LAPORAN AKHIR

PENELITIAN ILMU KEDOKTERAN

Analisa Kandungan Senyawa Kimia dan Uji Aktivitas Antikanker ekstrak Teripang pada Sel Kanker Serviks HeLa : Studi *In Vitro*

Oleh;
Dr. dr. Irena Ujianti, M. Biomed (0310108104)
dr. Bety Semara Lakshmi, MKM (8876999974)
Assyu'ara Al'asyi (2110015044)
Anik Khumairoh (2110015022)
Putri Salsabila Ediati (2110015048)

FAKULTAS KEDOKTERAN,
PENDIDIKAN DOKTER
UNIVERSITAS MUHAMMADIYAH PROF DR HAMKA
JAKARTA

2023

UNIVERSITAS MUHAMMADIYAH PROF. DR. HAMK
LEMBAGA PENELITIAN DAN PENGEMBANGA
Jl. Warung Buncit raya No. 17 Pancoran Jakarta Selatan
Lantai 6 Gedung Sekolah Pascasarjana

Nomor : 81/B.01.04/2023 — 23 Ramadha
Lamp. : satu berkas — 14 April
Perihal : Pencairan Tahap 1 Hibah Internal Simakip Batch 2 Tahun 2022/2023

Yang Terhormat,
Dr. dr. Wawang S Sukarya, Sp. OG (K), MARS, MH. Kes
Dekan Fakultas Kedokteran
Universitas Muhammadiyah Prof. DR. HAMKA

Assalamu'alaikum warahmatullahi wabarakatuh.

Ba'da salam semoga Bapak/Ibu senantiasa dalam keadaan sehat walafiat d dalam melaksanakan aktivitas sehari-hari. "Aamiin".

Berdasarkan hasil Reviu proposal dan surat pemberiaan Dana Hibah dari Fakultas/Sekolah Pascasarjana, bersama ini Kami sampaikan Daftar Pener Internal Simakip Batch 2 tahun 2022/2023. (daftar terlampir)
Ada beberapa catatan terkait administrasi yang masih belum diselesaikan d pengusul mohon menjadi perhatian pimpinan dalam pencairan dana peneli 1 (70%). Catatan tersebut sebagai berikut:

1. Dosen yang disetujuai oleh Fakultas/SPs tidak mengirimk proposal pada simakip (Warna Kuning)
2. Dosen Pengusul belum menyerahkan SPK ke Lemlitbang (Warna

Demikian kami sampaikan, atas perhatian Bapak/Ibu diucapkan terima kasih

Wabillahittaufiq wal hidayah.
Wassalamu'alaikum warahatullahi wabarakatuh.

K e t u a,

Dr. apt. Supandi, M. Si.

Tembusan :
1. Rektor
2. Wakil Rektor 1
3. Arsip

LAPORAN PENELITIAN

UNIVERSITAS MUHAMMADIYAH PROF DR. HAMKA Tahun 2023

Judul : **Analisa Kandungan Senyawa Kimia dan Uji Aktivitas Antikanker ekstrak H.scabra pada Sel Kanker Serviks HeLa : Studi *In Vitro***

Ketua Peneliti : Dr. dr. Irena Ujianti, M.Biomed
Skema Hibah :
Fakultas : Kedokteran
Program Studi : Pendidikan Dokter

Luaran Wajib

No	Judul	Nama Jurnal/ Penerbit/Prosiding	Level SCIMAGO /SINTA	Progress Luaran
1	**Network Pharmacology Analysis Reveals Bioactive Compounds and Potential Targets of Sea cucumber for Cervical Cancer Therapy**	F1000	Terindeks Scopus Q1	Review

Luaran Tambahan

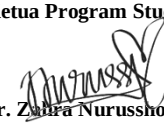
No	Judul	Nama Jurnal/ Penerbit/Prosiding	Level SINTA/SCIMAGO	Progress Luaran
1	Sea Cucumber (Schistopus hermanii) Compounds Targeting EGFR, PTGS2,	**ICNSSE 3**	Internasional conference	submit

	NF-?B in Cervical Cancer Treatment; In Silico Study			

.

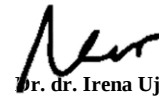
Mengetahui,

Ketua Program Studi



dr. Zahra Nurusshofa, Sp.PA.
NIDN. 0307028704

Ketua Peneliti



Dr. dr. Irena Ujianti, M.Biomed
NIDN.0310108104.

