



SEKOLAH TINGGI ILMU FARMASI "YAYASAN PHARMASI SEMARANG"
LEMBAGA PENELITIAN DAN PENGABDIAN KEPADA MASYARAKAT
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SURAT TUGAS

No. 013/AFM-SW/LPPM/STP/II/2019

Yang bertanda tangan dibawah ini :

Nama : Ahmad Fuad Masduqi, S.Si, M.Si.
NIY : 030813015
Jabatan : Ketua LPPM STIFAR "YAYASAN PHARMASI SEMARANG"

Memberi tugas kepada :



No.	Nama	NIY	Jabatan
1.	Ebta Narasukma A, S.Farm., M.Sc., Apt	YP. 040509009	Dosen

Untuk melaksanakan publikasi artikel ilmiah di *Indonesian Journal Of Pharmacy*, pada :

Periode : 2019 - 2020
Tempat : Universitas Gajah Mada

Demikian surat tugas dibuat kepada yang bersangkutan untuk dapat dilaksanakan dengan penuh rasa tanggung jawab.

Semarang, 6 Februari 2019
Ketua LPPM STIFAR
"YAYASAN PHARMASI SEMARANG"



Ahmad Fuad Masduqi, S.Si, M.Si.
NIY. YP. 030813015

LOGIN

The screenshot shows the login page of the Indonesian Journal of Pharmacy. The browser address bar displays the URL: <https://indonesianjpharm.farmasi.ugm.ac.id/index.php/3/login>. The page features a green navigation bar with links: Home, About, Login, Search, Current, Archives, Announcements, Indexing & Abstracting, Journal History, and Contact. The main content area is titled "Login" and includes a form with the following fields: Username (containing "ebtanarasukma"), Password (masked with dots), and a checkbox for "Remember my username and password". A "Login" button is positioned below the password field. A link for "Forgot your password?" is also present. To the right of the login form is a vertical green sidebar with a list of links: Focus & Scope, Author Guideline, Author Fees, Online Submission, Editorial Board, Peer Reviewer, Subscription Form, Screening for Plagiarism, and Visitor Statistics. Below the login form, a section titled "Indonesian J Pharm indexed by:" lists various academic databases and their logos, including Scopus, DOAJ, Dimensions, Google, Microsoft Academic, ProQuest, Copernicus, OneSearch, ISJD, WorldCat, getCITED, P.S.O.A.R. Resources, and Virtual Library: Pharmacy. At the bottom right, there is a "CITATION ANALYSIS" button and a note about the journal's publication by the Faculty of Pharmacy at Universitas Gadjah Mada in collaboration with IAI. The Windows taskbar at the bottom shows the system tray with the date 09/03/2022 and time 16:13.

USER HOME

The screenshot shows the user home page of the Indonesian Journal of Pharmacy. The browser address bar displays the URL: <https://indonesianjpharm.farmasi.ugm.ac.id/index.php/3/user>. The page features a green navigation bar with links: Home, About, User Home, Search, Current, Archives, Announcements, Indexing & Abstracting, Journal History, and Contact. The main content area is titled "User Home" and includes a section for "Indonesian Journal of Pharmacy" with a link to "Author" and statistics: "1 Active" and "0 Archive". There is also a "[New Submission]" link. Below this is a "My Account" section with links for "Edit My Profile", "Change My Password", and "Logout". The "Indonesian J Pharm indexed by:" section and the "CITATION ANALYSIS" button are also visible. The Windows taskbar at the bottom shows the system tray with the date 09/03/2022 and time 16:15.

PROFILE

The screenshot shows the 'Edit Profile' page for a user named 'ebtanarasukma'. The page is part of the Indonesian Journal of Pharmacy website. The user's profile information is as follows:

Username	ebtanarasukma
Salutation	Mrs.
First Name *	Ebta
Middle Name	Narasukma
Last Name *	Anggraeny
Initials	ENA Joan Alice Smith = JAS
Gender	F
Affiliation	College of Pharmaceutical Sciences "Yayasan Farmasi Semarang"
Signature	Ebta NA

The page also features a navigation menu with options like Home, About, User Home, Search, Current, Archives, Announcements, Indexing & Abstracting, Journal History, and Contact. A sidebar on the right contains links for Focus & Scope, Author Guideline, Author Fees, Online Submission, Editorial Board, Peer Reviewer, Subscription Form, Screening for Plagiarism, and Visitor Statistics. A 'CITATION ANALYSIS' button is visible at the bottom right.

SUBMISSIONS

The screenshot shows the '#1570 Summary' page for a submission. The submission details are as follows:

Authors	Ebta Narasukma Anggraeny
Title	THE EFFECT OF AQUEOUS FRACTION OF <i>Clinacanthus nutans</i> (Burm.f.) EXTRACT MICROCAPSULES ON RAT BLOOD GLUCOSE LEVEL
Original file	1570-2901-1-SM.DOCX 2019-07-03
Supp. files	1570-2903-1-SP.PDF 2019-07-03 ADD A SUPPLEMENTARY FILE
Submitter	Mrs. Ebta Narasukma Anggraeny
Date submitted	July 3, 2019 - 03:42 PM
Section	Articles
Editor	Editorial MFI

The status of the submission is 'In Editing', initiated on 2020-12-17, and last modified on 2020-12-17. The page also includes a 'Submission Metadata' section. The navigation and sidebar elements are consistent with the profile page.

