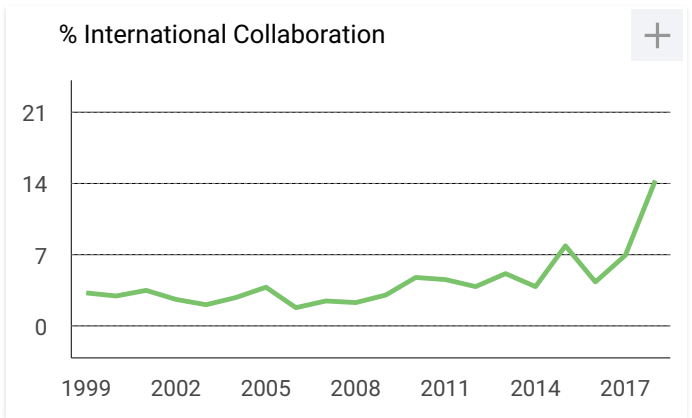
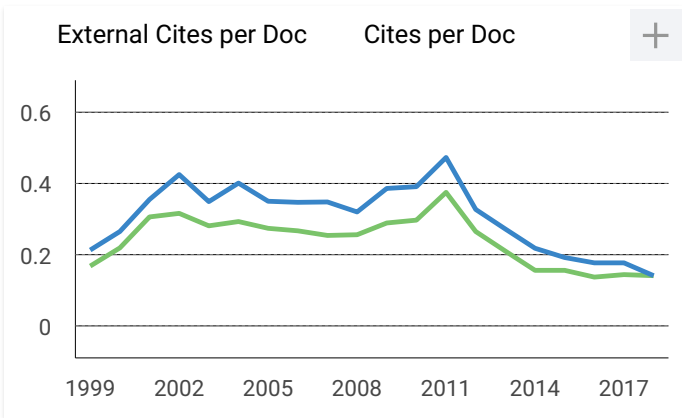
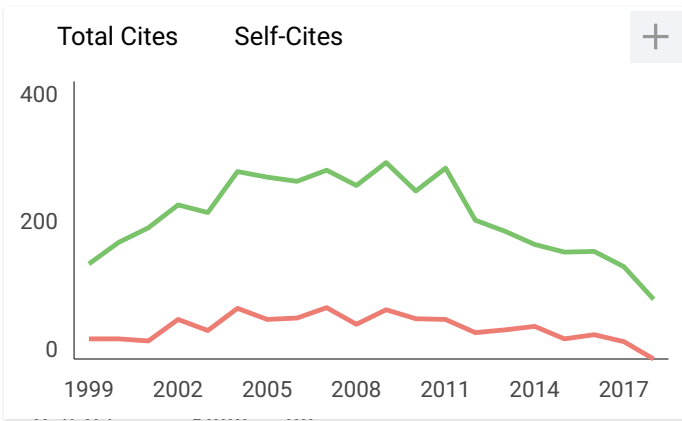
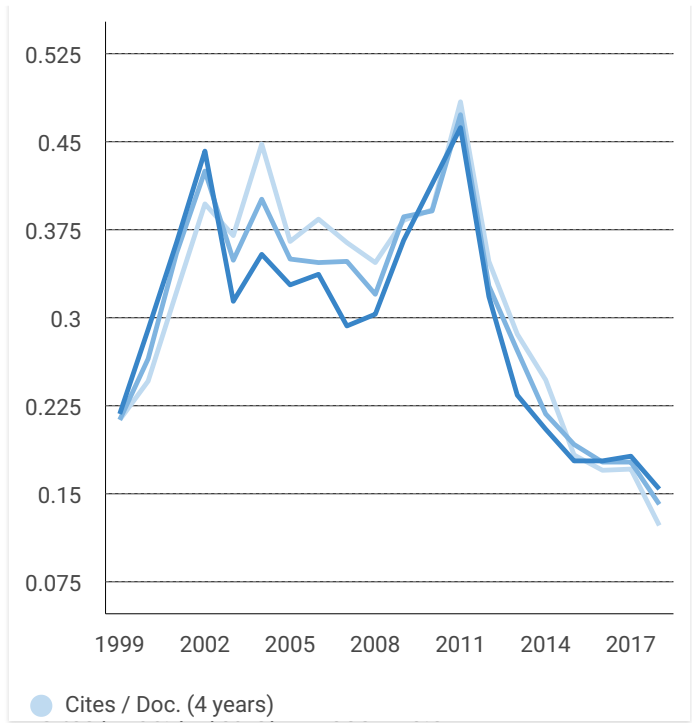
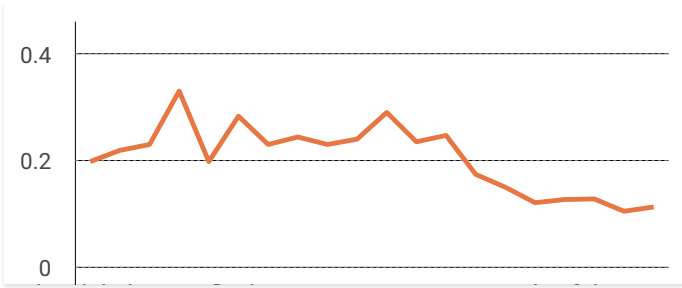