Menyetujui,

Dekan Fakultas Kedokteran

Dr. dr. Wawang S Sukarya, Sp.OG(K), MARS, MH.Kes.
NIDN.0030064701

Ketua Lemlitbang UHAMKA

Dr. apt. Supandi, M.Si

NIDN. 0319067801

LAPORAN AKHIR

Analisa Kandungan Senyawa Kimia dan Uji Aktivitas Antikanker ekstrak H.scabra pada Sel Kanker Serviks HeLa : Studi *In Vitro*

Latar Belakang (Background)

Kanker merupakan penyakit yang insidensinya semakin meningkat dari tahun ke tahun. Kanker serviks merupakan penyebab kematian ketiga akibat kanker di Amerika Serikat (National Cancer Institute, 2010). Pengobatan kanker menggunakan kemoterapi memberikan banyak efek samping, terutama pada sel normal. Efikasi agen kemoterapi juga diturunkan dengan adanya resistensi sel kanker (multi drug resistence mechanism). Untuk itu dikembangkan penelitian tentang penggunaan senyawa yang berasal dari alam sebagai agen kemoprevensi yang berpotensi sebagai agen pendamping kemoterapi.2 Agen kemoprevensi dimaksudkan untuk meningkatkan sensitivitas sel kanker dan mengurangi efek samping akibat agen kemoterapi. Agen kemoprevensi umumnya memiliki aktivitas menghambat pertumbuhan tumor melalui mekanisme cell cycle arrest, pemacuan apoptosis ataupun menghambat ekspresi protein yang berperan dalam Multi Drug Resistance.3
Salah satu bahan alam yang dapat digunakan sebagai agen antikanker serviks adalah holothuria scabra dipercaya mengandung tiga senyawa penting yang berperan dalam pengatasan kanker, yakni saponin, Echinoside A, Holothurin yang mampu menghambat perkembangan sel kanker.4 Saponin memiliki efek antitoksik, dalam hal ini sebagai antikanker dan juga sebagai antibiotik alami sehingga mampu menjaga organ tubuh yang belum terserang kanker untuk mencegah kanker.5 Agen antimetastatik mencegah penyebaran sel kanker, berfungsi sebagai pemutus hubungan pembuluh darah dan nutrisbi ke sel kanker atau tumor dan menyebabkan jaringan kanker akan kering kemudian mati juga sebagai anti apoptosis.6
Dalam penelitian ini akan diuji apakah ekstrak S. hermani mempunyai aktivitas sitotoksik terhadap sel kanker Serviks HeLa.7 HeLa merupakan sel kanker serviks yang diambil dari pasien kanker wanita berusia 76 tahun dan dikembangkan menjadi sel uji. Pengujian yang akan dilakukan adalah identifikasi kandungan senyawa kimia dan uji sitotoksik dari ekstrak tersebut pada sel kanker serviks

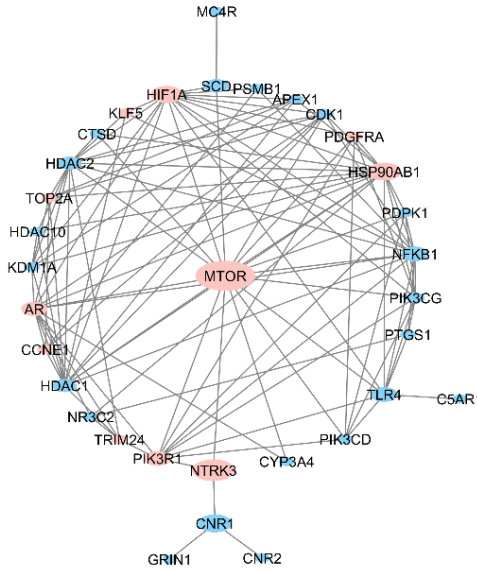
Tujuan Riset (Objective)

Penelitian ini merupakan penelitian awal guna mendapatkan informasi yang lebih mendalam mengenai aktivitas anti kanker ekstrak H.scabra sebagai agen kemopreventif. Untuk itu dipilih jenis sel kanker serviks. Penelitian ini

melengkapi data penggunaan ekstrak H.scabra sebagai agen kokemoterapi selain dengan doxorubicin. Hasil akhir penelitian ini adalah diperolehnya agen kokemoterapi yang poten dari bahan alam. Hasil penelitian ini akan memberikan informasi yang berharga bagi dunia sains penemuan obat karena memberikan pendekatan sistematik dalam pengembangan agen kokemoterapi di Indonesia. Pendekatan ini diharapkan dapat dilakukan juga di berbagai tempat riset di Indonesia.