Authors
 Name: Ebta Narasukma Anggraeny
 Affiliation: College of Pharmaceutical Sciences "Yayasan Pharmasi Semarang"
 Country: Indonesia
 Bio Statement: —
 Principal contact for editorial correspondence.

Title and Abstract
 Title: THE EFFECT OF AQUEOUS FRACTION OF *Clinacanthus nutans* (Burm.f.) EXTRACT MICROCAPSULES ON RAT BLOOD GLUCOSE LEVEL
 Abstract: **ABSTRACT**
 Diabetes mellitus is a common metabolic disease marked by hyperglycemia. Diabetes also can be caused by oxidative stress. The administration of antioxidants, whether it's from food or supplement can inhibit the formation of free radicals. *Clinacanthus nutans* is reported to possess various medicinal properties including blood glucose lowering effect, alpha-glucosidase inhibition activity, antioxidant activities, anti-cancer properties and anti-inflammatory effects. Based on research *Clinacanthus nutans* leaf extracts fractions has several shortcomings, amongst them are its viscosity that gave difficulties in formulation and its astringent and bitter taste that made it hard to use orally. To overcome these shortcomings, microencapsulation can be used.
 The purpose of this research is to find the blood glucose level lowering effect of the aqueous fraction of microcapsules and to find the effective dose on fructose and fat-induced Wistar strain rat. The induction using fructose and high-fat feed for 55 days was applied. Rats were divided into 8 groups: normal control (aquades), negative control (Arabic gum and maltodextrin), positive control (metformin), dose I 15,89 mg/kgBW (microcapsule of aqueous fraction of *Clinacanthus nutans* extract), dose II 31,78 mg/kgBW, dose III 47,67 mg/kgBW, dose IV 63,56 mg/kgBW, and dose V 79,45 mg/kgBW.
 The result showed that the microcapsules of the aqueous fraction of *Clinacanthus nutans* (Burm.f) extract could lower Wistar strain rat blood glucose level in the dose of 15.89 mg/kg of the rat's body weight.

Indexing
 Keywords: *Clinacanthus nutans* (Burm.f.), diabetes mellitus, fructose, high-fat feed.
 Language: en

TEMPLATE
 Journal Template

TOOLS
 MENDELEY
 grammarly
 EndNote
 zotero

NOTIFICATIONS
 View (13 new)
 Manage

USER
 You are logged in as...
 ebtanarasukma
 My Profile

PROSES EMAIL SEJAK JULI 2019

Gmail
 Search: IJP

Penelusuran lanjutan

1-16 dari 16

From	Subject	Date
Puma Arfah 2	[IJP] Password Reset Confirmation - We have received a request to reset your password for the Open Journal Sys...	19/05/20
Editorial, saya 6	[IJP] Editor Decision - : [IJP] Editor Decision To: Mrs. Ebta Narasukma Anggraeny Dear Mrs. Ebta Narasukma Ang...	18/02/20
Editorial, saya 4	[IJP] Editor Decision - : [IJP] Editor Decision To: Mrs. Ebta Narasukma Anggraeny Dear Ms Ebta Narasukma Anggr...	18/02/20
saya, Yaning 3	tambahan bahasan - terakhir dr [IJP] >> nt klo sudah di inggirkan di merah2in aja di artikel, nt kita kirim >> ke ba...	18/02/20
Puma Arfah	[IJP] Password Reset - Your password has been successfully reset for use with the Open Journal Systems web sit...	23/08/19
Puma Arfah	[IJP] Password Reset Confirmation - We have received a request to reset your password for the Open Journal Sys...	23/08/19
Editorial, saya 3	[IJP] Editor Decision - : [IJP] Editor Decision To: Mrs. Ebta Narasukma Anggraeny Dear Mrs. Ebta Narasukma Ang...	21/08/19
Puma Arfah 6	[IJP] Password Reset Confirmation - We have received a request to reset your password for the Open Journal Sys...	31/07/19
Puma Arfah	[IJP] Password Reset - Your password has been successfully reset for use with the Open Journal Systems web sit...	31/07/19

10,84 GB dari 15 GB (72%) telah digunakan

Persyaratan - Privasi - Kebijakan Program

Aktivitas akun terakhir: 1 hari yang lalu

PROSES EDITOR REVIEW AND REVISIONS

24 SEPTEMBER 2019

LLDIKTI Wilayah VI x #1570 Summary x Hasil penelusuran - ebtasukma x [IJP] Editor Decision - sukma.a... x

https://mail.google.com/mail/u/1/#search/IJP/FMfMCGxwDrRSHQXVQtsRZQxVdqZJLbd

Gmail IJP

Tulis

Kotak Masuk 416

Berbintang

Ditunda

Terkirim

Draf 7

Selengkapnnya

Meet

Rapat baru

Gabung ke rapat

Hangout

Sukma +

Tidak ada chat terbaru
Mulai yang baru

[IJP] Editor Decision Kotak Masuk x

Editorial MFI <mfi@ugm.ac.id>
kepada saya

Sel, 24 Sep 2019 08.59

Inggris > Indonesia > Terjemahkan pesan Nonaktifkan untuk: Inggris x

Dear Mrs. Ebta Narasukma Anggraeny

We have reached a decision regarding your submission to Indonesian Journal of Pharmacy: "THE EFFECT OF AQUEOUS FRACTION OF *Clinacanthus nutans* (Burm.f.) EXTRACT MICROCAPSULES ON RAT BLOOD GLUCOSE LEVEL". Please revise according to the review comment below.

