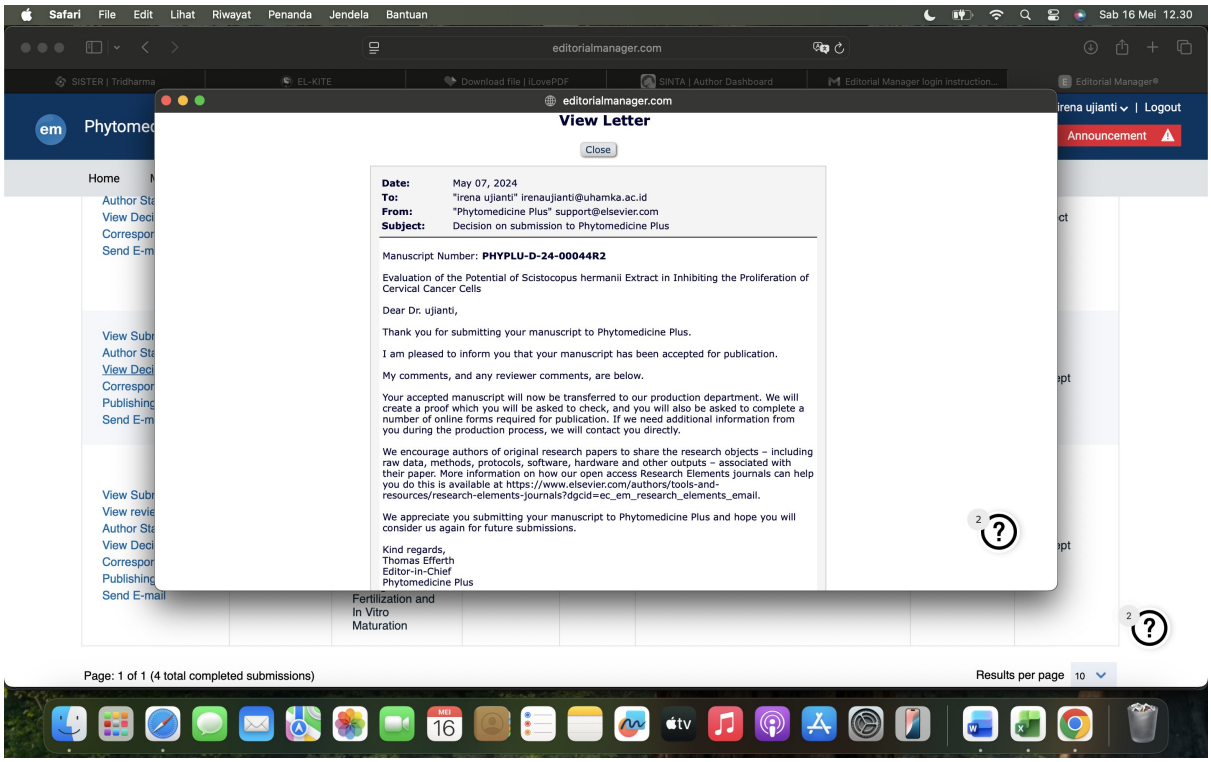
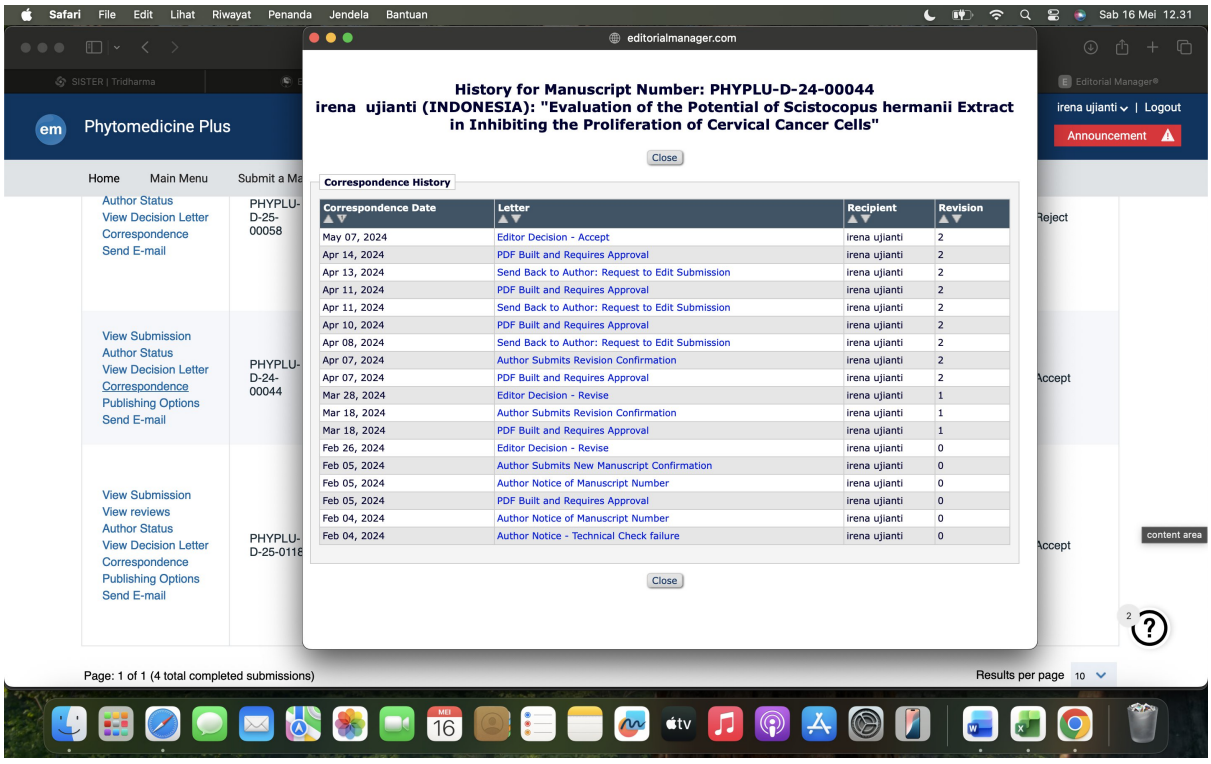
Accepted Letter



Bukti korespondensi



Reviewer 1,

Dear Reviewer,

Thank you for your insightful comments regarding the structure and clarity of our manuscript, particularly concerning the Results and Conclusion sections. We appreciate your feedback on the coherence of our storyline and the necessity to closely align our conclusions with the empirical results presented. In response to your valuable suggestions, we have undertaken a comprehensive revision to enhance the structure, clarity, and logical flow of our manuscript, with a keen focus on strengthening the linkage between our data presentation and the conclusions drawn. Below, we detail the measures taken to address your concerns

Comment 1: Clarify controversies and recent achievements, including publication years for novelty.

Response to Reviewer's Comment:

1. The emerging field of network pharmacology certainly has its areas of controversy. Critics often highlight the persistent challenge in validating the computational predictions through in vitro and in vivo experiments. This often sparks a debate on the reliability of identified novel targets and therapeutic indications. Moreover, concerns are raised about the vastness of data gathered in network pharmacology studies and the potential for false-positive results. Nevertheless, the field has made significant strides in recent years. Amongst the most noteworthy achievements is the successful identification of novel targets for existing drugs, facilitating their re-purposing for more efficacious treatment strategies. Furthermore, network pharmacology has expedited the drug discovery process by providing an efficient method of virtually screening prospective drug candidates
2. In relation to our study, one of the recent accomplishments includes the identification of unique bioactive compounds in the *Stichopus herrmanni* species and the formulation of a novel approach in treating cervical cancer. The success of this approach is not only limited to the context of cervical cancer but also holds potential for its application in the treatment of other cancer types.

Comment 2: The results and discussion section is very weak and no emphasis is given on the discussion of the results like why certain effects are coming into existence and what could be the possible reason behind them?

Response to Reviewer's Comment:

Updated Manuscript:

- **Discussion Enhancements**

    a. Our manuscript now provides a more comprehensive coverage of the underlying mechanisms at play. The ability of the bioactive compounds from Stichopus herrmanni: rengyol, eucommiol, ganoderic acid, and 6-isoinosine, to inhibit cell proliferation and prompt apoptosis offers critical insights into their prospective utility.

    b. These compounds, specifically, influence cell behavior through a variety of mechanisms. For instance, ganoderic acid, known from previous studies, can induce apoptosis by specifically enhancing the expression of the Bax protein and Caspase-3. Similarly, eucommiol notably inhibits cancer stem cells by targeting the JAK/STAT pathway, which plays a substantial role in cancer cell propagation. The compound 6-isoinosine also presents substantial potential by inhibiting DNA topoisomerase 1, an enzyme that modulates the topologic states of DNA during transcription, and acting as an immunomodulatory agent.

    c. Equally promising yet not extensively researched, rengyol, a molecule derived from cyclohexane, has been shown in certain studies to activate caspase-mediated apoptosis in human cancer cell lines.

    d. In addition, our research illustrates the selective mechanism employed by this extract, differing from the conventional chemotherapeutic, doxorubicin. Our findings reveal the extract's propensity for augmenting T lymphocyte generation and selectively modulating the PI3K-p53 signaling cascade. This alternative pathway could present valuable insights for developing new therapeutic strategies.

Comment 4: Results and conclusion: The section devoted to the explanation of the results suffers from the same problems revealed so far. Your storyline in the results section (and conclusion) is hard to follow. Moreover, the conclusions reached are really far from what one can infer from the empirical results.

Response to Reviewer's Comment:

1. **Clarification and Structuring of Results**: We recognized the necessity to present our results in a more organized and interpretable manner. To achieve this, we have:

    - **Rearranged the sequence** of our results to follow a logical flow that mirrors the progression of our research, starting from the identification of compounds in the LC-MS analysis to the exploration of their efficacy and mechanisms of action through in vitro and in silico studies.
    - **Added subheadings** within the Results section to clearly delineate each analysis type (e.g., LC-MS Analysis, In Silico Study, Toxicity Analysis, etc.), making it easier for readers to follow the narrative and understand the significance of each set of findings.
    - **Enhanced the narrative** by providing brief summaries at the beginning of each subsection, contextualizing the forthcoming data and its relevance to the study's overall aims.

2. **Consolidation of the Conclusion Section**: To ensure that our conclusions are directly inferable from the empirical results, we have:

    - **Tightly coupled our conclusions with our data**, explicitly highlighting how each finding supports our final interpretations and implications of the study.
    - **Streamlined the conclusions** to focus solely on the outcomes directly supported by the results, removing any far-reaching or unsubstantiated claims.

3. **Improvement of Storyline and Logical Flow**: We have revised the manuscript to enhance logical transitions and connections between sections, ensuring that:

    - The **discussion** directly feeds into the **conclusion**, with a clear and evident thread that ties our findings to their broader implications and potential applications in cancer therapy.
    - **Signposting and transitional phrases** have been strategically utilized to guide the reader through the narrative, thereby improving the manuscript's overall coherence and readability.

4. **Directly Addressing Discrepancies between Results and Conclusions**: We have conducted a thorough review to ensure that all conclusions are firmly rooted in our data, amending any overreaching or unsupported claims. This involved:

- **Revising our interpretation of the data** where necessary to ensure it aligns with what has been empirically demonstrated.
- **Discussing potential discrepancies** between our expectations and our findings in a transparent manner, including considering alternative explanations and suggesting directions for future research.
- **Conclusion :** By integrating data from both computational and laboratory-based investigations, we acquired a comprehensive understanding of Stichopus herrmanni's potential as an anticancer candidate. Our in-depth studies centered predominantly on analyzing cytotoxic effects of its bioactive compounds on the PI3K/AKT and p53 signaling pathways—key regulatory cascades in tumorigenesis. Moreover, the potential for immunomodulation implies the extract's capability to fine-tune the immune response, offering another vantage point in the battle against cancer. Nevertheless, while significant strides have been taken, more detailed investigations are required to substantiate these findings and illuminate the specific functions of Stichopus herrmanni extract in the oncological context. As we delve deeper into each underlying mechanism, we are optimistic that this may pave the way for innovative and effective therapeutic strategies in cancer treatment

In summary, our revisions aim to provide a clearer, more logical, and directly interpretable presentation of our research findings and their implications. We believe these changes have significantly improved the manuscript, making our storyline in the results and conclusion sections more coherent and ensuring our conclusions are directly supported by our results. We are grateful for the opportunity to enhance our manuscript and believe these amendments address the concerns you have raised.