Metodologi (Method)

Insilico study



Pembuatan ekstrak etanolik Scistocophus hermanii

S. hermani dikeringkan kemudian dibuat serbuk dengan blender dan diayak kemudian dilakukan perhitungan prosentase bobot kering terhadap bobot basah. Pembuatan ekstrak dengan metode maserasi dan uji KLT dimana

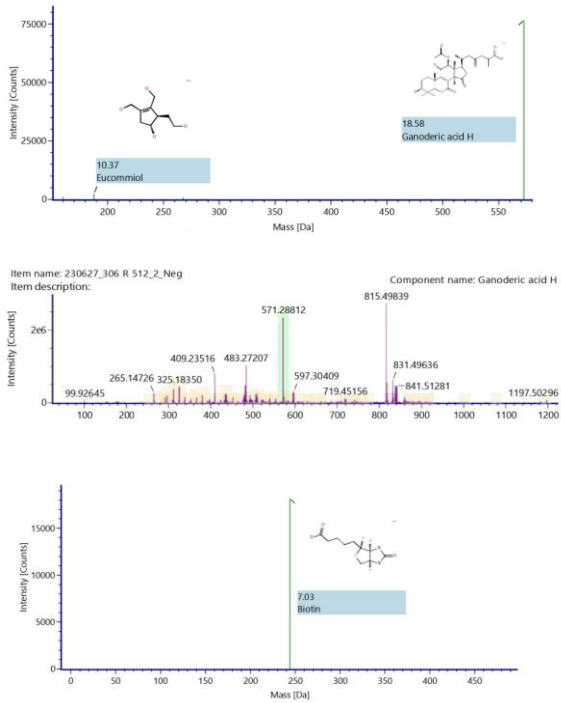
H.scabra ditimbang sebanyak 500,0 gram dimasukan kedalam bejana kemudian di rendam dengan 750 mL etanol 70%. Proses maserasi dilakukan selama ± 3 hari. Kemudian diekstrak dan diambil maseratnya. Sisa serbuk hasil penyaringan di remaserasi dengan 250 mL etanol 70% selama ± 2 hari. Ekstrak yang didapat dipekatkan dengan kompor dan rotary evaporator sampai kental dan bebas etanol. Setelah itu di lakukan pula kromatografi dengan kromatografi lapis tipis menggunakan fase diam berupa silika gel GF254 fase gerak berupa saponin, dan air dalam berbagai variasi perbandingan yang akan berfluoresensi pada uv 360 nm.

Pemeriksaan LCMS

RESULT OF ANALYSIS

Test Library : *Natural Product Library*
Compound Group : Phenol, Alkaloid, Terpenoid

No	ESI Mode	Compound Name	Result	Method
Phenol				
1	(+)	-	Positive	18-16-2/MU/SMM-SIG (LCMS/MS) QTOF
2	(-)	Rengyol	Positive	18-16-2/MU/SMM-SIG (LCMS/MS) QTOF
3	(-)	Eucommiol	Negative	18-16-2/MU/SMM-SIG (LCMS/MS) QTOF
Alkaloid				
1	(+)	-	Positive	18-16-2/MU/SMM-SIG (LCMS/MS) QTOF
2	(-)	6-Isoinosine	Negative	18-16-2/MU/SMM-SIG (LCMS/MS) QTOF
Terpenoid				
1	(+)	-	Positive	18-16-2/MU/SMM-SIG (LCMS/MS) QTOF
3	(-)	Ganoderic acid H	Negative	18-16-2/MU/SMM-SIG (LCMS/MS) QTOF