Title:
THE EFFECT OF AQUEOUS FRACTION OF *Clinacanthus nutans* (Burm.f.) EXTRACT MICROCAPSULES ON RAT BLOOD GLUCOSE LEVEL

Introduction:
Indonesia is on the 6th rank on the number of people with diabetes, with 10.3 million of patients. Diabetes can emerge from oxidative stress (U, K, & Ismail K. AO - Asmat, 2016). Oxidative stress happens occur when there is imbalance between oxidative system and antioxidants in the cells and tissues (ROS) (Pham-Huy, He, & Pham-Huy, 2008). The administration of antioxidants, whether it's from food or supplement can inhibit the formation of free radicals (Chikezie, Ojako, & Ogbuji, 2015).
C. nutans which is also called Sabah snake grass, having a place with plants of the group family of Acanthaceae (Kunson, Ruangrungrui, Lipipun, Khaiboon, & Rameshchirurat, 2013). *Clinacanthus nutans* is reported to

26°C Badai Petir 16:20 09/03/2022

9 OKTOBER 2019

LLDIKTI Wilayah VI x #1570 Summary x Hasil penelusuran - ebtasukma x [IJP] Editor Decision - sukma.a... x

https://mail.google.com/mail/u/1/#search/IJP/FMfMCGxwDrRSHQXVQtsRZQxVdqZJLbd

Gmail IJP

Tulis

Kotak Masuk 416

Berbintang

Ditunda

Terkirim

Draf 7

Selengkapnnya

Meet

Rapat baru

Gabung ke rapat

Hangout

Sukma +

Tidak ada chat terbaru
Mulai yang baru

[IJP] Editor Decision Kotak Masuk x

Editorial MFI <mfi@ugm.ac.id>
kepada saya

Rab, 9 Okt 2019 13.51

Inggris > Indonesia > Terjemahkan pesan Nonaktifkan untuk: Inggris x

Dear Ms Ebta Narasukma Anggraeny

If you have revised the text of the review results, please make the author respond according to the question from the reviewer and the answer from the author. to make it easier to proofreading

sincerely

INDONESIAN JOURNAL OF PHARMACY
Faculty of Pharmacy, Universitas Gadjah Mada
Skip Utara, 55281 Yogyakarta

Sukma Anggraeny <sukma.anggraeny@gmail.com>
kepada Editorial

Jum, 22 Nov 2019 12.39

Dear Editors,

We have sent the reply on the required revision via the web at October 10th, 2019. And the required revision have been sent via the web at October 1st. But Until now we haven't received any reply about the revision. We would like to inquire about the information on our submission, whether we need to submit more revisions.

Thank you for your attention and your help

26°C Badai Petir 16:21 09/03/2022

22 NOVEMBER 2019, 14 JANUARI 2020

The screenshot shows a Gmail inbox with three emails from Sukma Anggraeny. The first email, dated 22 Nov 2019 12.39, is addressed to 'kepada Editorial' and contains the text: 'Dear Editors, We have sent the reply on the required revision via the web at October 10th, 2019. And the required revision have been sent via the web ath October 1st. But Until now we haven't received any reply about the revision. We would like to inquire about the information on our submission, whether we need to submit more revisions. Thank you for your attention and your help. Sincerely, Ebta Narasukma.' The second email, dated 14 Jan 2020 14.55, is also to 'kepada Editorial' and says: 'Dear editors, From our correspondence so far, our latest correspondence was in November 2019. We would like to clarify whether we need to add more revisions for the paper we submit. If there are more revisions required, we would be happy to provide the revisions. Sincerely, Ebta NA.' The third email, dated 18 Feb 2020 10.06, is to 'kepada btmt.pharma'.

12 FEBRUARI 2020

The screenshot shows a Gmail inbox with one email from Editorial MFI, dated Rab, 12 Feb 2020 14.40, addressed to 'kepada saya'. The subject is '[IJP] Editor Decision'. The email body reads: 'Mrs. Ebta Narasukma Anggraeny, We have reached a decision regarding your submission to Indonesian Journal of Pharmacy: "THE EFFECT OF AQUEOUS FRACTION OF Cinacanthus nutans (Burm.f.) EXTRACT MICROCAPSULES ON RAT BLOOD GLUCOSE LEVEL." After we read your revision, your manuscript still needs to improve with some suggestion: 1. How did you prepare the aqueous fraction? 2. The objectives of this study are not clearly defined, what the relation between microcapsule (formulation), antioxidant and glucose level? 3. The English need to major improvement, we suggest that your manuscript sends to English expert.' The sender is Editorial MFI, Faculty of Pharmacy, Universitas Gadjah Mada, mfi@ugm.ac.id. The footer of the email identifies the journal as the Indonesian Journal of Pharmacy, Faculty of Pharmacy, Universitas Gadjah Mada, Skip Utara, 55281 Yogyakarta.

18 FEBRUARI 2020 DAN 28 FEBRUARI 2020

The screenshot shows a Gmail inbox on a Windows 10 desktop. The browser address bar displays the URL: <https://mail.google.com/mail/u/1/#search/IJP/FMfcgwGDNNldMqgHdThvkbQrFLISplc>. The search bar contains the text "IJP". The inbox list on the left shows 416 messages in the "Kotak Masuk" folder. The main view displays an email from Sukma Anggraeny (sukma.anggraeny@gmail.com) to IJP Editors, dated Wednesday, February 12, 2020, at 14:50. The email content reads: "Dear IJP Editors, Here by we attach the latest revision of our paper according to IJP's e-mail on February 12th. We have added data as requested. And we have posted paper in the IJP website on Februari 28. We hope this revision is acceptable. Best regards,". The Windows taskbar at the bottom shows the system tray with a temperature of 26°C and the date 09/03/2022.