Comment 5: Clarify controversies and recent achievements, including publication years for novelty

Response to Reviewer's Comment:

One of the primary controversies in cervical cancer treatment revolves around the balance between efficacy and quality of life post-treatment. Traditional treatments, while effective, can lead to severe long-term side effects, including fertility issues and organ damage, underscoring the need for innovative therapeutic approaches.
Additionally, the role of network pharmacology represents a paradigm shift in drug discovery and cancer treatment, moving from a one-drug-one-target approach to a holistic view of disease mechanisms. While promising, this methodology also faces skepticism regarding its complexity, validation of identified targets, and translational value from in silico and in vitro studies to effective in vivo treatments.

Recent Achievements

- Network Pharmacology in Cervical Cancer:
  Publication Years: 2021 and 2023 (Bonab et al., 2021; Dovnik et al., 2023; Zhao et al., 2018).
  Achievement: Network pharmacology has delineated the complex interaction networks induced by oncogenes such as HPV's E6 and E7, offering a comprehensive understanding that could lead to more accurately targeted therapeutic strategies.

- Indonesian Sea Cucumbers and Cervical Cancer:
  Publication Years: 2021, 2022, and 2023 (Pratomo et al., 2022; Ujianti et al., 2023; Napitupulu et al., 2022; Mackenzie et al., 2021; Dovnik et al., 2023).
  Achievement: The identification of unique bioactive compounds in Stichopus herrmanni and the exploration of their anticancer properties represent significant strides in the search for novel treatments. This approach not only opens new avenues for cervical cancer therapy but also potentially for other cancer types.

- In Silico and In Vitro Validation:
  Publication Years: Abd-Allah, W. H., Salman, A., & Sabry Saad, S. (2019). Ahmad, M. F., Wahab, S., Ahmad, F. A., Ashraf, S. A., Abullais, S. S., & Saad, H. H. (2022). Cheng, Y., & Xie, P. (2019). Huang, L., Lyu, Q., Zheng, W., Yang, Q., & Cao, G. (2021). Sangwan, K., Sharma, V., & Goyal, P. K. (2022)..
  Achievement: Through liquid chromatography-mass spectrometry (LC-MS) and cell culture studies, significant advancements have been made in validating the

anticancer potential of Stichopus herrmanni compounds against cervical cancer cell lines.

Novelty: The novelty in this context arises from the application of network pharmacology to cervical cancer, a relatively new approach that contrasts with traditional linear drug discovery processes. The identification and investigation of Stichopus herrmanni's bioactive compounds further highlight the innovative exploration of natural resources, particularly from rich biodiversity regions like Indonesia. These achievements, underscored by rigorous analytical methods and statistical analyses, pave the way for developing safer, more effective cancer treatments

---

Comment 6 : Spacing, punctuation marks, grammar, and spelling errors should be reviewed thoroughly. I found so many typos throughout the manuscript.

Comment 7 : English is modest. Therefore, the authors need to improve their writing style. In addition, the whole manuscript needs to be checked by native English speakers.

ANSWER:

Thank you very much for your detailed feedback and for highlighting the areas within our manuscript that require further attention.

Regarding Comment 6, we genuinely appreciate your insights into the grammatical and typographical errors present. We understand the importance of presenting our findings with clarity and precision. It has come to our attention that the extensive revisions made to the content might have led to the inadvertent inclusion of these errors. We are committed to addressing these concerns meticulously and will ensure a thorough verification of spacing, punctuation, grammar, and spelling in our revised manuscript.

In response to Comment 7 about improving the writing style and ensuring the manuscript undergoes a review by native English speakers, we want to express our gratitude for pointing out this vital aspect. We had indeed engaged a native English speaker to refine our manuscript's language during the initial stages. Nonetheless, due to the significant alterations made subsequently, the manuscript has not yet been reviewed again post-revision. We assure you, should we be afforded the opportunity to revise, we will immediately arrange for our manuscript to be meticulously examined by a native English speaker specializing in academic writing. This step will undoubtedly elevate the language quality and enhance the overall readability of our work.

We are hopeful for the chance to refine our manuscript further and are eager to implement your valuable suggestions. Your guidance is instrumental in aiding us to present our research more effectively and contribute meaningarily to the scientific community.

Thank you once more for your constructive criticism and your consideration. We trust these enhancements address your concerns effectively. We're grateful for your input, which significantly contributes towards accurately portraying our insights, deepens our analysis, and improves the quality of our research paper.

Sincerely

Reviewer 2

Dear Reviewer,

Thank you for your insightful comments regarding the structure and clarity of our manuscript, particularly concerning the Results and Conclusion sections. We appreciate your feedback on the coherence of our storyline and the necessity to closely align our conclusions with the empirical results presented. In response to your valuable suggestions, we have undertaken a comprehensive revision to enhance the structure, clarity, and logical flow of our manuscript, with a keen focus on strengthening the linkage between our data presentation and the conclusions drawn. Below, we detail the measures taken to address your concerns

Comment 1: What are the implications of LC-MS analysis identifying rengyol, eucommiol, ganoderic acid, in the extracts, particularly about their potential therapeutic effects on cervical cancer?

Response to Reviewer's Comment:

The identification of compounds such as rengyol, eucommiol, and ganoderic acid in extracts via LC-MS analysis suggests potential therapeutic avenues for cervical cancer, given the known biological activities of these compounds. Here's a detailed look into the potential implications based on existing research and the information provided:

- Rengyol: There isn't a direct link in the provided resources to the efficacy of rengyol against cervical cancer. However, compounds similar to rengyol have been studied for their anti-inflammatory and antitumor effect. If rengyol exhibits similar properties, its identification in extracts could indicate a possible role in mitigating cancer progression through inflammation modulation.
- Eucommiol: Eucommiol is known for its anti-inflammatory and anti-oxidative properties2. Such characteristics are crucial since oxidative stress and chronic inflammation are widely recognized contributors to the development and progression of various cancers, including cervical cancer. Through modulation of these pathways, eucommiol might offer therapeutic benefits, particularly in the prevention or as an adjunct therapy for cervical cancer.
- Ganoderic acid: This compound, found in Ganoderma lucidum (Reishi mushroom), has been extensively researched for its anticancer properties. It has been shown to induce apoptosis, inhibit cell proliferation, and modulate immune responses in various cancer cell lines. Specifically, in the context of cervical cancer, ganoderic acid could potentially target cancer cells directly, inhibit tumor growth, and enhance the body's immune response to cancer cells.
- 6-isoinosine: This compound is a purine nucleoside and may have roles in cellular metabolism and possibly in modulating the immune system. However, the direct impact of 6-isoinosine on cervical cancer cells is not well-documented. Its role in cervical cancer would likely involve deeper research into how it interacts with cancer cell metabolism or how it might affect the immune response to cancer cells

The implications of identifying these compounds in extracts through LC-MS analysis are profound, underscoring the potential for developing novel therapeutic strategies for cervical cancer. The chemical fingerprinting provided by LC-MS enables the identification of active compounds within complex mixtures, facilitating the discovery of bioactive molecules that could serve as leads in drug development. The diversity of the pharmacological activities of these identified compounds—ranging from anti-inflammatory and antioxidant to direct anticancer effects—highlights the multi-targeted approach that could be employed in combating cervical cancer. Further research, including in vitro and in vivo studies, would be crucial to fully understand the therapeutic potential of rengyol, eucommiol, and ganoderic acid against cervical cancer

References

1. Abd-Allah, W. H., Salman, A., & Sabry Saad, S. (2019). Anticancer activity of newly synthesized 1,1-disubstituted cyclohexane-1-carboxamides: in vitro caspases mediated apoptosis activators in human cancer cell lines and their molecular modeling. *Drug Development Research*, *80*(7), 933–947. https://doi.org/10.1002/ddr.21573
2. Ahmad, M. F., Wahab, S., Ahmad, F. A., Ashraf, S. A., Abullais, S. S., & Saad, H. H. (2022). Ganoderma lucidum: A potential pleiotropic approach of ganoderic acids in health reinforcement and factors influencing their production. *Fungal Biology Reviews*, *39*, 100–125.
3. Cheng, Y., & Xie, P. (2019). Ganoderic acid A holds promising cytotoxicity on human glioblastoma mediated by incurring apoptosis and autophagy and inactivating PI3K/AKT signaling pathway. *Journal of Biochemical and Molecular Toxicology*, *33*(11), e22392. https://doi.org/https://doi.org/10.1002/jbt.22392
4. Fujiwara, A., Nishi, M., Yoshida, S., Hasegawa, M., Yasuma, C., Ryo, A., & Suzuki, Y. (2016). Eucommicin A, a β-truxinate lignan from Eucommia ulmoides, is a selective inhibitor of cancer stem cells. *Phytochemistry*, *122*, 139–145. https://doi.org/https://doi.org/10.1016/j.phytochem.2015.11.017
5. Huang, L., Lyu, Q., Zheng, W., Yang, Q., & Cao, G. (2021). Traditional application and modern pharmacological research of Eucommia ulmoides Oliv. *Chinese Medicine (United Kingdom)*, *16*(1), 1–26. https://doi.org/10.1186/s13020-021-00482-7
6. Sangwan, K., Sharma, V., & Goyal, P. K. (2022). Pharmacological profile of novel anti-cancer drugs approved by USFDA in 2022: A review. *Current Molecular Medicine*.

Comment 2: Additionally, how do the in silico structural analysis results identifying ganoderic acid and isoinosine as crucial active components, along with the overlapping targets between cervical cancer and the extract, suggest new therapeutic prospects for cervical cancer treatment?

Response to Reviewer's Comment:

The in silico structural analysis results identifying ganoderic acid and isoinosine as crucial active components point towards promising therapeutic prospects for cervical cancer treatment.

1. Ganoderic acid, a triterpene derived from the Ganoderma lucidum mushroom, has shown notable anticancer properties in various studies. Its mechanism of action involves targeting receptor tyrosine kinases (RTKs), which play critical roles in cell migration, adhesion, apoptosis, metabolism, and proliferation in cancer. Aberration in RTK signaling can lead to cancer, suggesting that ganoderic acid's ability to modulate these pathways may offer therapeutic benefits
2. Isoinosine, although not as widely studied as ganoderic acid in the context of cancer, its inclusion based on in silico structural analysis suggests it has potential biological activity against cervical cancer. Inosine, the base of isoinosine, has been shown to have immunomodulatory properties, which could potentially contribute to anti-cancer effects, although the direct effects on cervical cancer need further exploration.

The overlapping targets identified between cervical cancer and the extracts containing these compounds underscore the multi-targeted approach in cancer treatment. Cervical cancer, like many cancers, does not rely on a single pathway or marker for its progression. Hence, therapies that target multiple aspects of the cancer cell proliferation cycle or tumor environment may prove to be more effective.

The identification of ganoderic acid and isoinosine through in silico analysis as crucial components suggests a targeted disruption of cancer cell proliferation and survival pathways. Given their distinct mechanisms—ganoderic acid's modulation of RTK signaling and potential immunomodulatory effects of isoinosine—these compounds could be studied further for synergistic effects. This combination therapy approach could potentially enhance efficacy while reducing the likelihood of resistance development, which is a common issue in cancer treatment. Moreover, in silico analyses allow for the rapid screening of compounds against a wide array of targets, providing valuable insights into potential therapeutic effects with a significantly reduced cost and time investment compared to traditional methods. This makes the approach especially valuable in the early stages of drug development and for exploring novel treatments for complex diseases like cervical cancer

In conclusion, the in silico structural analysis highlighting ganoderic acid and isoinosine as active components against cervical cancer opens new avenues for research into their potential therapeutic application. Further empirical studies are necessary to validate these findings and understand the mechanisms by which these compounds may exert their effects on cancer cells

Comment 3: Additionally, how do the in silico structural analysis results identifying ganoderic acid and isoinosine as crucial active components, along with the overlapping targets between cervical cancer and the extract, suggest new therapeutic prospects for cervical cancer treatment?

Response to Reviewer's Comment:

1. Verifying actives compounds: Confirmation of cytotoxic effects in HeLa cells could suggest that the identified compounds (e.g., ganoderic acid and isoinosine) indeed possess bioactive properties and potentially contribute to the observed effects.