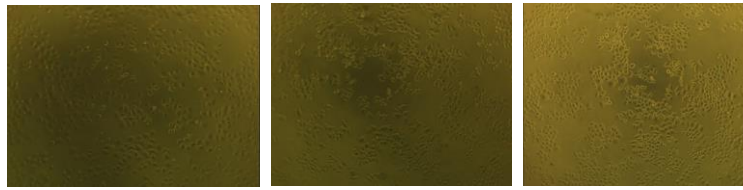
Uji Sitotoksisitas dan apoptosis sel

Uji sitotoksisitas menggunakan metode MTT

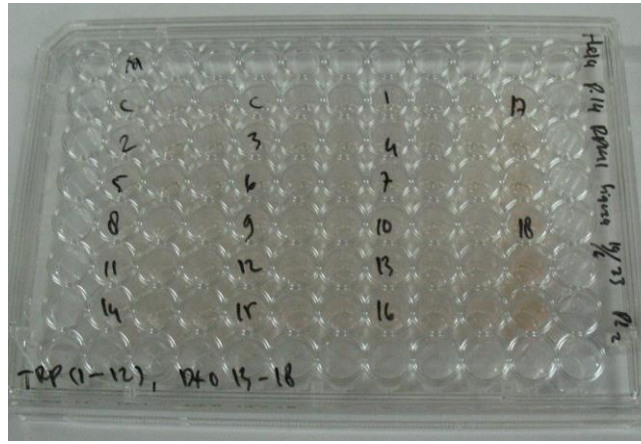
Sel dengan kepadatan 5x103 sel/sumuran didistribusikan ke dalam plate 96 sumuran dan diinkubasi selama 48 jam untuk beradaptasi dan menempel di dasar sumuran. Keesokannya media diambil, dicuci PBS kemudian ditambahkan 100 µL media kultur yang mengandung DMSO 0,2% saja (kontrol) atau sampel uji dalam bentuk tunggal (ekstrak H.scabra) diinkubasi selama 48 jam. Pada akhir inkubasi, media kultur yang mengandung sampel dibuang, dicuci dengan 100 µL PBS. Kemudian ke dalam masing-masing sumuran ditambahkan 100 µL media kultur yang mengandung 5 mg/ml MTT, inkubasi lagi selama 4 jam pada suhu 37°C. Sel yang hidup akan bereaksi dengan MTT membentuk kristal formazan berwarna ungu. Setelah 4 jam, media yang mengandung MTT dibuang, dicuci PBS kemudian ditambahkan

larutan stopper SDS dalam HCl 0,1% 200 µL untuk melarutkan kristal formazan. Digoyang di atas shaker selama 10 menit kemudian dibaca dengan dengan ELISA reader pada panjang gelombang 595 nm.

Teripang Doxo Kontrol



MTT Assay



Pembahasan

Background: Seacucumber is a marine natural product that has shown efficacy in medical treatments. However, the bioactive compounds, potential targets, and underlying mechanisms of Sea cucumber in cervical cancer are not well understood.

Methods: The active ingredients and targets of S. hermanii were extracted from the CMNPD database. Bioinformatics analysis was used to determine the

core ingredients, potential targets, and signaling pathways of S.hermanii, including constructed Drug-Ingredient-Gene symbols-Disease (D-I-G-D), protein interaction (PPI), super-PRED, and The Open Target Platform. Toxicity of the compounds was predicted using the Protox II platform.
Results: In this study, exploration of the active compounds and molecular mechanisms of Sea cucumber in the treatment of cervical cancer was conducted through network pharmacology analysis. Variegatuside C and Variegatuside D are the core active ingredients of seacucumber, which can regulate most of the targets related to cervical cancer. Through PPI network screening, we found that 11 targets, which overlap between cervical cancer and Sea cucumber, namely MTOR, Platelet-Derived Growth Factor Receptor Alpha(PDGFRA), Phosphoinositide-3-Kinase Regulatory Subunit 1 (PIK3R1), Krüppel-like Factor 5 (KLF5), Neurotrophic Receptor Tyrosine Kinase 3 (NTRK3), Hypoxia-Inducible Factor 1 Alpha (HIF1A), Cyclin E1 (CCNE1), Androgen Receptor (AR), Tripartite Motif Containing 24 (TRIM24), Heat Shock Protein 90 Alpha Family Class B Member 1 (HSP90AB1), and Topoisomerase II Alpha (TOP2A), may provide new ideas for the treatment of cervical cancer. The PI3K/AKT/mTOR cascade signalling pathway, which can affect various cellular functions such as cell survival, growth, proliferation, migration, and energy metabolism, was identified as the potential mechanism of Sea cucumber against cervical cancer.
Conclusions: The network pharmacology analysis demonstrated the potential of Seacucumber specifically its active compounds Variegatuside C and Variegatuside D, in regulating targets associated with cervical cancer via the PI3K/AKT/mTOR signalling pathway.