15 JULI 2020

The screenshot shows a Gmail inbox on a Windows 10 desktop. The browser address bar displays the URL: <https://mail.google.com/mail/u/1/#search/IJP/FMfcgwGDNNldMqgHdThvkbQrFLISplc>. The search bar contains the text "IJP". The inbox list on the left shows 416 messages in the "Kotak Masuk" folder. The main view displays a reply email from Sukma Anggraeny (sukma.anggraeny@gmail.com) to IJP Editors, dated Wednesday, July 15, 2020, at 14:41. The email content reads: "Dear IJP Editors, Here by we attach the latest revision of our paper according to IJP's e-mail on February 12th. We have added data as requested. And we have posted paper in the IJP website on Februari 28. We hope this revision is acceptable. Best regards,". Below the text is a PDF attachment titled "1570-3026-1-ED F...". At the bottom of the email content, there are buttons for "Balas" and "Teruskan". The Windows taskbar at the bottom shows the system tray with a temperature of 26°C and the date 09/03/2022.

19 AGUSTUS 2020

LLDIKTI Wilayah VI x #1570 Summary x Hasil penelusuran - ebtasukma x AUTHOR RESPON - EBTA NARASUKMA x

https://mail.google.com/mail/u/1/#search/IJP/QgrCjHSNjpkptfcDvrRZNGfChGZQtqTZV

Gmail IJP

Tulis

Kotak Masuk 416

Berbintang

Ditunda

Terkirim

Draf 7

Selengkapnya

Meet

Rapat baru

Gabung ke rapat

Hangout

Sukma +

Tidak ada chat terbaru
Mulai yang baru

4 dari 16

AUTHOR RESPON - EBTA NARASUKMA ANGGRAENY

Sukma Anggraeny <sukma.anggraeny@gmail.com>
kepada Editorial

Rab, 19 Agu 2020 13.23

Dear, Editors MFI

heres our author response in the form of tables to answer your questions.
Also hereby attached the manuscript which English has been improved. We hope this answer is acceptable.

**"THE EFFECT OF AQUEOUS FRACTION OF *Clinacanthus nutans* (Burm.f.)
EXTRACT MICROCAPSULES ON RAT BLOOD GLUCOSE LEVEL".**

Question	Answer
1. How did you prepare the aqueous fraction?	Microcapsules were made from 8 grams of aqueous fraction of <i>C. nutans</i> extract. To obtain the aqueous fraction, 200 g of <i>C. nutans</i> leaves powder were macerated with 2 liters of ethanol 96% in 5 days. Then the aqueous extract was separated from ethanol solvent using a rotary vacuum evaporator at 40°C. The extract was fractionated in stages with the liquid-liquid partition method using a separating funnel. The fractionation process was carried out by dissolving 10 g of concentrated ethanol extract with 100 mL of distilled water and stirring the mixture until all extracts were dissolved. Then, the mixture was put into a separating funnel with a capacity of 250 mL and fractionated using 100 mL of n-hexane and then the mixture was shuffled. Two phases of mixture was

Type here to search

26°C Badai Petir 16:28 09/03/2022

29 AGUSTUS 2020

LLDIKTI Wilayah VI x #1570 Summary x Hasil penelusuran - ebtasukma x AUTHOR RESPON - EBTA NARASUKMA x

https://mail.google.com/mail/u/1/#search/IJP/QgrCjHSNjpkptfcDvrRZNGfChGZQtqTZV

Gmail IJP

Tulis

Kotak Masuk 416

Berbintang

Ditunda

Terkirim

Draf 7

Selengkapnya

Meet

Rapat baru

Gabung ke rapat

Hangout

Sukma +

Tidak ada chat terbaru
Mulai yang baru

4 dari 16

AUTHOR RESPON - EBTA NARASUKMA ANGGRAENY

Sukma Anggraeny <sukma.anggraeny@gmail.com>
kepada Editorial

Sab, 29 Agu 2020 16.24

Dear, Editors Journal Indonesian Of Pharmacy

I send my manuscript improvement. The revisions in highlight color.
I hope the manuscript is acceptable.

Sincerely,
Ebta Narasukma A

1570-3026-1-ED F...

Type here to search

26°C Badai Petir 16:28 09/03/2022

17 DESEMBER 2020

The screenshot shows a Gmail interface with the search bar containing "IJP". The left sidebar shows folders like "Kotak Masuk" (416), "Berbintang", "Ditunda", "Terkirim" (7), "Draf", and "Selengkapny". The main content area displays an email from "Editorial MFI" (mfi@ugm.ac.id) dated "Kam, 17 Des 2020 09:51". The email subject is "[IJP] Editor Decision". The body text reads: "We have reached a decision regarding your submission to Indonesian Journal of Pharmacy, 'THE EFFECT OF AQUEOUS FRACTION OF Clinacanthus nutans (Burm.f.) EXTRACT MICROCAPSULES ON RAT BLOOD GLUCOSE LEVEL.' We are pleased to confirm that your manuscript entitled Indonesian Journal of Pharmacy, 'THE EFFECT OF AQUEOUS FRACTION OF Clinacanthus nutans (Burm.f.) EXTRACT MICROCAPSULES ON RAT BLOOD GLUCOSE LEVEL,' has been accepted for publication in Indonesian journal of Pharmacy. However, please make payment to MANDIRI UGM Branch (Account holder: UGM.FA.KAF PENERIMAAN LANGGANAN JURNAL No. VA: 8888803014100003) before we could send you the official letter of acceptance. Thank you very much for submitting your manuscript to the Indonesian Journal of Pharmacy. Sincerely, Editorial MFI, Faculty of Pharmacy, Universitas Gadjah Mada, mfi@ugm.ac.id".