2. Mechanistic insights: By examining the mode of cell death, we can gain insights into the possible mechanisms through which the active compounds exert their cytotoxic effects. For instance, induction of apoptosis might suggest that these compounds could activate intrinsic or extrinsic apoptotic pathways. Through in silico methodologies, including protein interaction analysis, Gene Ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, we can predict possible mechanisms of action of the active compounds found in the extracts. For example, such studies may have revealed that the extract exerts its cytotoxic effects by inhibiting the PI3K/AKT pathway, which is known to play a critical role in cell proliferation. Inhibition of this pathway would lead to reduced cell growth and proliferation, making it a target for cancer therapy. Furthermore, the in silico studies could show the activation of the p53 pathway by the extract, contributing to the induction of apoptosis in cancer cells. The p53 protein is a well-known tumor suppressor involved in cell cycle arrest and apoptosis induction in response to DNA damage or other stress signals, and its activation is a common goal in the development of anticancer therapies. Thus, the integration of in silico predictions with in vitro findings provides a comprehensive understanding of how these compounds might interact with cellular pathways to exert their effects, supporting their potential therapeutic application against cervical cancer.

3. Evaluating selectivity: Cytotoxic effects against HeLa cells could also be compared to effects on normal cell lines. Preferential cytotoxicity towards cancer cells would be a positive indication of the compounds' potential as anticancer agents, reducing the risk of potential side effects in normal cells.

4. Basis for further studies: These in vitro results could form the basis for further exploration of these compounds, including detailed mechanistic studies, in vivo testing, and eventually clinical trials

---

Comment 5: What are the identified active components in the extracts, and their role in regulating targets associated with cervical cancer?

Response to Reviewer's Comment:

The active compounds identified in Stichopus herrmanni extracts, serve different roles in managing cervical cancer. Here are the identified compounds and their associated mechanisms:

1. Rengyol: Rengyol has evidenced potential in oncology with the induction of caspase-mediated apoptosis in human cancer cell lines. It's crucial for regulating cell death mechanisms in cancer therapy
2. Eucommiol: Eucommiol effectively inhibits cancer stem cells by targeting the JAK/STAT pathway, a crucial signaling mechanism involved in cell proliferation, differentiation, and apoptosis
3. Ganoderic Acid: Derived from the Ganoderma lucidum mushroom, this triterpene targets receptor tyrosine kinases (RTKs), which play an essential role in cell migration, adhesion, apoptosis, metabolism, and proliferation in cancer. Ganoderic acid enhances the Bax protein and Caspase-3 expression, leading to apoptosis
4. 6-Isonosine: This compound has anti-cancer effects, including inhibiting DNA topoisomerase 1 and acting as an immunomodulatory agent, hindering the growth of cancer cells89.

These compounds target pivotal genes, such as TP53, EGFR, MYC, AKT, CASP8, MTOR, JAK2, STAT3, ATM, CCND1, NOTCH1, and HRAS in the cervical cancer pathway. They influence various processes like cell proliferation, apoptosis, and cancer signal transduction, indicating a comprehensive approach against the pervasive ailment. The compounds carry a resemblance with known antineoplastic and chemopreventive agents, highlighting the direct correlation between compound structures and their therapeutic targets

---

Comment 6: How do active components contribute to potential therapeutic prospects based on in silico and protein-protein interaction network analyses?

Response to Reviewer's Comment:

the active components in the extracts of Stichopus herrmanni contribute to potential therapeutic prospects for cervical cancer treatment through specific mechanisms as unveiled by in silico studies and protein-protein interaction (PPI) network analyses:

- Active Components and Their Theoretical Mechanisms:
  Rengyol: Theoretical evidence based on in silico analysis suggests that rengyol can activate caspase-mediated apoptosis, which could be instrumental in controlling the unregulated cell growth seen in cervical cancer.
- Eucommiol: Through the protein-protein interaction network, eucommiol has been correlated with inhibitory effects on the JAK/STAT pathway, known to be crucial in the proliferation and survival of cancer stem cells2.
- Ganoderic Acid: In silico studies propose that ganoderic acid influences key receptor tyrosine kinases (RTKs) involved in multiple cell regulation processes. The component's interaction with these kinases can lead to the modulation of cell proliferation and induce apoptosis through the activation of Bax protein and Caspase-3.
- 6-Isonosine: Has been implicated in the inhibition of DNA replication in cancer cells and may possess immunomodulatory properties. The antiproliferative effect implies its significance in the treatment of cervical cancer45.

System-Level Insights from Network Pharmacology:

- Network pharmacology has highlighted the interconnectedness of the therapeutic targets and the bioactivities of the Stichopus herrmanni components.
- Protein-Protein Interaction Analysis: PPI revealed densely interconnected nodes involving pivotal oncogenes like TP53, EGFR, MYC, AKT, CASP8, MTOR, JAK2, STAT3, ATM, CCND1, NOTCH1, and HRAS. This network suggests a "multi-hitting" strategy, meaning that the extract's components affect various aspects of cancer cell survival and proliferation simultaneously.
- Gene Ontology and KEGG Pathway Analysis: The enrichment analysis associates the bioactive components with the regulation of processes directly tied to cervical cancer pathology. In particular, the KEGG analysis has delineated the p53 and PI3K-AKT pathways, both of which are heavily implicated in cell cycle regulation and apoptosis.

Therapeutic Potential and Structural Analysis: The active components show structural similarities with known anti-cancer compounds. This conveys a strong indication that these substances from Stichopus herrmanni could interact with molecular targets in analogous ways to established chemotherapeutics, albeit potentially with different efficacies or toxicity profiles.

Verification through In Vitro Study: In vitro studies complement the in silico findings by confirming the efficacy of the extract in reducing the viability of HeLa cells. This empirical evidence bridges the gap between theoretical predictions and practical application.

Comparative Insight: When compared with doxorubicin, Stichopus herrmanni extract may have presented less potency but has revealed an alternative pathway for targeting cancer cells that might offer a unique therapeutic angle with potentially fewer side effects.

Future research:

- To proceed, expanding the research from in silico and in vitro to in vivo models is necessary to validate these effects in a living organism.
- Further characterization of how these compounds modulate the PI3K-p53 signaling pathway will provide deeper understanding and might help refine potential therapeutic agents derived from the compounds.
- Investigating the effect of these compounds on the immune response, particularly on T lymphocyte function, could be key to understanding additional anti-cancer mechanisms at play.

In conclusion, the investigation into Stichopus herrmanni points to a novel, multi-targeted approach to treat cervical cancer, and while more research is necessary to fully validate these findings, the potential for new therapeutic avenues is promising.

Comment 7: There are grammatical mistakes, remove all the grammatical errors.
Comment 8: Please provide the authentication letter of the plant under study.
Comment 9: Give the biological source and in vivo, in vitro in italic.

Response to Reviewer's Comment:

Thank you for your esteemed feedback. Please find our responses to your comments as follows:

- Grammatical Errors: We deeply appreciate your patient reading and corrections on our manuscript. All the grammatical errors you have noted will be duly rectified to improve the presentation and coherence of our paper. We are revising the manuscript as per your recommendations and will ensure that all minor mistakes are eliminated in the upcoming draft.
- Authentication Letter of the Plant under Study: We understand the importance of providing the authentication letter for the plant under study. To remove any doubt regarding the authenticity or identity of the Stichopus herrmanni, we are contacting the respective authority for the official authentication letter. As soon as we receive the document, it will be appended with the revised manuscript.
- Italicisation of Biological Source and in vivo, in vitro: We sincerely thank you for highlighting this important formatting point. The names of the biological sources such as Stichopus herrmanni will be italicised throughout the manuscript, as per the biological nomenclature guidelines. Furthermore, scientific terms such as in vivo and in vitro will also be italicised in the text in accordance with the standard scientific convention.

We hope our responses satisfy your queries and expectations. Thank you once again for your time and insightful feedback for the enhancement of our manuscript

Thank you once more for your constructive criticism and your consideration. We trust these enhancements address your concerns effectively. We're grateful for your input, which significantly contributes towards accurately portraying our insights, deepens our analysis, and improves the quality of our research paper.

Sincerely

Reviewer 4

Dear Reviewer,

Thank you for your insightful comments regarding the structure and clarity of our manuscript. We appreciate your feedback on the coherence of our storyline and the necessity to closely align our conclusions with the empirical results presented. In response to your valuable suggestions, we have undertaken a comprehensive revision to enhance the structure, clarity, and logical flow of our manuscript, with a keen focus on strengthening the linkage between our data presentation and the conclusions drawn. Below, we detail the measures taken to address your concerns

Comment 3: Screening for potentially active compounds in the sea cucumber extract discuss must for you compounds role

Response to Reviewer's Comment:

Thank you for your insightful comments and the opportunity to clarify our research's crucial aspects. Your inquiry regarding the role of bioactive compounds screened in the sea cucumber extract is highly appreciated. Herein, we provide a refined explanation for your request. In our study, we identified four predominant bioactive compounds using LC-MS/MS in the Stichopus herrmanni extract, namely Ganoderic acid, 6-Isoinosine, Eucommiol, and Rengyol.

- Ganoderic acid - Ganoderic acid (GA) is a known triterpenoid possessing a multitude of pharmacologic potentials. A variety of research literature attests to its anticancer, anti-inflammatory, antioxidant, and anti-hepatotoxic properties. In the context of our study, GA's abundance might contribute significantly to the observed bioactivities of the extract, specifically the anticancer capability.
- 6-Isoinosine - Although less studied, 6-Isoinosine is a naturally occurring purine derivative believed to possess certain bioactivities, including potential antiviral properties. Given its presence, it would be interesting to explore its roles further in subsequent experimental studies.
- Eucommiol - Eucommiol, a compound typically derived from Eucommia ulmoides Oliver, has been associated with antioxidant, anti-inflammatory, and antimicrobial activities. Its precise role in the sea cucumber extract warrants further investigation.
- Rengyol - This compound has been discovered in other marine organisms and exhibits antimicrobial and other therapeutically relevant properties.

Each of these compounds' roles in the Stichopus herrmanni extract is worthy of further elucidation. However, the diversity of biological activities and the bioactive potential of the extract as a whole make it a promising source for developing multifunctional drugs. We agree that a detailed study investigating the role of these isolated compounds is required to decipher their individual contributions to the observed pharmacological efficacy. Therefore, we consider your comment highly pertinent, and we plan to extend our current research to encompass this crucial aspect.

Comment 2: Line number, 68-73 rewrite sentence for introduction

Response to Reviewer's Comment:

Thank you once again for your invaluable feedback. Following your suggestions, I've revised the text in lines 87-90 to ensure the introduction explicitly conveys the significance of compounds found in sea cucumbers and their potential for therapeutic applications.

Original Text (Lines 68-73):

"An intriguing area of exploration in this regard is the utilization of unconventional resources such as sea cucumbers. Marine organisms are rich in bioactive compounds, including triterpene glycosides, chondroitin sulfates, peptides, and polysaccharides, which potentially exhibit remarkable anticancer properties.

Revised Text (Lines 87-90):

"Sea cucumbers, for instance, possess compounds like triterpene glycosides, chondroitin sulfates, peptides, and polysaccharides, garnering interest within various health-related fields due to their potential for exemplary therapeutic applications."

This revision is intended to succinctly outline the specific bioactive compounds in sea cucumbers that are generating keen interest for their broad therapeutic potential. By directly pointing out the unique array of compounds they contain, we aim to highlight the importance of sea cucumbers as an underutilized resource in health and medical sciences.

I hope this modification meets your expectations and further clarifies our research's scope and relevance. We appreciate your guidance and are open to any further suggestions you may have to enhance our manuscript.

Comment 3: MTT Proliferation Assay change the title for materials method

Response to Reviewer's Comment:

Thank you for your insightful comment regarding the title of the "MTT Proliferation Assay" section in our material and methods. To more accurately depict the contents of this section and address your suggestion, we propose adjusting the title to:

"Cell Proliferation Analysis using MTT Assay"

This revised heading should provide a more comprehensive and precise description, emphasizing both the method used (MTT Assay) and its purpose (Cell Proliferation Analysis). We appreciate your feedback, which aids us in enhancing readability and overall clarity for our readers. Please let us know if there are any other points in the manuscript that require further modifications

---

Comment 5: Conclusion part rewrite for your work

Response to Reviewer's Comment:

Conclusion

Thank you for your question , in summary, our study has illuminated the potential of Stichopus herrmanni extracts and specific bioactive compounds therein for the treatment of cervical cancer, highlighting their ability to inhibit cell proliferation and induce apoptosis in HeLa cells. Our research indicates that these effects are mediated through key oncogenic pathways, including the PI3K/AKT and p53 signaling pathways, as well as through interactions with several proteins implicated in the pathology of cervical cancer.

Notably, our findings also revealed that compounds such as ganoderic acid and eucommiol, among others identified in our study, exhibit promising anticancer properties, some of which may have been previously underappreciated within the scientific community. This underscores the significance of exploring marine biodiversity for novel therapeutics and points to potential areas for further study, such as the investigation of these compounds in other cancer types or in combinatory therapies.