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With best regard,
Nanik

On Tue, May 21, 2019 at 6:32 PM INDIAN CHEMICAL SOCIETY <indi3478@dataone.in> wrote:
[Quoted text hidden]

[Quoted text hidden]

2 attachments

 **Draft for JICS.doc**
143K

 **Supporting information for JICS.doc**
550K

nanik siti aminah <nanik-s-a@fst.unair.ac.id>
To: khun nay <khun.nay.win-2017@fst.unair.ac.id>

Wed, May 22, 2019 at 7:00 PM

[Quoted text hidden]

2 attachments

 **Draft for JICS.doc**
143K

 **Supporting information for JICS.doc**
550K

Proof (URGENT)

10 messages

INDIAN CHEMICAL SOCIETY <indi3478@dataone.in>
To: nanik siti aminah <nanik-s-a@fst.unair.ac.id>

Fri, Jun 7, 2019 at 4:20 PM

To

Dr. Nanik Siti Aminah

Department of Chemistry

Faculty of Science and Technology

Universitas Airlangga

Komplek Kampus C UNAIR

[Jl. Mulyorejo, Surabaya](#)[Indonesia](#)

Dear Dr. Aminah,

Attached please find the proof of your manuscript entitled "**Coumarins from Myanmar edible fruit tree (*Casimiroa edulis*)**" for your kind perusal.

Please acknowledge the receipt of the same and do the needful in this regard at your earliest convenience and send the same back to us by tomorrow along with the Pictorial Abstract for the Contents page of the Journal.

Please note that the cost of **PDF** of your manuscript is 50 US Dollar only. Payment should be made by CTS DD or multicity CTS cheque drawn in favour of Indian Chemical Society, payable at Kolkata.

With best regards

Dr. Rahul Bhattacharya
Executive Officer
Indian Chemical Society

 **PROOF.pdf**
255K

nanik siti aminah <nanik-s-a@fst.unair.ac.id>

Sat, Jun 8, 2019 at 4:36 PM

To: khun nay <khun.nay.win-2017@fst.unair.ac.id>, alfinda novi kristanti <alfinda-n-k@fst.unair.ac.id>

Dear Khun

Congrat, one of your manuscript accepted.

Please learn carefully how to pay?

The day after tomorrow we will discuss

Thank you

[Quoted text hidden]

--

Dr. Nanik Siti Aminah

Assoc. Professor on Natural Product Chemistry

Dept. of Chemistry

Fac. of Science and Technology

Universitas Airlangga

Vice Dean on Research and Partnership

Faculty of Science and Technology

Universitas Airlangga

Komplek Kampu C UNAIR

Jl. Ir. Soekarno

Surabaya-East Java

Indonesia

email address : nanik-s-a@fst.unair.ac.id

 **PROOF.pdf**
255K

nanik siti aminah <nanik-s-a@fst.unair.ac.id>

Mon, Jun 10, 2019 at 1:52 PM

To: INDIAN CHEMICAL SOCIETY <indi3478@dataone.in>

Dear

Dr. Rahul Bhattacharya

Executive Officer

Indian Chemical Society

We are very happy to receive the good news from you that our manuscript accepted.

Would you like to teach me, the procedure for the payment?