20 JANUARI 2021

The screenshot shows a Gmail interface with the search bar containing "IJP". The left sidebar shows folders like "Kotak Masuk" (416), "Berbintang", "Ditunda", "Terkirim", "Draf", and "Selengkapny". The main content area displays an email from "Sukma Anggraeny" (sukma.anggraeny@gmail.com) dated "Rab, 20 Jan 2021 12:11". The email subject is "INVOICE Ebita Nara...". The body text reads: "Dear, Editorial Thank you very much for accepted my manuscript in Indonesian Journal Of Pharmacy Here i send the author's name. Sincerely, Ebita Narasukma". There are two attachments: "INVOICE Ebita Nara..." and "Title Page.docx".

"THE EFFECT OF AQUEOUS FRACTION OF *Clinacanthus nutans* (Burm.f.)
EXTRACT MICROCAPSULES ON RAT BLOOD GLUCOSE LEVEL".

Question	Answer
<p>1. How did you prepare the aqueous fraction?</p>	<p>Microcapsules were made from 8 grams of aqueous fraction of <i>C. nutans</i> extract. To obtain the aqueous fraction, 200 g of <i>C.nutans</i> leaves powder were macerated with 2 liters of ethanol 96% in 5 days. Then the aqueous extract was separated from ethanol solvent using a rotary vacuum evaporator at 40°C. The extract was fractionated in stages with the liquid-liquid partition method using a separating funnel. The fractionation process was carried out by dissolving 10 g of concentrated ethanol extract with 100 mL of distilled water and stirring the mixture until all extracts were dissolved. Then, the mixture was put into a separating funnel with a capacity of 250 mL and fractionated using 100 mL of n-hexane and then the mixture was shuffled. Two phases of mixture was produced and the aqueous layer was taken out. This process was repeated for 3 times. The aqueous residue obtained was then fractionated further with similar process using 100 mL ethyl acetate. The mixture then was shaken and left to form two layers, namely the ethyl acetate fraction and the aqueous fraction. The two layers then were separated while, the n-hexane fraction, ethyl acetate fraction, and the water fraction obtained were each evaporated on waterbath at temperature of 80°C.</p> <p>Maltodextrin and Arabic gum were weighed according to the ratio, and then were mixed until they were homogenously dissolved. Then aqueous fraction of <i>C. nutans</i> extract was added, and then distilled water was added to the volume of 200 ml. The mixture was put into the freezer for 24 hours, and then the frozen mixture was freeze-dried in the temperature of -100°C and then the mixture was allowed to stand for 72 hours. The resulting microcapsules were sieved with no. 24 mesh sieve to form micro-sized microcapsules.</p>
<p>2. The objectives of this study are not clearly defined, what the relation between microcapsule (formulation),</p>	<p>This research was a follow up of previous research by Retraningsih, aqueous fraction of <i>C.nutans</i> leaves rat blood glucose</p>

antioxidant and glucose level?	<p>lowering activity in the level of 15.89 mg/kg BB (Retnaningsih et al., 2019).</p> <p>The previous study for the aqueous fraction showed that the aqueous fraction reduced 41.39% of blood glucose level (Retnaningsih et al., 2019). The microcapsules from the same aqueous fraction was able to reduce 59.06% of blood glucose level. Then, it can be concluded that microencapsulation improved the efficacy of aqueous fraction of <i>C. nutans leaves</i>.</p>
3. The English need to major improvement, we suggest that your manuscript sends to English expert.	File attached with the name of 1570-3026-1-ED Feb 28 2020 IJP

THE EFFECT OF AQUEOUS FRACTION OF *Clinacanthus nutans* (Burm.f.) EXTRACT MICROCAPSULES ON RAT BLOOD GLUCOSE LEVEL

ABSTRACT

Diabetes mellitus is a common metabolic disease marked by hyperglycemia which is also caused by oxidative stress. The administration of antioxidants, whether it's from food or supplements can inhibit the formation of free radicals. *Clinacanthus nutans* has been reported to possess various medicinal advantages such as blood glucose lowering effect, alpha-glucosidase inhibition activity, antioxidant activities, anti-cancer properties, and anti-inflammatory effects. However, *Clinacanthus nutans* leaf extracts fractions has several limitations, i.e. highly viscous that may constraint its pharmaceutical formulation as well as the astringent and bitter taste which diminish its oral-based formulation. To solve so, microencapsulation strategy during formulation can be used here in.

The purpose of this research is to find the blood glucose level lowering effect of the aqueous fraction of microcapsules and to find the effective dose on fructose and fat-induced Wistar strain rat. The induction was done using fructose and high-fat feed for 55 days. Rats were divided into 8 groups: normal control (aquadest), negative control (Arabic gum and maltodextrin), positive control (metformin), dose I (15.89mg/kg BW microcapsule of aqueous fraction of *Clinacanthus nutans* extract), dose II (31.78mg/kg BW), dose III (47.67mg/kg BW), dose IV (63.56 mg/kg BW), and dose V (79.45 mg/kg BW).