Daftar Pustaka (Voncoover)

1. Goyal R, Jialal I. Diabetes Mellitus Type 2. Verdure: Health Science Journal [Internet]. 2022 Jun 19 [cited 2022 Nov 1];3(1):8–17. Available from: https://www.ncbi.nlm.nih.gov/books/NBK513253/

2. Hall JE, Hall ME. Guyton and Hall Textbook of Medical Physiology. 14th ed. Philadelphia: Elsevier; 2021.

3. International Diabetes Federation. IDF Diabetes Atlas 2021 [Internet]. 2021 [cited 2022 Sep 13]. Available from: https://diabetesatlas.org/atlas/tenth-edition/

4. Kementrian Kesehatan RI. CEGAH, CEGAH, dan CEGAH: Suara Dunia Perangi Diabetes [Internet]. 2018 [cited 2022 Nov 1]. Available from: https://www.kemkes.go.id/article/view/18121200001/prevent-prevent-and-prevent-the-voice-of-the-world-fight-diabetes.html

5. Sapra A, Bhandari P. Diabetes Mellitus. StatPearls [Internet]. 2022 Jun 26 [cited 2022 Nov 1]; Available from: https://www.ncbi.nlm.nih.gov/books/NBK551501/

6. Tan SY, Mei Wong JL, Sim YJ, Wong SS, Mohamed Elhassan SA, Tan SH, et al. Type 1 and 2 diabetes mellitus: A review on current treatment approach and gene therapy as potential intervention. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2019 Jan 1;13(1):364–72.

7. Suzuki T, Kanamori T, Inouye S. Quantitative visualization of synchronized insulin secretion from 3D-cultured cells. Biochem Biophys Res Commun. 2017 May 13;486(4):886–92.

8. Ningsih SS, Avissa R, Stujanna EN, Listyaningsih E, Yashiro T, Sukarya WS. Evaluation of morphology and viability of spheroid derived from Insulin-GLase cell line: A model system to understand Type 2 Diabetes Mellitus. J Exp Clin Med. 2021 Apr 23;38(3):211–5.

9. Miranda MA, Macias-Velasco JF, Lawson HA. Pancreatic β-cell heterogeneity in health and diabetes: Classes, sources, and subtypes. Am J Physiol Endocrinol Metab [Internet]. 2021 Apr 1 [cited 2023 Jun 1];320(4):E716–31. Available from: https://journals.physiology.org/doi/10.1152/ajpendo.00649.2020

10. Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, et al. Pathophysiology of Type 2 Diabetes Mellitus. Int J Mol Sci [Internet]. 2020 Sep 1 [cited 2022 Nov 1];21(17):1–34. Available from: /pmc/articles/PMC7503727/

11. Kaneto H, Matsuoka TA. Role of Pancreatic Transcription Factors in Maintenance of Mature β-Cell Function. International Journal of Molecular Sciences 2015, Vol 16, Pages 6281-6297 [Internet]. 2015 Mar 18 [cited 2022 Nov 1];16(3):6281–97. Available from: https://www.mdpi.com/1422-0067/16/3/6281/htm

12. Mao X, Chen H, Tang J, Wang L, Shu T. Hepcidin links gluco-toxicity to pancreatic beta cell dysfunction by inhibiting Pdx-1 expression. Endocr Connect. 2017 Feb 9;6(3):121–8.

13. Nichols CG, Remedi MS. The diabetic β-cell: hyperstimulated vs. hyperexcited. Diabetes Obes Metab [Internet]. 2012 [cited 2023 Apr 16];14 Suppl 3(0 3):129–35. Available from: https://pubmed.ncbi.nlm.nih.gov/22928573/

14. Park SH, Park JH, Shim HM, Na AY, Bae KC, Lim JG, et al. Protection of pancreatic β-cells against glucotoxicity by short-term treatment with GLP-1. Biochem Biophys Res Commun. 2015 Apr 17;459(4):561–7.