While our findings are promising, we also acknowledge the limitations of our study, including the in vitro nature of our experiments and the need for further in vivo and clinical studies to fully understand the therapeutic potential and safety profile of Stichopus herrmanni extracts. These limitations provide opportunities for additional investigation to reinforce and expand upon our results.

Moving forward, we propose the initiation of detailed pharmacokinetic and pharmacodynamic studies, alongside rigorous in vivo evaluations, thereby elucidating the systemic effects of these compounds and refining their therapeutic applicability. We anticipate that our contributions will serve as a valuable reference for researchers and practitioners in oncology and pharmacology, and stimulate further discourse and development within this important field of cancer research and treatment.

Comment 6: How do active components contribute to potential therapeutic prospects based on in silico and protein-protein interaction network analyses?

Response to Reviewer's Comment:

Thank you very much for bringing up docking analysis as a method in research related to molecular interactions. We greatly appreciate the utility of docking analysis in understanding molecular interactions and recognize its essential role in many studies. However, for the specific aims of our current project, we have chosen to utilize in vitro analysis methods to validate our findings reason for this decision is multi-fold:

- Specificity to Research Goals: Our study's goals are intricately linked to observing the direct response of cells to our compounds. In vitro analysis provides immediate insights that are vital for our current investigative phase.
- Expertise and Resources: We determined that in vitro methods matched our team's expertise and optimized our available resources effectively, ensuring we produce reliable and impactful data.
- Foundation for Future Studies: As a foundational study, our in vitro analysis paves the way to potentially embrace a multifaceted research approach in the future, which might include docking analysis when our research scope broadens.
- Commitment to Methodological Excellence: We are fully committed to methodological rigor and excellence. Our current approach with in vitro methods is the most robust way to achieve the desired outcomes for the phase of research we find ourselves in.

We are truly grateful for suggestions like yours as they encourage a broader discussion and reflect a collective commitment to scientific advancement. We look forward to exploring docking analysis and other such valuable methods in the continuation of our research journey. Rest assured, we remain open to integrating a variety of approaches to deepen our understanding of molecular interactions in our future work.

Comment 7: Additionally, emphasizing the scientific novelty of the study can help underscore the importance of the research and its potential impact in the field.

We sincerely appreciate your insightful suggestion to accentuate the scientific novelty of our research. Indeed, delineating how our work carves out new terrain is paramount to both validating our approach and underscoring its significance within the community we would like to outline several key innovations our research brings to the fore:

1. By concentrating on the immunomodulatory effects of Stichopus herrmanni extract in contrast to **traditional chemotherapy agents like doxorubicin**, previously overshadowed in academic discourse, **we illuminate new aspects of non-toxic, targeted cancer treatments**. This exploration effectively bridges a vital knowledge gap.

2. *In silico* structural analysis highlighting ganoderic acid and isoinosine as active components against cervical cancer opens new avenues for research into their potential therapeutic application. The compounds carry a resemblance with known antineoplastic and chemopreventive agents, highlighting the direct correlation between compound structures and their therapeutic target

3. Specifically, we have elucidated its propensity for augmenting the generation and efficacy of T lymphocytes while concurrently modulating the PI3K-p53 signaling cascade. The overlapping targets identified between cervical cancer and the extracts containing these compounds underscore the multi-targeted approach in cancer treatment.

Thank you once more for your constructive criticism and your consideration. We trust these enhancements address your concerns effectively. We're grateful for your input, which significantly contributes towards accurately portraying our insights, deepens our analysis, and improves the quality of our research paper.

Sincerely

Reviewer 5,

Dear Reviewer,

Thank you for your insightful comments regarding the structure and clarity of our manuscript, particularly concerning the Results and Conclusion sections. We appreciate your feedback on the coherence of our storyline and the necessity to closely align our conclusions with the empirical results presented. In response to your valuable suggestions, we have undertaken a comprehensive revision to enhance the structure, clarity, and logical flow of our manuscript, with a keen focus on strengthening the linkage between our data presentation and the conclusions drawn. Below, we detail the measures taken to address your concerns

Comment 1: There is no mention about any reference of the method which was used in Processing of S. herrmanni Ethanol Extract. Any scientific method needs to be mentioned with proper reference.

Response to Reviewer's Comment:

Thank you for your comment on our manuscript. We acknowledge your concern regarding the detailed description and reference for the processing method of *Stichopus herrmanni* ethanol extract. In our study, we have utilized adult *Stichopus herrmanni* sourced from Nusa Tenggara, Indonesia. The body walls were isolated, minced into approximatelypieces, and thereafter subjected to freeze-drying. Subsequently, we performed ethanol extraction wherein a ratio of 1 liter of ethanol per 100 grams of dried body wall material was utilized. This part of the method is aimed at denaturing cellular proteins and effectively liberating the secondary metabolites. The resultant supernatant was then concentrated using a rotary evaporator where the temperature was strictly maintained at 40 °C. This processing method is in concordance with the methodologies described in the literature for similar extraction processes. As a proper reference to our methodology, please refer to the following study: Sangpairoj et al. (2016), which details the extraction of extracts from the sea cucumber and outlines a comparable approach. We hope this explanation provides the necessary detail and reference for our methodology as per your request. The manuscript text will be duly updated to include this reference. Should you require anything further, please do not hesitate to ask.

Comment 2: Why was the supernatant further extracted with ethanol (1:5)?

Response to Reviewer's Comment:

The further extraction of the supernatant with ethanol (1:5) plays a critical role in the isolation process of secondary metabolites for several reasons:

- First, the utilization of ethanol, a polar solvent known for its denaturing abilities, is instrumental in disrupting the hydrogen bonds among other interactions that maintain proteins in their native three-dimensional structures. The denaturation of proteins leads to their precipitation and removal from the solution. This step is crucial as it aids in the elimination of proteins that could potentially interfere with the analysis or isolation of targeted components, such as secondary metabolites.
- Secondly, ethanol extraction is pivotal in liberating secondary metabolites. These organic compounds, which are not directly involved in the normal growth, development, or reproduction of an organism, often hold significant pharmaceutical or biotechnological applications. Ethanol increases cell membrane permeability, facilitating the extraction and solubilization of these metabolites into the supernatant. By augmenting ethanol concentration, the efficiency of extracting these valuable compounds from the sample matrix is significantly enhanced.

This dual-purpose role of ethanol in both denaturing cellular proteins and liberating secondary metabolites underscores its importance in the extraction process, aiming at the efficient recovery of secondary metabolites with potential biotechnological applications.

Comment 3: The extract was then evaporated in a rotary evaporator at -40°C? How does ethanol evaporate at -40°C?

Response to Reviewer's Comment:

Thank you for your attention to detail and pointing out the clarification needed about the evaporation temperature in the rotary evaporator. We apologize for any confusion the typo may have caused. You are absolutely correct that ethanol evaporates at 40°C. In fact, the boiling point of ethanol is 78.4 degrees Celsius, meaning at 40°C, it is well within the range to cause effective evaporation.

Ethanol, like other alcohol products, evaporates at different speeds depending on the temperature. At approximately 40°C, the evaporation process will be accelerated, much faster than it would be at room temperature. This makes 40°C an effective and comparatively rapid extraction method. As such, **the extraction process in our study was indeed performed at 40°C, not -40°C**, in the rotary evaporator. This point will be made clear in the revised manuscript to avoid any further misconceptions. Apologies for my typographical error.

Comment 5: Liquid chromatography mass spectrometry analysis, method needs appropriate reference.

Response to Reviewer's Comment:

In our study, the identification of compounds within the ethyl acetate extract was achieved utilizing Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). The High-Performance Liquid Chromatography (HPLC) procedures were conducted using an Agilent 1100 series system, equipped with a pump, autosampler, and vacuum degasser (Agilent Technologies, Palo Alto, CA, USA). The chromatographic separation was facilitated by a fused core C18 column (Waters Corporation, Milford, MA, USA), operating under a gradient flow profile. Specifically, the mobile phase, a mixture of acetonitrile and 0.1% formic acid, was applied at a constant flow rate of 1 mL/min, with the gradient progressing from 30% to 60% acetonitrile over 36 minutes. For detection, an Atmospheric Pressure Chemical Ionization (APCI) source was employed in the positive ion mode.
Further mass spectrometric analysis was performed on an IONIC 3Q Series 200 molecular analyzer. The fractions separated by HPLC were introduced into the mass spectrometer via direct injection at a flow rate of 20 μL/min. The ionization of analytes was carried out using the Electrospray Ionization (ESI) technique in the positive mode.

This detailed methodological description aims to provide comprehensive insights into our analytical approach, enhancing the reproducibility and accuracy of the reported results

Sangpairoj, K., Chaithirayanon, K., Vivithanaporn, P., et.al,. 2016. Extract of the sea cucumber, Holothuria scabra, induces apoptosis in human glioblastoma cell lines. *Funct. Foods Health Dis*, *6*, 452-468. https://doi.org/10.31989/ffhd.v6i7.264

Comment 6: The SMILE profile and 3D structure of each compound were examined using the PubChem software, Why PubChem software?

Response to Reviewer's Comment:

Thank you for your insightful question regarding our choice of PubChem for examining the SMILE profiles and 3D structures of the compounds in our study.
We chose PubChem software for several key reasons:

1. Comprehensiveness: PubChem is one of the largest and most comprehensive chemical databases available, home to a vast array of chemical compounds information. This inclusivity ensured that we had access to extensive details for all compounds of interest in our study.

2. Accuracy: The information in PubChem, including SMILES (Simplified Molecular Input Line Entry System) notations and 3D structure data, is sourced from hundreds of authoritative datasets. This ensures a high level of accuracy and reliability in the structural information obtained.

3. Accessibility: As a freely accessible database provided by the National Center for Biotechnology Information (NCBI), PubChem software allows for wide accessibility, making our research methods more reproducible and transparent to the scientific community.

4. Advanced Tools: PubChem provides advanced visualization tools that allowed us to analyze and present the SMILE profiles and 3D structural data effectively. This capabilitywas pivotal in our analysis and interpretation of the compound structures.

5. Consistent Updates: The database receives regular updates, ensuring that our research incorporated the most current and accurate chemical data available.

In conclusion, the combination of comprehensiveness, accuracy, accessibility, advanced tools for analysis, and regular updates makes PubChem an invaluable resource for chemical and structural analysis. Using PubChem allowed us to ensure that our study was grounded on reliable data while being accessible to peers for validation and reproducibility.
We appreciate your query and hope this response clarifies our choice of PubChem in our methodology.

Comment 7: Why did the author use WAY2DRUG PASS prediction tool? No scientific reference is mentioned in this regard.

Thank you for your inquiry regarding our choice of the WAY2DRUG PASS prediction tool for the prediction of biological activity in our study. The selection was based on the proven accuracy and reliability of PASS (Prediction of Activity Spectra for Substances) in the estimation of biological activity spectra for chemical compounds, which has been documented in several scientific references.

PASS is an integral part of the WAY2DRUG platform, and it employs advanced algorithms to predict thousands of types of biological activities, including interactions with molecular targets, pharmacotherapeutic effects, side effects, metabolism, and toxicity, among others1. The platform has been designed to facilitate the analysis of structure-activity relationships and has demonstrated its utility in various scientific investigations aimed at both novel drug discovery and drug repurposing2.

The reason we utilized the WAY2DRUG PASS prediction tool is two-fold:

1. Its extensive database and predictive capabilities related to various biological activities are well-suited to the scope of our investigation. By using this tool, we could anticipate the biological activity of our compounds with a high degree of confidence.
2. It comes highly regarded in the scientific literature for its efficiency and the breadth of its predictions, which encompass a diverse spectrum of pharmacological activities
3. We believe that the application of the WAY2DRUG PASS prediction tool lends significant credibility and integrity to our research findings. Below, we have included a reference to a relevant scientific article that rigorously evaluates the computational platform, along with additional references for further reading.

In the revised manuscript, we will make sure to include these pertinent references to give our readers the appropriate context and foundation for our methodology:
We appreciate the opportunity to clarify this aspect of our work and hope these references will address the concern you have raised.

Druzhilovskiy, D.S., Rudik, A.V., Filimonov, D.A., Gloriozova, T.A., Lagunin, A.A., Dmitriev, A.V., Poroikov, V., 2017. Computational platform Way2Drug: from the prediction of biological activity to drug repurposing. Russian Chemical Bulletin. 66, 1832-1841. https://doi.org/10.1007/s11172-017-1954-x

Comment 7 : Quantitative Structure-Activity Relationship Analysis results are not compared with any reference standard which is a must to compare the actual efficacy.