The result showed that the microcapsules of the aqueous fraction of *Clinacanthus nutans* (Burm.f) extract could lower Wistar strain rat blood glucose level in the dose of 15.89 mg/kg of the rat's body

weight.

Keyword: Clinacanthus nutans (Burm.f), diabetes mellitus, fructose, high-fat feed.

INTRODUCTION

Indonesia is on the 6th rank on the number of people with diabetes, with 10.3 millions of patients. Diabetes can be induced due to oxidative stress (Asmat et al., 2016). Oxidative stress occurs when there is imbalance between oxidative system and antioxidants in the cells and tissues (ROS) (Pham-Huy et al., 2008). Antioxidants can be obtained either from food or supplements in order to inhibit the formation of free radicals (Chikezie et al., 2015).

Clinacanthus nutans which is also called *Sabah snake grass*, is from family of *Acanthaceae* (Kunsorn et al., 2013). *C.nutans* is reported to have various medicinal properties, including blood glucose lowering effect, alpha-glucosidase inhibition, antioxidant activities, anti-cancer properties, and anti-inflammatory (Sakdarat et al., 2009).

The aqueous extract of *C.nutans* leaves had antioxidant activity of 532.24 µg/mL (Nugraheni et al., 2017). The usage of *C.nutans* leaf extracts fractions has several problems, amongst them is its viscosity that gives difficulties in formulation and its astringent and bitter taste that makes it hard to be delivered orally. Herein, in order to solve these problems microencapsulation can be used (Munin & Edwards-Lévy, 2011).

Microencapsulation is a microscopic encapsulation process of

drug particles with special coatings that improves physical and chemical properties of these particles (Cahyanii et al., 2018). Optimization of maltodextrin and Arabic gum on the microencapsulation of aqueous fraction of *C. nutans* leaf extracts is carried out using simplex lattice design. The microcapsules can be produced using the ratio of 0.804 part maltodextrin and 0.194 part Arabic gum (0.804:0.194). The resulting microcapsules produced had 1.49% moisture level of 1.49%, the solubility of 97.47%, and the antioxidant activity was on of 842.499 ppm (Cahyani et al., 2018). Therefore, based on the previous research, the aim of this study is to find the hypoglycemic effect of the microcapsules of aqueous fraction of *C.nutans* leaf extract.

MATERIALS AND METHODS

Materials

The sample used in this research is in the form of microcapsules form, which was made from the aqueous fraction of *C. nutans* leaves. The plant material of *C. nutans* leaves has been collected from Temu Kencono, Semarang. The test animals used were male Wistar strain rats aged 2–3 months, weighed 150–250 grams. Other materials used were fructose with the dose of 1.8 g/kg BW of mice body weight, HFF (combination of lard and duck egg yolk with the ratio of (3:1)), metformin as a positive control with the dose of 0.126 g/kg, glibenclamide with the dose of 0.63

mg/kg, and glucose with the dose of 6.75 g/kg.

Methods

a. Sample preparation

Microcapsules were made from 8 grams of aqueous fraction of *C. nutans* extract. To obtain the aqueous fraction, 200 g of *C. nutans* leaves powder were macerated with 2 liters of ethanol 96% in 5 days. Then the aqueous extract was separated from ethanol solvent using a rotary vacuum evaporator at 40°C. The extract was fractionated in stages with the liquid-liquid partition method using a separating funnel. The fractionation process was carried out by dissolving 10 g of concentrated ethanol extract with 100 mL of distilled water and stirring the mixture until all extracts were dissolved. Then, the mixture was put into a separating funnel with a capacity of 250 mL and fractionated using 100 mL of n-hexane and then the mixture was shuffled. Two phases of mixture was produced and the aqueous layer was taken out. This process was repeated for 3 times. The aqueous residue obtained was then fractionated further with similar process using 100 mL ethyl acetate. The mixture then was shaken and left to form two layers, namely the ethyl acetate fraction and the aqueous fraction. The two layers then were separated while, the n-hexane fraction, ethyl acetate fraction, and the water fraction obtained were each evaporated on waterbath at temperature of 80°C.

Maltodextrin and Arabic gum were weighed according to the ratio, and then were mixed until they were homogeneously dissolved. Then aqueous fraction of *C. nutans* extract was added, and then distilled water

was added to the volume of 200 ml. The mixture was put into the freezer for 24 hours, and then the frozen mixture was freeze-dried in the temperature of -100°C and then the mixture was allowed to stand for 72 hours. The resulting microcapsules were sieved with no. 24 mesh sieve to form micro-sized microcapsules.

b. Test Animal Treatment

Ten rats were not induced and 35 rats were induced with fructose and HFF for 55 days. Insulin resistance test was carried out on the 54th day (the day before induction ended). After animal group test was done for insulin resistant, the 35 induced rats were further divided into seven groups, where each group consists of 5 rats. The groups received treatments according to the methods described below:

Group I (negative control)

Induced and administered with suspension of Arabic gum and maltodextrin orally from 56th to 70th day.

Group II (positive control)

Induced and administered with metformin orally with a dose of 0.126 g/kg from 56th to 70th day.