Target Jurnal Internasional (Output)

Lampiran Log Book

No	Kegiatan
1	**Persiapan alat dan bahan kultur s**
2	**Pembuatan medium kultur s**
3	**Mulai *thawing cell* iGL**
4	**Proses subkultur sel dan pengamatan morfologi**
5	**Pembuatan laporan 70%**
6	**Perlakuan pada medium sel kultur iGL**
7	**Panen/*harvesting* sel dan pengamatan morfologi sel iGL**
8	**Pengukuran kadar insulin sel iGL dan pengolahan data insulin**
9	**Pembuatan laporan akhir dan draft jurnal**

Lampiran LuaranWajib

Network Pharmacology Analysis Reveals Bioactive Compounds and Potential Targets of Sea cucumb for Cervical Cancer Therapy

Authors

Irena Ujianti[1], Bety Semara Lakhsmi[2], Zahra Nurusshofa[3], Leli H indriyanti[4], Wawang S Sukarya[5]

[1]Department of Medical Physiology, Faculty of Medicine, Universitas Prof.Dr. Hamka, Jakarta, Indones
[2]Department of Public Health, Faculty of Medicine, Universitas Prof.Dr. Hamka, Jakarta, Indonesia
[3]Department of Pathology Anatomy, Faculty of Medicine, Universitas Prof.Dr. Hamka, Jakarta, Indones
[4]Department of Occupational Health, Faculty of Medicine, Universitas Prof.Dr. Hamka, Jakarta, Indone
[5]Department of Obstetric and Ginecology, Faculty of Medicine, Universitas Prof.Dr. Hamka, Jakarta, Indonesia

Corresponding author: Irena Ujianti (irenaujianti@uhamka.ac.id)

Abstract

Background: Seacucumber is a marine natural product that has shown efficacy in medical treatme However, the bioactive compounds, potential targets, and underlying mechanisms of Sea cucumb cervical cancer are not well understood.

Methods: The active ingredients and targets of S. hermanii were extracted from the CMNPD datab Bioinformatics analysis was used to determine the core ingredients, potential targets, and signa pathways of S.hermanii, including constructed Drug-Ingredient-Gene symbols-Disease (D-I-G-D), pro protein interaction (PPI), the Gene Ontology (GO), and the Kyoto Encyclopedia of Genes and Geno (KEGG).

Results: In this study, exploration of the active compounds and molecular mechanisms of Sea cucu in the treatment of cervical cancer was conducted through network pharmacology analysis. Variegatu C and Variegatuside D are the core active ingredients of seacucumber, which can regulate most o targets related to cervical cancer. Through PPI network screening, we found that 11 targets, which ov between cervical cancer and Sea cucumber, namely MTOR, Platelet-Derived Growth Factor Rece Alpha(PDGFRA), Phosphoinositide-3-Kinase Regulatory Subunit 1 (PIK3R1), Krüppel-like Factor 5 (KL Neurotrophic Receptor Tyrosine Kinase 3 (NTRK3), Hypoxia-Inducible Factor 1 Alpha (HIF1A), Cyclin (CCNE1), Androgen Receptor (AR), Tripartite Motif Containing 24 (TRIM24), Heat Shock Protein 90 A Family Class B Member 1 (HSP90AB1), and Topoisomerase II Alpha (TOP2A), may provide new idea the treatment of cervical cancer. The PI3K/AKT/mTOR cascade signalling pathway, which can a various cellular functions such as cell survival, growth, proliferation, migration, and energy metabolism, identified as the potential mechanism of Sea cucumber against cervical cancer.

Lampiran Luaran Tambahan

LETTER OF ACCEPTANCE

Dear,

Irena Ujianti

Assalamu 'alaikum wr wb.

Greetings from ICNSSE

We are pleased to inform you that your fullpaper entitled

Sea Cucumber (Schistopus hermanii) Compounds Targeting EGFR, PTGS2, NF-?B in Cervical Cancer Treatment; In Silico Study

has been **ACCEPTED** for:

ICNSSE 2023

After completing the payment please upload your payment proof and final draf (in a pdf format) to your account as soon as possible. Your presentation schedule will be added to the conference program upon completion of the payment process.
Please kindly completed your payment : **Rp.200.000** transfer to:

Account Name : **Universitas Muhammadiyah Prof.Dr.HAMKA**
Account Number : **17366489**
Swift Code : **BNINIDJA**
Nama Bank : **Bank Nasional Indonesia - BNI**