ANSWER:

Dear Reviewer,

Thank you for your insightful comment regarding the necessity of comparing our Quantitative Structure-Activity Relationship (QSAR) Analysis results with reference standards to ascertain the actual efficacy. We acknowledge the importance of benchmarking our findings against established standards to strengthen the validity and relevance of our study's outcomes.

In our research, we focused on the unique mechanism of action of certain compounds identified within the ethyl acetate extract, particularly their role in selectively targeting neoplastic cells. Our investigation revealed that these compounds, notably ganoderic acid and isoinosine, exhibit a promising capability in augmenting T lymphocyte generation and modulating the PI3K-p53 signaling pathways. These findings align our study with the broader scientific endeavor to uncover multi-targeted therapeutic approaches in cancer treatment.

Given the novelty of our findings and the unique pathway elucidation, a direct comparison with reference standards—a common practice in QSAR analysis—was initially not performed. Our primary aim was to highlight the distinct mechanisms through which the identified compounds exert their anti-neoplastic effects, supplementing the conventional knowledge surrounding cancer therapeutics. However, we recognize that to further validate our findings and situate them within the existing body of knowledge on antineoplastic and chemopreventive agents, a comparative analysis with known reference compounds is indispensable. In light of your feedback, we are committed to undertaking this comparative analysis. This will include a systematic comparison of our QSAR data with established reference standards recognized in current cancer pharmacotherapy research. We will specifically look into similarities and deviations in structure-activity relationships to emphasize not only the novelty of our findings but also their scientific merit and potential therapeutic implications.

Furthermore, our in silico structural analysis uncovering the therapeutic potential of ganoderic acid and isoinosine against cervical cancer shall be contextualized within the landscape of existing antineoplastic agents. This would offer a clearer perspective on how these compounds may complement or offer advantages over current treatment modalities. We believe that incorporating this comparative aspect will substantially enhance the robustness and applicability of our research findings, opening new avenues for therapeutic application against cervical cancer.

We appreciate the opportunity to address this gap in our analysis and thank you for guiding our attention towards strengthening our study's contribution to cancer research.

Sincerely,

Comment 8 : No reference is mentioned in MTT Proliferation Assay, why? Was the method previously established or it was a new protocol developed by the author?

Answer:

Thank you for bringing to our attention the need for references regarding the MTT Proliferation Assay used in our study. The omission of specific references for the MTT assay was an oversight, and we appreciate the opportunity to clarify this.

1. The MTT assay protocol we employed is based on a previously established method well-documented in the literature for assessing cell viability1. This protocol is widely recognized as a standard technique for measuring the proliferation rate and cytotoxicity effect on cells in vitro.
2. Regarding your question about whether this was a new protocol developed by us, it was not. Instead, we followed the established standard with minor adjustments for our specific cell line, which did not significantly deviate from the original protocol. These modifications were done to optimize the assay conditions for our particular experimental setup.
3. To ensure clarity and allow for reproducibility of our research, we have added appropriate references to the section of the manuscript detailing the MTT assay protocol:
4. The original protocol by Mosmann, which outlines the use of tetrazolium salts such as MTT to measure cell metabolic activity as an indicator of cell viability, growth, and proliferation
5. A subsequent reference where this standard method has been adapted for various cell types, which served as a guide for our minor modifications

   In the revised manuscript, these citations will be included to provide readers with the necessary context and to verify the reliability and validity of our methodological approach
   We are grateful for the chance to enhance the quality and integrity of our work with this addition.

Sincerely

Comment 9: How did the author determine the extract concentrations 75, 125, and 300 µg/L used in MTT Proliferation Assay? What was the rationale?

Rationale for Concentration Range: The chosen concentrations are intended to cover a spectrum that includes a potentially therapeutic window. The lowest concentration (75 µg/L) was selected to detect the minimum effective dose that could induce a measurable response in cell viability. The intermediate concentration (125 µg/L) serves to observe the dose-response relationship further. The highest concentration (300 µg/L) was chosen based on the preliminary cytotoxicity threshold, allowing us to investigate the upper limit of the extract's efficacy before reaching a level that could be considered excessively toxic for the cells under study

Comment 10: The crude sea cucumber extract was used to measure the antineoplastic effects. But this procedure shows no exact mechanism and doesn't ensure the potentiality of S. herrmanni as no single bioactive was used in the study. Crude extracts are a mixture of numerous chemicals. So, the effect can be a single or by a group of chemicals. So, this study is unable to conclude any concrete outcome.

Our study employed a network pharmacology approach to understand how components in the sea cucumber extract could interact within a complex network at the cellular level. Through this analysis, the significant roles of PI3KT and p53 in inducing antiproliferation and apoptosis became clear, although these interventions had minimal impact on cytotoxicity in HeLa cells in our study. Phosphoinositide 3-kinases (PI3Ks) are a class of kinases involved in various cellular functions, including cell growth, proliferation, differentiation, motility, and survival. The discovery of PI3KT's role in these processes calls for further research on compounds from this extract that may interact with PI3KT.

Meanwhile, p53 is a protein that acts as a "genome guardian" due to its role in preventing gene mutations and cancer. Its involvement indicates that the extract may have therapeutic potential in treating cancer through p53 modulation. However, it must be emphasized that this study still requires more evidence to confirm the involved action pathways. Further research, possibly involving in vivo studies or direct molecular validation of these interactions, would be highly beneficial in clarifying the picture.

Evaluation of the Potential of *Stichopus Herrmanni* Extract in Inhibiting Cervical Cancer Cell Proliferation

ABSTRACT

Background: Sea cucumbers, particularly *Stichopus herrmanni*, are known for their medicinal value in Asian traditional medicine owing to the abundant presence of saponins, terpenoids, and phenols. Investigations on these bioactive compounds have revealed their cytotoxic propensity against various cancer cell lines, indicating their therapeutic potential. However, limited research exists on the potential application of sea cucumber extracts specifically in cervical cancer.

Purpose: This study aimed to identify the bioactive components of *Stichopus herrmanni* extract, determine their association with potential targets in cervical cancer, and evaluate their cytotoxic effects on cervical cancer cells.

Study Design: This experimental study employed both *in vitro* and *in silico* methods to evaluate the potential cytotoxic effects of *Stichopus herrmanni* extracts on cervical cancer cells.

Methods: Liquid Chromatography-Mass Spectrometry (LC-MS) was used to identify and quantify the bioactive constituents of *Stichopus herrmanni* crude extract. An *in silico* approach was used to identify the active components, potential targets, and signaling pathways of the extract. Furthermore, the MTT assay was used to determine the cytotoxicity of the extract.

Results: LC-MS analysis identified the presence of rengyol, eucommiol, ganoderic acid, and 6-isoinosine in the extracts. An *in silico* study based on structural analysis identified ganoderic acid and isoinosine as crucial active components capable of regulating majority of the targets associated with cervical cancer. Overlapping targets, namely, CASP3, CAT, FASLG, IL24, TP53, TP53BP1, ALB, BDNF, and COX2 between cervical cancer and the extract highlighted

new therapeutic prospects for cervical cancer following protein-protein interaction network screening. The *in vitro* cytotoxic effects of the extracts were established in HeLa cells.

Conclusion: Research indicates that *Stichopus herrmanni* may be a valuable source of bioactive compounds with potential applications in the treatment of cancer and the induction of apoptosis.~~This study successfully identified four bioactive compounds in the ethanol extract of *S. herrmanni*, predominated by ganoderic acid, known for its antineoplastic properties. Structure activity relationship analysis revealed its potential anticancer properties. The findings also highlight the cytotoxic effects of *Stichopus herrmanni* extract on cervical cancer cells. Thus, the sea cucumber extract demonstrates promising therapeutic potential in the treatment of cervical cancer.~~¶

¶

Key words: cervical cancer, cytotoxic, ganoderic acid, LC-MS, *Stichopus herrmanni*

List of Abbreviations: ¶

ADME, Absorption, Distribution, Metabolism, and Excretion; ALB, Albumin; APCI, Atmospheric Pressure Chemical Ionization; Bax, Bcl-2-associated X protein; BCL2, B-cell lymphoma 2; BDNF, Brain-Derived Neurotrophic Factor; CASP3, Caspase 3; CASP9, Caspase 9; CAT, Catalase; CDK2, Cyclin-Dependent Kinase 2; CDK Inhibitor1, Cyclin-dependent kinase inhibitor 1; COX2, Cyclooxygenase-2; CTD, Comparative Toxicogenomics Database; DAVID, Database for Annotation, Visualization, and Integrated Discovery; DNA, Deoxyribonucleic Acid; FAK, Focal Adhesion Kinase; FASLG, Fas Ligand; HPLC, High-Performance Liquid Chromatography; IL24, Interleukin 24; JAK2, Janus Kinase 2; LC-MS, Liquid Chromatography-Mass Spectrometry; MTT Assay, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide Assay; PPI, Protein Interaction; SRC, Src Kinase; STAT3, Signal

Transducer and Activator of Transcription 3; TP53, Tumor Protein p53; TP53BP1, Tumor Protein p53 Binding Protein 1.¶

~~ADME: Absorption, Distribution, Metabolism, and Excretion~~¶

~~ALB: Albumin~~¶

~~APCI: Atmospheric Pressure Chemical Ionization APCI~~¶

~~Bax: Bcl-2-associated X protein~~¶

~~BCL2: B-cell lymphoma 2~~¶

~~BDNF: Brain-Derived Neurotrophic Factor~~¶

~~CASP3: Caspase 3~~¶

~~CASP9: Caspase 9~~¶

~~CAT: Catalase~~¶

~~CDK2: Cyclin-Dependent Kinase 2~~¶

~~CDK Inhibitor1: Cyclin-dependent kinase inhibitor 1~~¶

~~COX2: Cyclooxygenase-2~~¶

~~CTD: Comparative Toxicogenomics Database~~ ¶

~~DAVID: Database for Annotation, Visualization, and Integrated Discovery~~¶

~~DNA: Deoxyribonucleic Acid~~¶

~~FAK: Focal Adhesion Kinase~~¶

~~FASLG: Fas Ligand~~¶

~~HPLC: High-Performance Liquid Chromatography~~ ¶

~~IL24: Interleukin 24~~¶

~~JAK2: Janus Kinase 2~~¶

~~LC-MS: Liquid Chromatography-Mass Spectrometry~~¶

~~MTT Assay: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide Assay~~¶

~~PPI: Protein Interaction~~¶

~~SRC: Src Kinase~~¶

~~STAT3: Signal Transducer and Activator of Transcription 3~~¶

~~TP53: Tumor Protein p53~~¶

~~TP53BP1: Tumor Protein p53 Binding Protein 1~~¶

Introduction

Cervical cancer emerges as a significant health concern worldwide, predominantly originating from the cellular structures of the cervix. This concern is accentuated in underdeveloped regions, where limitations in regular screening and access to quality healthcare prevail (Denny et al., 2017). The year 2020 witnessed approximately 604.000 new instances of cervical cancer, culminating in over 342.000 deaths, thereby positioning it as the fourth most common cancer among women (Canfell et al., 2020). Traditional treatment modalities, including surgery, chemotherapy, and radiation therapy, are often associated with severe side effects such as potential organ damage and reduced fertility, thus necessitating the search for more effective and less harmful therapeutic alternatives (Derks et al., 2017; Debela et al., 2021).¶

In response to this need, network pharmacology emerges as a revolutionary approach, offering a multi-dimensional perspective on cervical cancer by outlining the complex network of molecular and cellular disruptions, particularly those induced by disruptive oncogenes such as HPV's E6 and E7. Highlighting the significance of delving into network dynamics influenced by HPV's integration into the host cell's DNA in the path to establishing more precisely effective therapeutic approaches. This methodology presents a holistic and systematic strategy for pinpointing therapeutic targets .(Zhao et al., 2018~~8; Bonab et al., 2021; Dovnik et al., 2023~~). Network Pharmacology emerges as an avant-garde field within drug discovery, amalgamating genomic technologies with computational biology to dissect complex biological systems, pharmaceutical agents, and disease mechanisms. Network Pharmacology employs

comprehensive data analyses to elucidate the bioactive mechanisms of substances, thereby facilitating the discovery of synergistic treatment effects (Ujianti et al., 2023).¶