Group III, IV and V, VI, VII

Induced and administered with microcapsule of aqueous fraction of *C. nutans* extract orally with the dose of 15.89; 31.78; 47.67; 63.56; and 79.45 mg/kg from 56th to 70th day.

c. Evaluation of insulin resistance

Ten animals from the sample groups were induced. The test animals induced were divided into these two following groups:

- a) Group 3 = test animals induced without glibenclamide administration (n = 5)
- b) Group 4 = test animals induced with glibenclamide 0.63 mg/Kg BW administration (n = 5)

The test animals without induction (normal feed) were also divided into two groups.

- c) Group 1 = normal test animals without glibenclamide administration (n = 5)
- d) Group 2 = normal test animals with glibenclamide 0.63 mg/kg BW) administration (n = 5)

On the 54th day, the test animals were fasted for 8-10 hours and later their blood was taken, and then their blood glucose level were measured. Insulin resistance test **was** carried out by administering glucose with the dose of 6.75 g/kg orally and 30 minutes later the test animals were given glibenclamide. The test animals were given glibenclamide after 30 minutes treatment with glucose with the dose of 6.75 g/kg orally. Two hours later, the blood of test animals were taken and the blood sugar glucose level was measured.

d. Blood glucose measurement

The blood of the test animals was taken through the ocular vein. The rat blood serum was obtained by centrifuging the rat blood at 3000 rpm for 10 minutes, then 1000 µl glucose PAP reagent was added to 10 µl of the serum and the resulting mixture was measured using Microlab 300. Blood glucose level was read in the unit of mg/dl.

e. Ethical clearance

Prior to the start of research, the proposed method was submitted to Komisi Etik Fakultas Kesehatan Masyarakat Universitas Diponegoro (FKM UNDIP). The certificate of ethical clearance was numbered No.023/ BC/FKM/2018.

RESULTS AND DISCUSSION

This research was a follow up of previous research by Retraningsih, aqueous fraction of *C.nutans* leaves rat blood glucose lowering activity in the level of 15.89 mg/kg BB (Retnaningsih et al., 2019). This study was a continuation of previous studies, which showed that aqueous fraction of ethanolic extracts of *C.nutans* leaves in the dose of 15.89 mg/kg was effective in reducing blood glucose levels in rats induced by fructose and high-fat feed. The microcapsule dose was taken from the effective dose of aqueous fraction of *C.nutans* leaves. The aqueous fraction dose was then converted to microcapsule dose. The microcapsule dose obtained was 85.2072 g, equal to 8 grams of aqueous fraction. The aqueous fraction was selected for the microcapsules preparation because the aqueous fraction was found to have the highest antioxidant activity among the extracts and fractions (Retnaningsih et al., 2019)

At the beginning of this study, the **tested** animals **s** were orally induced to be hyperglycemic with fructose and HFF high-fat feed. The feeding of high-fat feed was expected to increase glucose level and free fatty acids in the cell plasma **that will** decrease the capability of the insulin receptor substrate in activating P13-kinase causing the decrease**s** of the expression of GLUT4 and the activity of glucose transport, **along with** the blood glucose level increases

(Shulman, 2000). In the liver, fructose is metabolized into free fatty acids that will be delivered in blood disrupting the metabolism and transport of glucose (Sun & Empie, 2012).

The result of insulin resistance evaluation showed that in the induced group, the group that received glibenclamide treatment still showed high blood glucose level. From this result, it can be concluded that the fructose and HFF can induce insulin resistance. The result of insulin resistance evaluation can be seen in Table 1.

Table 1. Insulin resistance test results with glibenclamide hypoglycemic effect test

Group	Blood Glucose Level (mg/dL) Day 54	
	Without Glibenclamide (n=5)	With Glibenclamide (n=5)
Normal	147,8 ± 3,56	123,60 ± 9,10
Induced	328,6 ± 38,61	240 ± 31,83

This result showed that even with the insulin production stimulating effect of glibenclamide, the blood glucose level in the induced test animal was in the range of diabetic blood glucose level. Thus, it can be concluded that the fructose and HFF was capable of inducing insulin resistance. In the normal group, there was no any blood glucose level decrease observed because of the bodies' homeostasis mechanism in regulating blood glucose level. Figure 1 shows the hypoglycemic effect of glibenclamide in the normal group and in the induced group.

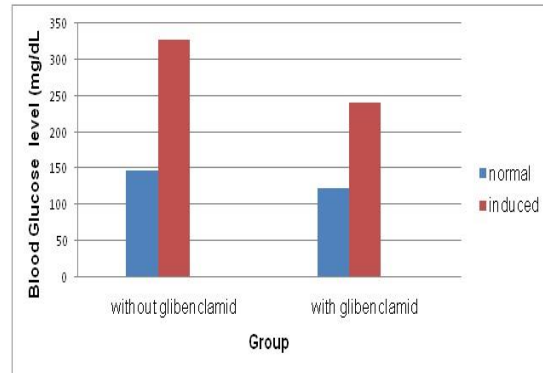


Figure 1. Hypoglycemic Effect of Glibenclamide on Normal Group and Induced Group

The rats' blood glucose level was measured in the 56th day, after the induction ended. From the result, paired-samples t-test showed $p < 0.05$, which means there was significant difference. This result showed the success of the induction process.

The test animals then were treated with the microcapsules of aqueous fraction *C.nutans* leaf extract for 14 days. In the 70th day, the blood glucose level of all groups was measured to observe the blood glucose level decreasing effect of microcapsules of aqueous fraction of *C.nutans* leaf extract. The blood glucose level measurement result in the average number ± SD can be seen in Table 2 and Figure 2.