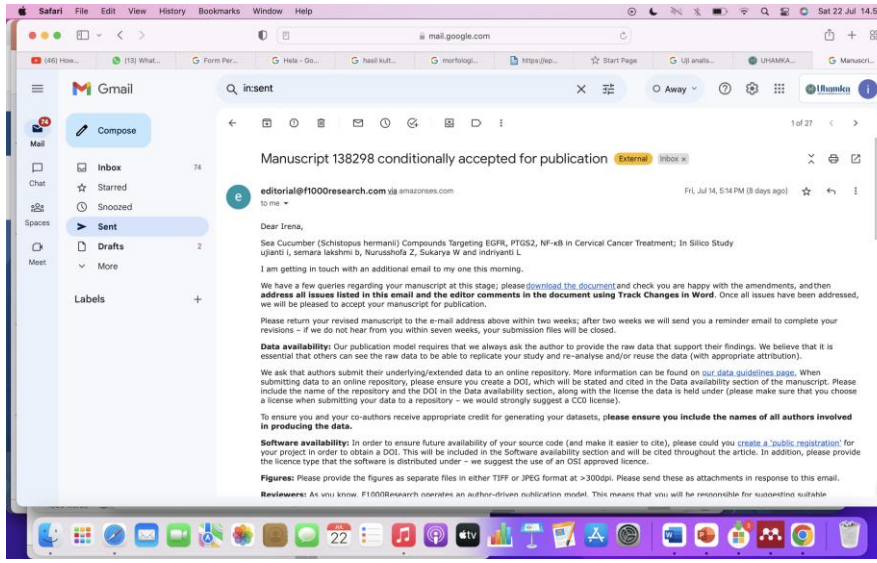
We are looking forward for welcoming you on ICNSSE 2023.

Sincerely,

Dr. Apt. Supandi, M.Si

Chairman ICNSSE 3 2023

Bukti Indexed


Safari File Edit View History Bookmarks Window Help
Sat 22 Jul 14.59
mail.google.com
Gmail
in:sent
Away
Compose
Inbox 74
Starred
Snoozed
Sent
Drafts 2
More
Labels
Mail
Chat
Spaces
Meet
1 of 27
Manuscript 138298 conditionally accepted for publication
External
Inbox ×
editorial@f1000research.com via amazonses.com
to me
Fri, Jul 14, 5:14 PM (8 days ago)
Dear Irena,
Sea Cucumber (Schistopus hermanii) Compounds Targeting EGFR, PTGS2, NF-κB in Cervical Cancer Treatment; In Silico Study
ujianti i, semara lakshmi b, Nurusshofa Z, Sukarya W and indriyanti L
I am getting in touch with an additional email to my one this morning.
We have a few queries regarding your manuscript at this stage; please download the document and check you are happy with the amendments, and then address all issues listed in this email and the editor comments in the document using Track Changes in Word. Once all issues have been addressed, we will be pleased to accept your manuscript for publication.
Please return your revised manuscript to the e-mail address above within two weeks; after two weeks we will send you a reminder email to complete your revisions – if we do not hear from you within seven weeks, your submission files will be closed.
Data availability: Our publication model requires that we always ask the author to provide the raw data that support their findings. We believe that it is essential that others can see the raw data to be able to replicate your study and re-analyse and/or reuse the data (with appropriate attribution).
We ask that authors submit their underlying/extended data to an online repository. More information can be found on our data guidelines page. When submitting data to an online repository, please ensure you create a DOI, which will be stated and cited in the Data availability section of the manuscript. Please include the name of the repository and the DOI in the Data availability section, along with the license the data is held under (please make sure that you choose a license when submitting your data to a repository – we would strongly suggest a CC0 license).
To ensure you and your co-authors receive appropriate credit for generating your datasets, please ensure you include the names of all authors involved in producing the data.
Software availability: In order to ensure future availability of your source code (and make it easier to cite), please could you create a 'public registration' for your project in order to obtain a DOI. This will be included in the Software availability section and will be cited throughout the article. In addition, please provide the licence type that the software is distributed under – we suggest the use of an OSI approved licence.
Figures: Please provide the figures as separate files in either TIFF or JPEG format at >300dpi. Please send these as attachments in response to this email.
Reviewers: As you know, F1000Research operates an author-driven publication model. This means that you will be responsible for suggesting suitable