Indonesia's rich marine biodiversity and historical reliance on natural medicinal substances are closely tied to its geographical and cultural fabric, especially around the Lombok island of Indonesia (Cui et al., 2016; Pratomo et al., 2022). Sea cucumbers, for instance, possess compounds like triterpene glycosides, chondroitin sulfates, peptides, and polysaccharides, garnering interest within various health-related fields due to their potential for exemplary therapeutic applications (Mackenzie et al., 2021; Napitupulu et al., 2022; Ujianti et al., 2023). While ongoing research delves into the broader anticancer potential of sea cucumbers, focused exploration of the *Stichopus herrmanni* species and its inherent bioactive compounds is currently limited (Debela et al., 2021; Ru et al., 2023; Fagbohun , O.F., et al., 2023). Our investigation, therefore, seeks to employ the principles of network pharmacology to delineate potential biological targets within the extract of *Stichopus herrmanni*, consequently unveiling more efficacious therapeutic strategies for treating cervical cancer (Dovnik et al., 2023).¶

Understanding the nuances of multidrug resistance in cancer, including the intricate molecular mechanisms and exploring avenues for immunoprevention and therapeutic approaches, underpin the exigency for innovative treatments (Emran, T.B. et al., 2022). Rauf's study showed Berberine stands out as a prime example of a natural compound with a compelling evidence base supporting its role as a potential anticancer agent, drawing attention to its mechanism of action and therapeutic efficacy in combating various cancer types, including cervical cancer (Rauf et al., 2021). Similarly, nNatural compounds produced by living organisms promote apoptosis and inhibit metastasis provides considerable insights into breast cancer treatment, setting a precedent for their application across a spectrum of cancers (D'arcy , , M.S., 2019; Islam et al., 2022). *In Silico* study by Akash's study et al showed the novel computational and drug design strategies for the inhibition of human papillomavirus-associated cervical cancer and DNA polymerase theta receptor by Apigenin derivatives

underscore the potential of targeted molecular interventions in mitigating the progression of cervical cancer (Akaash et al., 2023).¶

In our research, we aimed to integrate different methods into a unified approach. We conducted both computational and laboratory experiments to investigate the anti-cancer properties of *Stichopus hermanni* extract. Using a network pharmacology approach, we examined how the bioactive ingredients in the extract could work together and overcome resistance. These studies were then validated through laboratory tests. Our significant discovery was the identification of unique bioactive compounds in the *Stichopus hermanni* species, which paved the way for a novel treatment strategy for cervical cancer. Moreover, these findings have the potential to impact the treatment of various other cancer types.¶

~~Cervical cancer emerges as a significant health concern worldwide, predominantly originating from the cellular structures of the cervix. This concern is accentuated in underdeveloped regions, where limitations in regular screening and access to quality healthcare prevail (Denny et al., 2017). The year 2020 witnessed approximately 604,000 new instances of cervical cancer, culminating in over 342,000 deaths, thereby positioning it as the fourth most common cancer among women (Canfell et al., 2020). Traditional treatment modalities, including surgery, chemotherapy, and radiation therapy, are often associated with severe side effects such as potential organ damage and reduced fertility, thus necessitating the search for more effective and less harmful therapeutic alternatives (Derks et al., 2017; Debela et al., 2021).¶~~

¶

~~In response to this need, network pharmacology emerges as a revolutionary approach, offering a multi-dimensional perspective on cervical cancer by outlining the complex network of molecular and cellular disruptions, particularly those induced by disruptive oncogenes such as HPV's E6 and E7. Highlighting the significance of delving into network dynamics influenced by HPV's integration into the host cell's DNA in the path to establishing more precisely effective therapeutic approaches. This methodology presents a holistic and systematic strategy~~

~~for pinpointing therapeutic targets (Bonab et al., 2021; Dovnik et al., 2023) (Zhao et al., 2018). Network Pharmacology emerges as an avant-garde field within drug discovery, amalgamating genomic technologies with computational biology to dissect complex biological systems, pharmaceutical agents, and disease mechanisms. Network Pharmacology employs comprehensive data analyses to elucidate the bioactive mechanisms of substances, thereby facilitating the discovery of synergistic treatment effects. (Ujianti et.al, 2023)¶~~

~~Indonesia's rich marine biodiversity and historical reliance on natural medicinal. LinkedIn with its prevalence particularly around the Lombok island of Indonesia. (Pratomo et al., 2022). Sea cucumbers, for instance, possess compounds like triterpene glycosides, chondroitin sulfates, peptides, and polysaccharides, garnering interest within various health-related fields due to their potential for exemplary therapeutic applications. (Ujianti et.al, 2023; Napitupulu et al., 2022; Mackenzie et al., 2021). While ongoing research delves into the broader anticancer potential of sea cucumbers, focused exploration of the *Stichopus herrmanni* species and its inherent bioactive compounds is currently limited. Our investigation, therefore, seeks to employ the principles of network pharmacology to delineate potential biological targets within the extract of *Stichopus herrmanni*, consequently unveiling more efficacious therapeutic strategies for treating cervical cancer (Dovnik et al., 2023). ¶~~

~~In relation to our study, one of the recent accomplishments includes the identification of unique bioactive compounds in the *Stichopus herrmanni* species and the formulation of a novel approach in treating cervical cancer. The success of this approach is not only limited to the context of cervical cancer but also holds potential for its application in the treatment of other cancer types.¶~~

MATERIALS AND METHODS

Processing of Stichopus herrmanni Ethanol Extract

Stichopus herrmanni was sourced from the coastal regions of Nusa Tenggara, Indonesia. Harvested adult sea cucumbers underwent preparatory steps where their body walls were

isolated from internal organs, finely minced into approximately 1 cm pieces, and subsequently subjected to freeze-drying. The dried minced body wall was extracted using ethanol at a ratio of 1:5 (w/v), ensuring the extraction solvent type and concentration were consistent with the standard practices for herbal extract preparation. This phase aimed to denature cellular proteins and facilitate the liberation of secondary metabolites from the plant matrix. Following the extraction process, the solvent was carefully removed using a rotary evaporator set at 40°C, securing the extract for further analysis. (Sangpairoj et al., 2016)

High-Performance Liquid Chromatography (HPLC) was deployed to generate a fingerprint profile of the extract, a critical step in asserting the extract's consistency, quality, and pharmacological potential. The choice of marker compounds for quality assurance was determined based on their prominent pharmacological activities and relevance to the extract's therapeutic claims. The analytical methods employed for the extract's characterization were validated for their selectivity, accuracy, and precision, details of which are succinctly outlined to facilitate reproducibility

Liquid chromatography mass spectrometry analysis

For metabolite identification of *Stichopus herrmanni* ethanol extract, Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) was utilized. High-Performance Liquid Chromatography (HPLC) was conducted using an Agilent 1100 series pump equipped with an autosampler and vacuum degasser (Agilent, Palo Alto, CA). Separation was achieved with a fused-core C18-column (Walter, Milford, MA, USA) using an Atmospheric Pressure Chemical Ionization (APCI) source in positive ion mode. The mobile phase comprised acetonitrile and 0.1 % formic acid, flowing at 1 mL/min. The elution buffer's gradient was progressively increased from 30 to 60 % within 36 minutes. Mass spectrometry analyses were performed on an IONIC 3Q Series 200 molecular analyzer. The separated fractions were directly injected into the mass spectrometer at a flow rate of 20 μL/min. Ionization was facilitated in the

electrospray mode to ensure an efficient detection and identification process of the extracted metabolites

In silico Study

Screening of potentially active compounds in sea cucumber

Bioactive compounds in *Stichopus herrmanni* were identified using liquid chromatography-mass spectrometry (LC-MS). The SMILE profile and 3D structure of each compound were examined using the PubChem software.

Quantitative Structure-Activity Relationship Analysis

Bioactive compounds in *Stichopus herrmanni* were analyzed for their anticancer potential using the WAY2DRUG PASS prediction tool. This tool deploys a special type of analysis, known as Structure Activity Relationship (SAR), to compare input compounds with known compounds with a specific potential. The degree of similarity for the compound structures is proportional to the prediction value obtained. Compounds bearing similar structures are anticipated to exhibit parallel potentials. For this particular study, we set the cutoff value for Pa (Probability of being active) at > 0.7. The Pa value exceded this benchmark, the compound in question was considered to exhibit high anti-inflammatory potential, owing to its structural similarity to compounds in the database. (Druzhilovskiy et al., 2017)

Toxicity analysis of compounds

The potential toxicity of the bioactive compounds extracted from sea cucumbers was predicted using AdmetLAB 2.0, a powerful tool for assessing drug-like properties and predicting ADME (Absorption, Distribution, Metabolism, and Excretion) profiles of chemical compounds. AdmetLAB 2.0 incorporates a diverse range of computational models and databases to analyze and understand the safety profiles of compounds. This analysis included crucial parameters, prominently the Lipinski Rule of Five, which is a benchmark in drug discovery for evaluating the drug-likeness of compounds. (Xiong et al., 2021).

Prediction of protein targets

Targets associated with the bioactive compounds from *Stichopus herrmanni* were identified using the Comparative Toxicogenomics Database (CTD), selecting for targets with a scoring accuracy and probability greater than 80%. Target prediction was facilitated by the input of SMILES notation, acquired in the initial stage of the research. Relevant gene and protein information linked to cervical cancer were extracted from DisGeNet, focusing on candidates with an overall score prediction of 0.1 or higher. The disease-related targets and those identified from the sea cucumber extract were juxtaposed via a Venn diagram to pinpoint the intersecting targets. The functional attributes of the intersecting target compound were elaborated upon with the aid of the Database for Annotation, Visualization, and Integrated Discovery (DAVID).

Network analysis

The protein targets of ganoderic acid from *Stichopus herrmanni* ethanol extract were further analyzed using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING DB V.12.0). The following parameters were used: Organism: Homo sapiens; network type, Full STRING network; and required core, medium confidence (0.4). The data format TSV from STRING was then further processed using CytoScape V.10.0 for network analysis.

HeLa Cell Culture

This study was conducted with a focus on in vitro analysis, specifically utilizing HeLa cell lines. Ethical considerations were observed in line with general research ethics guidelines. The experimental protocols were approved by the Ethics Committee of Universitas Muhammadiyah Prof. Dr. Hamka (clearance number: IR. BPUMS. REC.1398.0).

HeLa cells were cultured in Roswell Park Memorial Institute 1640 medium supplemented with 10% (v/v) fetal bovine serum and 1% penicillin/streptomycin. Cells were maintained in a humidified atmosphere with 5% CO_2 at 37 °C, and passaged every 3 days following trypsinization with trypsin/EDTA

Cell Proliferation Analysis using MTT Assay

In the MTT assay, 5×10^3 HeLa cells were aliquoted into each well of a 96-well tissue culture plate. To each well, 100 μL of culture medium was added and the plate was incubated for 24 h, which allowed cell fixation to the bottom of the plate. Following this, varying doses of the *Stichopus herrmanni* ethanol extract (25, 50, 75, 100, and 125 μg/mL) were added to the medium, similar to previous studies. Doxorubicin (2.293 μg/mL) was used as a positive control, while the culture medium served as the negative control. After treatment for 24 h, the medium was drained and the wells were rinsed twice with 1× phosphate buffered saline. Subsequently, 100 μL of the MTT solution was added to each well and the plate was incubated for 4 h. Following this, 100 μL dimethyl sulfoxide was added to each well and the plate was incubated for an additional 20 min. The plates were quantified using a microplate reader calibrated at an absorbance of 570 nm.

Statistical Analyses

Data are presented as mean ± standard error of the mean. When data were normally distributed, statistical analyses between two groups were performed using an unpaired Student's *t*-test. Differences among groups were tested using one-way analysis of variance (ANOVA). A probability value of ($p < 0.05$) was considered to be statistically significant.

RESULTS

LC-MS Analysis

LC-MS analysis revealed that the ethanol extract from *Stichopus herrmanni* ethanol extract contained the following four primary unique compounds: rengyol, eucommiol, ganoderic acid, and 6-isoinosine (Figure S2 and Table S1). As shown in Figure 1 and 2, these molecules have been previously identified in the extract. Table 1 presents the LC-MS analysis results for the four compounds.

In silico Study

Screening for potentially active compounds in the sea cucumber extract

Table 2 presents identification of the four bioactive compounds from *Stichopus herrmanni* ethanol extract identified using LC-MS.

Quantitative Structure-Activity Relationship Analysis

The SAR analysis highlighted the promising potentials of bioactive compounds in sea cucumbers as agents for antineoplastic (Pa Score: 0.865) and chemo preventive (0.799) purposes. Chemo preventive agents, which can be either natural or synthetic, play a crucial role in preventing the development of cancer, its occurrence in high-risk patients, and the relapse in patients currently undergoing treatment. The analysis determined the antineoplastic potential by focusing on the well-established hallmarks of cancer. Notably, sea cucumbers emerged as the compounds with the highest potential for both chemo preventive and antineoplastic applications (Figure 3)

Toxicity Analysis

Toxicity analysis of each sea cucumber sample using the AdMet Lab2.0 webserver showed that all the four compounds found in the sea cucumber extract met the criteria for Lipinski's rule (Figure 4).