Table 2. Average Blood Glucose Levels of All Test Animal Groups and Percentage of Decrease in Blood Glucose Levels

Group	Blood Glucose Level (mg/dL)			Decrease (%) Day 56-day 70	Differences with negative control
	Day 1	Day 56	Day 70		
Normal	119.2 ± 26.0	121.6 ± 18.8	120.6 ± 18.1	1.26 ^b	-149.0
Negative control: gum + maltodextrin	133.2 ± 56.1	266.8 ± 31.7	269.6 ± 32.4	-1.05 ^b	0
Positive Control: metformin	115 ± 14.7	302.4 ± 31.6	163.72 ± 11.8	61.96 ^a	154.6
Microcapsule dose I (15.89 mg/Kg BB)	96 ± 16.4	325.6 ± 57.5	228.35 ± 38.1	59.06 ^a	140.4
Microcapsule dose II (31.78 mg/Kg BB)	107 ± 16.8	276.8 ± 52.5	228.76 ± 54.3	60.75 ^a	163.4
Microcapsule dose III (47.67 mg/kg BB)	104 ± 15.0	281.8 ± 58.5	257.81 ± 101.53	61.79 ^a	164.4
Microcapsule dose IV (63.56 mg/kg BB)	117.6 ± 8.85	288.8 ± 40.0	199.67 ± 31.8	62.11 ^a	162.8
Microcapsule dose V (79.45 mg/kg BB)	96.8 ± 26.0	266.8 ± 28.1	233.69 ± 67.88	63.57 ^a	174.4

^aDifferent letters show significant difference (p<0.05) with the normal group

^bDifferent letters show significant difference with control positive group

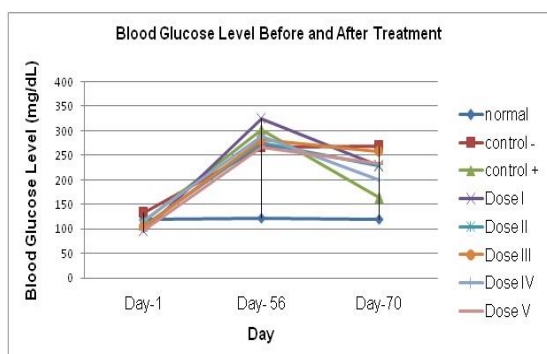


Figure 2. Average blood glucose level before and after treatment

The decrease of blood glucose levels percentage were examined using one-way ANOVA. The result obtained showed the p value of 0.000. This p value, which is less than 0.05, indicates that there is significant difference between the treatment groups. The next test was the post-hoc test. The post-hoc test showed the percentage of blood glucose level decrease of the normal group which was showed by significant difference from the decrease of blood glucose level percentage in the negative group. From this result, it showed that maltodextrin and Arabic gum did not affect the rats' blood glucose level. The positive control group showed significant difference from the normal group, which means the research method is correct, since metformin is an antidiabetic agent which has already been proven in decreasing blood glucose level. The positive control group did not show significant difference with the treatment group with the doses 15.89 mg/kg, 31.78 mg/kg, 47.67 mg/kg, 63.56 mg/kg, and 79.45 mg/kg BW. This result means that the microcapsules of aqueous fractions of *C.nutans* leaf extract was proven to be capable of decreasing rats' blood glucose level and has the comparable effectivity with metformin.

The use of metformin was based on the latest American Diabetes Association/European Association for the Study of Diabetes (ADA/EASD) management guidelines for diabetes mellitus and the Association of Clinical Endocrinologists/American College of Endocrinology (AAACE/ACE) recommendation. The AAACE recommends metformin as the first line monotherapy. The mechanism of metformin is by inhibiting glucose

production in the liver, decreasing the absorption of glucose in the digestive tract, and increasing insulin sensitivity (Abrori et al., 2017).

The previous study for the aqueous fraction showed that the aqueous fraction reduced 41.39% of blood glucose level (Retnaningsih et al., 2019). The microcapsules from the same aqueous fraction was able to reduce 59.06% of blood glucose level. Then, it can be concluded that microencapsulation improved the efficacy of aqueous fraction of *C. nutans* leaves.

The capability of the microcapsules in decreasing blood glucose level is related to the flavonoid content in *C. nutans* leaves. In Anggraeny's previous research, it was stated that the aqueous fraction of the *C. nutans* leaves contained flavonoid from the group S. The flavonoid compound found in *C. nutans* was 5,6,7,4',5' pentahydroxy flavone (Anggraeny et al., 2017).

The flavonoids contained in *C. nutans* have antioxidant and antidiabetic activity. The mechanism as antioxidants is by the flavonoids' capability of suppressing the apoptosis of β Langerhans cells without altering the proliferations of those cells (Ruhe & McDonald, 2001). The *C. nutans* flavonoids mechanism as antidiabetic is by acting as glucosidase inhibitor. The α -glucosidase inhibitors delay absorption of carbohydrates obtained from food, which can reduce blood glucose levels after eating (Ross et al., 2004).

CONCLUSION

From the result of this study, it can be concluded that

1. The treatment of microcapsules of aqueous fraction of *C. nutans* leaf extract to male Wistar strain rats was proven to decrease blood glucose level significantly.
2. The effective dose of microcapsules of aqueous fraction of *C. nutans* leaf extract was found to be 15.89 mg/kg BW because in small dose it was able to give comparable effect to metformin.

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