Thank you for your kindness help and cooperation

With best regard

[Quoted text hidden]

--

Dr. Nanik Siti Aminah

Assoc. Professor on Natural Product Chemistry

Dept. of Chemistry

Fac. of Science and Technology

Universitas Airlangga

Vice Dean on Research and Partnership

Faculty of Science and Technology

Universitas Airlangga

[Quoted text hidden]

INDIAN CHEMICAL SOCIETY <indi3478@dataone.in>

Tue, Jun 11, 2019 at 1:48 PM

To: nanik siti aminah <nanik-s-a@fst.unair.ac.id>

Dear Sir,

Attached please find the necessary information regarding the online payment.

Please send the correction, if any along with the Pictorial Abstract at the earliest.

[Quoted text hidden]

 **RTGSCurrent.pdf**
239K

nanik siti aminah <nanik-s-a@fst.unair.ac.id> Thu, Jun 13, 2019 at 4:10 PM
To: INDIAN CHEMICAL SOCIETY <indi3478@dataone.in>, khun nay <khun.nay.win-2017@fst.unair.ac.id>, alfinda novi kristanti <alfinda-n-k@fst.unair.ac.id>

Dear Dr. Rahul Bhattacharya
Executive Officer
Indian Chemical Society

Greeting from Universitas Airlangga

I have check the manuscript that you send to me. There is a little correction from me. I also have send the payment via bank.

Attached file, I send to you :

- 1. The correction of manuscript.**
- 2. The receipt of the payment from the Bank.**

Hopefully we will get the good news from you for the next process.

If you need further information, please don't hesitate to tell me.

**With best regard,
Nanik**

[Quoted text hidden]

2 attachments

 **PROOF_REV NANIK_JUNE 13, 2019.pdf**
252K

 **IJCS_RECEIPT OF PAYMENT.pdf**
226K

INDIAN CHEMICAL SOCIETY <indi3478@dataone.in> Thu, Jun 13, 2019 at 4:42 PM
To: nanik siti aminah <nanik-s-a@fst.unair.ac.id>

Please send the Graphical abstract for the contents page of the journal.

[Quoted text hidden]

nanik siti aminah <nanik-s-a@fst.unair.ac.id> Thu, Jun 13, 2019 at 4:55 PM
To: khun nay <khun.nay.win-2017@fst.unair.ac.id>

Khun

Please prepare the graphical abstract to me and I will send it to IJCS.

Thank you.

----- Forwarded message -----
From: **INDIAN CHEMICAL SOCIETY** <indi3478@dataone.in>

Date: Thu, Jun 13, 2019 at 4:43 PM
Subject: RE: Proof (URGENT)
To: nanik siti aminah <nanik-s-a@fst.unair.ac.id>

[Quoted text hidden]
[Quoted text hidden]

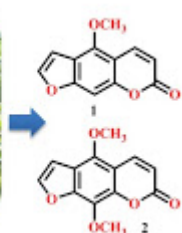
nanik siti aminah <nanik-s-a@fst.unair.ac.id>
To: INDIAN CHEMICAL SOCIETY <indi3478@dataone.in>

Thu, Jun 13, 2019 at 8:12 PM

Attached file, I send three types of graphical abstract


Thank you
[Quoted text hidden]

3 attachments



Graphical abstract for IJCS.jpg
47K

 **Graphical abstract for JICS.doc**
646K

 **Graphical Abstract for JICS.pptx**
213K

nanik siti aminah <nanik-s-a@fst.unair.ac.id>
To: INDIAN CHEMICAL SOCIETY <indi3478@dataone.in>

Thu, Jun 20, 2019 at 1:30 PM

Dear
Dr. Rahul Bhattacharya
Executive Officer
Indian Chemical Society

Good Day

Base on your email,

We already sent :

1. [The graphical abstract on](#) Jun 13, 2019, 8:12 PM (7 days ago)
2. [The receipt of the payment from the Bank on](#) Jun 13, 2019, 4:10 PM (7 days ago)

Would you like to give the update the progress of our manuscript.

If you still more data, please don't hesitate to tell me.

Thank you for your kindness help and cooperation.

Sincerely yours,
Nanik

On Fri, Jun 7, 2019 at 4:21 PM INDIAN CHEMICAL SOCIETY <indi3478@dataone.in> wrote:
[Quoted text hidden]

--
Dr. Nanik Siti Aminah