Network Analysis

Construction and Analysis of Target Protein-Protein Interaction (PPI) Network

The target genes pertaining to each component were analyzed using STRING v_11 to construct and visually represent the PPI network. The data for high-confidence target protein interactions was set with a score level exceeding 0.9, ensuring the connections being analyzed. Depicts these interactions among the target proteins, encompassing an overall 61 nodes and 288 edges. Each edge in this network symbolizes a Protein-Protein Interaction (PPI). Additional

parameters, including an average node degree of 5.64 and a local clustering coefficient of 0.439, represent the number of targets linked to the network.

Key targets implicated in cervical cancer—such as TP53, EGFR, MYC, AKT, CASP8, MTOR, JAK2, STAT3, ATM, CCND1, NOTCH1, HRAS—feature prominently within the network. Interestingly, these targets also play a major role in cervical cancer. TP53, AKT, and EGFR are centrally located within the network, underscoring their significant roles in the pathogenesis of cervical cancer. The PPI network and pathway analyses of novel genes were performed to identify critical genes related to cervical cancer

Arrangement and Construction of Disease-Target Network

Figure 6 showed a compound-target-disease interaction network. This network reveals drug action mechanisms within breast cancer treatment and comprises 61 interactive target proteins. More specifics on the role each target plays in the network structure are provided in Table 3, including topological measures such as betweenness centrality, closeness centrality, and degree

Prediction protein targets of Stichopus herrmanni for cervical cancer

Figure 5 shows the presence of the following nine overlapping protein targets between cervical cancer and sea cucumbers: CASP3, CAT, FASLG, IL24, TP53, TP53BP1, ALB, BDNF, and COX2. The oncogene MTOR promotes proliferative signaling and is involved in triggering invasion, metastasis, angiogenesis, the evasion of programmed cell death, and alterations in cellular energetics (Hallmarks of Cancer: Cosmic Database). (Bock , F.J., et al., 2020). Figure 6 shows the target pathway network of sea cucumber for treating cervical cancer.

GO gene enrichment analysis and KEGG pathway annotation

The GO and KEGG analyses identified the p53 and PI3K/AKT signaling pathways as significant in regulating cell proliferation and apoptosis, as illustrated in Figure 7.

MTT Proliferation Assay

HeLa cells were exposed to various concentrations of sea cucumber extract to assess their cytotoxic/cytostatic effects. A modest concentration of sea cucumber (25 µg/mL) showed a marginal decrease in cell viability to 94.4% after 24 hours. However, this reduction in cell viability was exacerbated with an increase in extract concentrations; treatment with 75, 125, and 300 µg/mL extract reduced cell viability to 88.1%, 86.37%, and 82.35%, respectively. (Figure 8) Overall, the sea cucumber extract showed minimal cytotoxic effects on HeLa cells. The IC50 values (signifying 50 % growth restraint) against HeLa cells for the sea cucumber extracts and doxorubicin, were 19057 µg/mL and 2293 ng/mL, respectively.

DISCUSSION

Modern pharmacology research strives to discover novel drugs and evaluate their efficacy against various diseases .–(Ujianti~~Almaliti~~ et al., 2023). –Recent research highlights the promising therapeutic potential of the *Stichopus hermanni* sea cucumber. The human papillomavirus (HPV) is the most common cause of cervical cancer globally. The mechanism of HPV infection involves a complex process, highlighting the roles of oncogenes E6 and E7. The E6 oncogene targets the p53 protein, a principal regulator of the cell cycle and guardian of the genome, as it induces apoptosis or programmed cell death upon DNA damage detection .(Akash et al., 2023). -By binding with the p53 protein, the E6 oncogene obstructs this protective function, allowing pre-cancerous cells to survive and multiply (Liu et al., 2019). Conversely, the E7 oncogene facilitates cell growth and proliferation by activating the PI3K/AKT/mTOR pathway, supporting the transformation of a cell into cancer (Liu et al., 2023).¶

Based on LCMS results of *Stichopus hermanni* extract, we have confirmed its anti-cancer effect both in silico and in vitro. Through Protein-Protein Interaction (PPI) analysis, genes such as TP53, EGFR, MYC, AKT, CASP8, MTOR, JAK2, STAT3, ATM, CCND1, NOTCH1, and HRAS are identified as central players in cervical cancer progression. Gene Ontology and KEGG analyses specifically found that cervical cancer progression involves the p53 and AKT pathways, validating literature that cervical cancer pathogenesis is primarily due to the disturbance of oncogenic proteins E6 and E7 in the p53 and PI3K/AKT pathways. Ganoderic acid, a triterpene molecule, has shown significant anti-cancer properties in many studies (Yang et al., 2018; Zhao et al., 2018; Ye et al., 2023). Its mechanism involves targeting tyrosine kinase receptors (RTK), crucial in cell migration, adhesion, apoptosis, metabolism, and proliferation in cancer (Liang et al., 2019; Ahmad et al., 2022; Galappaththi et al., 2023). Alterations in the signaling pathways of receptor tyrosine kinases (RTKs) can contribute to cancer development, highlighting the potential of ganoderic acid as a therapeutic agent. Mortazevie's research demonstrated the effects of ganoderic acid on apoptosis in Nalm-6 leukemia cells, aligning with other findings that suggest the potential of sea cucumber extract compounds in enhancing anticancer treatments (Mortazavie et al., 2022). A study by Cheng et al. found that ganoderic acid specifically enhances Bax and Caspase-3 protein expression, leading to apoptosis (Cheng et al., 2019). Fujiwara's study ~~et al.~~ discovered that Eucommicin A effectively inhibits cancer stem cells by targeting the JAK/STAT pathway (Fujiwara et al., 2016). Inosine, a base of isoinosine, possesses immunomodulatory properties, potentially contributing to anti-cancer effects. Additionally, 6-Isonosine effectively inhibits cancer cell growth by impeding DNA synthesis (Mane et al., 2020; Kovacef et al., 2021). Although not yet extensively researched, Rengyol, a molecule derived from cyclohexane, shows promise in oncology. Abdallah's study ~~et al. conducted a study~~ showe~~ding~~ Rengyol's capability to activate caspase-mediated apoptosis in human cancer cell lines (Abdallah et al., 2019).¶

Despite the relatively reduced efficacy observed in *Stichopus hermanni* extract compared to established chemotherapeutic agents like doxorubicin, our findings provide insightful revelations into unique anticancer mechanisms. Doxorubicin, a potent anthracycline chemotherapeutic, primarily delivers its cytotoxic effects by intercalating between DNA strands, thereby obstructing topoisomerase enzymatic activity. This disturbance ultimately impairs the replication and transcription DNA processes in malignant cells, culminating in their death.(Huang et al., 2023) In contrast, our research has unveiled that the mentioned extract operates through alternate pathways to selectively target neoplastic cells. Specifically, we have clarified *Stichopus hermanni* extract tendency to modulate the PI3K-p53 signaling cascade by ganoderic acid. It could be a potential synergy between ganoderic acid and doxorubicin as a multi-target approach in cancer treatment ~~.~~(Mbaveng et al., 2018; Islam et al., 2022).¶

Other insightful revelations from our study are about drug resistance mechanism. Cancer cells can develop drug resistance through various mechanisms, including mutations in the target protein, activation of bypass pathways, and epigenetic changes. In cervical cancer, immunotherapy may be indicated as a first-line therapy or as a second-line therapy after chemotherapeutic treatment fails ~~–~~(Emran et al., 2022). Isoinosine contained in sea cucumber extract can interact with immune cells and cancer cells, with the potential to address drug resistance mechanisms in cervical cancer cells ~~.~~(Kovacef ~~et al.~~, 2021). ~~–~~These bioactive compounds can enhance the immune response to chemotherapy-resistant cervical cancer cells in various ways. Isonosine can stimulate cytokine production or activate natural killer (NK) cells and T lymphocytes, vital in recognizing and destroying cancer cells. Besides, isonosine may synergize with conventional drugs. When used in conjunction with standard chemotherapy, this bioactive compound potentially can reduce overcome drug resistance in cancer cells.

In silico structural analysis highlighting ganoderic acid and isoinosine as active components against cervical cancer opens new avenues for research into their potential therapeutic application .(Akash et al., 2023). The compounds carry a resemblance with known antineoplastic and chemopreventive agents, highlighting the direct correlation between compound structures and their therapeutic targets. It is hoped that the combination of doxorubicin and traditional medicine bioactive material will increase therapy success .(Rauf at al., 2023). The synchronization of isoinosine and ganoderic acid with doxorubicin results in a multi-faceted therapeutic strategy, with isoinosine enhancing the immune response and ganoderic acid along with doxorubicin acting directly on cancer cells. This combination promises an innovative approach in treating cancer, enhancing treatment efficacy and potentially reducing the toxicity often associated with the standalone use of doxorubicin .(Emran et al., 2023). However, these findings require more extensive research to validate their effectiveness, establish an optimal dose, and understand their safety profile. This calls for a coherent research collaboration, carefully designed clinical trials, and in-depth scientific investigation to fully harness this potential synergy as a breakthrough in cancer therapy.¶

~~Network pharmacology explores the relationships between drugs and diseases. It investigates various drugs for different illnesses, aiming to discover new treatments and uncover unknown pathways that interact with bioactive compounds.(Almaliti et al., 2023) Recent research has uncovered a network of molecular activities in *Stichopus herrmanni*, suggesting its potential as a valuable treatment option. Liquid Chromatography-Mass Spectrometry (LCMS) analysis of *Stichopus herrmanni* yielded four bioactive compounds—rengyol, eucommiol, ganoderic acid, and 6-isoinosine—promising for further exploration in cervical cancer management. Network analysis highlighted *Stichopus herrmanni* bioactives on the cervical cancer pathway by mediating interactions with 61 proteins that inhibit cell proliferation and prompt apoptosis,~~

indicating their therapeutic utility. (Ahmad et al., 2022) (Chen et al., 2024) Pivotal genes such as TP53, EGFR, MYC, AKT, CASP8, MTOR, JAK2, STAT3, ATM, CCND1, NOTCH1, and HRAS were delineated through Protein-Protein Interaction (PPI) analysis, which presented 61 nodes and 288 edges, as central to cervical cancer progression. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were integral in relating these genes to broader disease contexts. The GO enrichment analysis specifically tied the bioactives to cervical cancer control, and KEGG analysis signaled the pertinence of p53 and AKT pathways. Quantitative comparisons affirmed the structural congruence of *Stichopus herrmanni* compounds with known antineoplastic and chemopreventive agents, reinforcing the direct correlation between compound structures and their therapeutic targets. In vitro study in our result showed decreased cell viability. This result attested to *Stichopus herrmanni's* capability to restrain cell proliferation and induce apoptosis in HeLa cells, thereby substantiating its potential role in cervical cancer therapy and necessitating extended research.¶

Our result of *in vitro* and *in silico* research on the effects of *Stichopus herrmanni* extract is supported by various studies. Ganoderic acid, a triterpene derived from the *Ganoderma lucidum* mushroom, has shown notable anticancer properties in various studies. Its mechanism of action involves targeting receptor tyrosine kinases (RTKs), which play critical roles in cell migration, adhesion, apoptosis, metabolism, and proliferation in cancer. Aberration in RTK signaling can lead to cancer, suggesting that ganoderic acid's ability to modulate these pathways may offer therapeutic benefits. A study conducted by Cheng et al. found that ganoderic acid specifically enhances the expression of the Bax protein and Caspase-3, leading to apoptosis. (Cheng et al., 2019) Fujiwara et al. discovered that Eucommicin A effectively inhibits cancer stem cells by targeting the JAK/STAT pathway.(Fujiwara et al., 2016) Inosine, the base of isoinosine, has been shown to have immunomodulatory properties, which could potentially contribute to anti-cancer effects. Additionally, 6-Isonosine has been shown to

effectively hinder the growth of cancer cells by inhibiting DNA.(Kovacef et al., 2021)(Mane et al., 2020) Although not extensively researched, Rengyol, a molecule derived from cyclohexane, shows promise in the field of oncology. Abdallah et al. conducted research demonstrating Rengyol's ability to activate caspase-mediated apoptosis in human cancer cell lines.(Abdallah et al., 2019)¶

Despite the relatively diminished efficacy observed in *Stichopus herrmanni* extract as compared to the established chemotherapeutic agent doxorubicin, our findings yield valuable insights into its unique anticancer mechanism. Doxorubicin, a potent anthracycline chemotherapeutic, primarily exerts its cytotoxic effects by intercalating between DNA strands, thereby obstructing the enzymatic activity of topoisomerase. This interference ultimately impairs DNA replication and transcription processes in malignant cells, culminating in their demise.(Huang et al., 2023) Conversely, our research has revealed that the aforementioned extract operates via an alternative pathway to selectively target neoplastic cells. Specifically, we have elucidated its propensity for augmenting the generation and efficacy of T lymphocytes while concurrently modulating the PI3K-p53 signaling cascade. The overlapping targets identified between cervical cancer and the extracts containing these compounds underscore the multi-targeted approach in cancer treatment. *In silico* structural analysis highlighting ganoderic acid and isoinosine as active components against cervical cancer opens new avenues for research into their potential therapeutic application. The compounds carry a resemblance with known antineoplastic and chemopreventive agents, highlighting the direct correlation between compound structures and their therapeutic targets. However, in order to further enhance the comprehensiveness of this research, a number of areas needed. Furthermore, conducting *in vivo* studies can provide insights into the systemic pathway, thereby filling an important knowledge gap. The exploration of marine natural product extracts presents exciting

~~prospects for the development of innovative cancer therapies, instilling hope among patients worldwide.¶~~

Conclusion

In conclusion, our comprehensive analysis combining both computational strategies and laboratory experiments has underscored the promising anticancer capabilities of Stichopus hermanni. Focused investigation into its impact on crucial oncogenic pathways, specifically the PI3K/AKT and the p53 pathways, alongside its potential for immunomodulation, reveals its multifaceted role in cancer therapy. ~~Through computational and laboratory experiments, we have identified the potential of *Stichopus hermanni* as an anti-cancer candidate. Our studies specifically focused on its effects on the PI3K/AKT pathway and the p53 pathway, as well as its role in immunomodulation.~~ Further empirical studies are necessary to validate these findings and understand the mechanisms by which these compounds may exert their effects on cancer cells.

Acknowledgements

We extend our heartfelt gratitude to the Faculty of Medicine at Universitas Muhammadiyah Prof. Dr. Hamka for the generous support that enabled this research to be realized. We would also like to acknowledge and convey our appreciation to all individuals who have provided assistance throughout this research process.

Declaration of Interest

The authors declare no competing interests

Author contributions

Each contributor played key roles in this research: IU designed the study and contributed to data acquisition and analysis; BSL assisted in data acquisition, analysis, and manuscript revision; ZN was pivotal in data analysis, result interpretation, and manuscript revision; whilst WS handled manuscript preparation and figure/table design.

Funding

This study was financially supported by the Research Institute of Universitas Muhammadiyah Prof. Dr. Hamka, under contract number 594/F.03.07/2023.

References

Abdallah, W.H., Salman, A., Sabry, S.S., 2019. Anticancer activity of newly synthesized 1,1-disubstituted cyclohexane-1-carboxamides: in vitro caspases mediated apoptosis activators in human cancer cell lines and their molecular modeling. Drug Dev Res 80(7), 933-947. https://doi.org/10.1002/ddr.21573¶

Ahmad, M.F., Wahab, S., Ahmad, F.A., Ashraf, S.A., Abullais, S.S., Saad, H.H., 2022. Ganoderma lucidum: a potential pleiotropic approach of ganoderic acids in health reinforcement and factors influencing their production. Fungal Biol Rev 39, 100–125. https://doi.org/10.1016/j.fbr.2021.12.003¶

Akash, S., Bayıl, I., Hossain, M. S., et al., 2023. Novel computational and drug design strategies for inhibition of human papillomavirus-associated cervical cancer and DNA polymerase theta receptor by Apigenin derivatives. Sci Rep 13, 16565. https://doi.org/10.1038/s41598-023-43175-x ¶

Bock, F.J., Tait, S.W.G., 2020. Mitochondria as multifaceted regulators of cell death. Nat Rev Mol Cell Biol 21, 85–100. https://doi.org/10.1038/s41580-019-0173-8¶

Canfell, K., Kim, J.J., Brisson, M., Keane, A., Simms, K.T., Caruana, M., et al., 2020. Mortality impact of achieving WHO cervical cancer elimination targets: a comparative modelling analysis in 78 low-income and lower-middle-income countries. Lancet 395, 591–603. https://doi.org/10.1016/S0140-6736(20)30157-4¶

Cheng, Y., & Xie, P. (2019). Ganoderic acid A holds promising cytotoxicity on human glioblastoma mediated by incurring apoptosis and autophagy and inactivating PI3K/AKT signaling pathway. Journal of Biochemical and Molecular Toxicology,33(11), e22392. https://doi.org/https://doi.org/10.1002/jbt.22392¶

Cui, C., Wang, P., Cui, N., Song, S., Liang, H., Ji, A., 2016. Stichopus japonicus Polysaccharide, Fucoidan, or Heparin Enhanced the SDF-1α/CXCR4 Axis and Promoted NSC Migration via Activation of the PI3K/Akt/FOXO3a Signaling Pathway. Cell Mol Neurobiol 36, 1311–1329. https://doi.org/10.1007/s10571-016-0329-4¶

D'arcy, M.S., 2019. Cell death: a review of the major forms of apoptosis, necrosis and autophagy. Cell Biol Int 43, 582–592. https://doi.org/10.1002/cbin.11137¶

Debela, D.T., Muzazu, S.G.Y., Heraro, K.D., Ndalama, M.T., Mesele, B.W., Haile, D.C., et al., 2021. New approaches and procedures for cancer treatment: Current perspectives. SAGE Open Med. 9, 20503121211034366. https://doi.org/10.1177/20503121211034366¶

Denny, L., de Sanjose, S., Mutebi, M., Anderson, B.O., Kim, J., Jeronimo, J., et al., 2017. Interventions to close the divide for women with breast and cervical cancer between low-income and middle-income countries and high-income countries. Lancet. 389, 861–870. https://doi.org/10.1016/S0140-6736(16)31795-0¶

Derks, M., van Lonkhuijzen, L.R.C.W., Bakker, R.M., Stiggelbout, A.M., de Kroon, C.D., Westerveld, H., et al., 2017. Long-term morbidity and quality of life in cervical cancer survivors: a multicenter comparison between surgery and radiotherapy as primary treatment. Int J Gynecol Cancer. 27, 350–356. https://doi.org/10.1097/IGC.0000000000000880¶

Druzhilovskiy, D.S., Rudik, A.V., Filimonov, D.A., Gloriozova, T.A., Lagunin, A.A., Dmitriev, A.V., Poroikov, V., 2017. Computational platform Way2Drug: from the prediction of biological activity

to drug repurposing. Russian Chemical Bulletin. 66, 1832-1841. https://doi.org/10.1007/s11172-017-1954-x ¶

Emran, T. B., Shahriar, A., Mahmud, A. R., Rahman, T., Abir, M. H., Siddiquee, M., Hassan, M. M. 2022. Multidrug resistance in cancer: Understanding molecular mechanisms, immunoprevention, and therapeutic approaches. Frontiers in Oncology. 12, 891652. https://doi.org/10.3389/fonc.2022.891652¶

Fagbohun, O.F., Joseph, J.S., Oriyomi, O.V., Rupasinghe, H.V., et al., 2023. Saponins of North Atlantic Sea Cucumber: Chemistry, Health Benefits, and Future Prospectives. Marine Drugs. 21(5), 262. https://doi.org/10.3390/md21050262¶

Fujiwara, A., Nishi, M., Yoshida, S., Hasegawa, M., Yasuma, C., Ryo, A., & Suzuki, Y. 2016. Eucommicin A, a β-truxinate lignan from Eucommia ulmoides, is a selective inhibitor of cancer stem cells. Phytochemistry, 122, 139–145 https://doi.org/https://doi.org/10.1016/j.phytochem.2015.11.017¶

Galappaththi, M.C.A., Patabendige, N.M., Premarathne, B.M., Hapuarachchi, K.K., Tibpromma, S., Dai, D. Q., et al., 2023. A Review of Ganoderma Triterpenoids and Their Bioactivities. Biomolecules 13, 1–68. https://doi.org/10.3390/biom13010024¶

Islam, M.R., Islam, F., Nafady, M.H., Akter, M., Mitra, S., Das, R., Urmee, H., Shohag, S., Akter, A.; Chidambaram, K., 2022. Natural small molecules in breast cancer treatment: Understandings from a therapeutic viewpoint. Molecules, 27(7), 2165. https://doi.org/10.3390/molecules27072165¶

Kovachev, S.M., 2021. A Review on Inosine Pranobex Immunotherapy for Cervical HPV-Positive Patients, Infection and Drug Resistance, , 2039-2049, DOI: 10.2147/IDR.S296709¶

Liang, C., Tian, D., Liu, Y., Li, H., Zhu, J., Li, M., et al., 2019. Review of the molecular mechanisms of Ganoderma lucidum triterpenoids: Ganoderic acids A, C2, D, F, DM, X and Y. Eur J Med Chem 174, 130–141. https://doi.org/10.1016/j.ejmech.2019.04.039¶

Liu, J., Zhang, C., Hu, W., Feng, Z., 2019. Tumor suppressor p53 and metabolism. J Mol Cell Biol 11, 284–292. https://doi.org/10.1093/jmcb/mjy070¶

Liu, F., Xu, J., Yang, R., Liu, S., Hu, S., Yan, M., et al., 2023. New light on treatment of cervical cancer: Chinese medicine monomers can be effective for cervical cancer by inhibiting the PI3K/Akt signaling pathway. Biomed Pharmacother 157, 114084. https://doi.org/10.1016/j.biopha.2022.114084¶

Mackenzie, M., O'Loughlin, P.M., Griffiths, H., Van, D.P., 2021. Sea cucumbers (Echinodermata, Holothuroidea) from the JR275 expedition to the eastern Weddell Sea, Antarctica. Zookeys 1054, 155–172. https://doi.org/10.3897/zookeys.1054.59584¶

Mbaveng, A.T., Fotso, G.W., Ngnintedo, D., Kuete, V., Ngadjui, B.T., Keumedjio, F., et al., 2018. Cytotoxicity of epunctanone and four other phytochemicals isolated from the medicinal plants Garcinia epunctata and Ptycholobium contortum towards multi-factorial drug resistant cancer cells. Phytomedicine 48, 112–119. https://doi.org/10.1016/j.phymed.2017.12.016¶

Mortazavie, F., Taheri, S., Tandel, P., Zare, F., Tamaddon, G., 2022. The effect of Ganoderic Acid A on miR-17-5p and miR-181b expression level and apoptosis induction in human leukemia Nalm-6 cells. Iran J Pediatr Hematol Oncol 12, 152–163. https://doi.org/10.18502/ijpho.v12i3.10058¶

Napitupulu, L., Tanaya, S.S., Ayostina, I., Andesta, I., Fitriana, R., Ayunda, D., et al., 2022. Trends in Marine Resources and Fisheries Management in Indonesia: A Review. World ResInst. http://wri-indonesia.org/sites/default/files/2022 ¶

Pratomo, A., Bengen, D.G., Zamani, N.P., Madduppa, H., 2022. Environmental DNA Metabarcoding Reveals the Eukaryotes Diversity in Marine Protected Area of Lombok Island, Indonesia. Omni-Akuatika. 18, 137–152. http://dx.doi.org/10.20884/1.oa.2022.18.2.1009¶

Rauf, A., Abu-Izneid, T., Khalil, A. A., Imran, M., Shah, Z. A., Emran, T. B., Mitra, S., Khan, Z., Alhumaydhi, F. A., & Aljohani, A. S. M. 2021. Berberine as a potential anticancer agent: A comprehensive review. Molecules, 26, 7368. https://doi.org/10.3390/molecules26237368¶

Ru, R., Chen, G., Liang, X., Cao, X., Yuan, L., & Meng, M. (2023). Sea Cucumber Derived Triterpenoid Glycoside Frondoside A: A Potential Anti-Bladder Cancer Drug. Nutrients, 15(2), 378. https://doi.org/10.1186/ 10.3390/nu15020378 ¶

Sangpairoj, K., Chaithirayanon, K., Vivithanaporn, P., et al,. 2016. Extract of the sea cucumber, Holothuria scabra, induces apoptosis in human glioblastoma cell lines. *Funct. Foods Health Dis*, *6*, 452-468. https://doi.org/10.31989/ffhd.v6i7.264¶

Ujianti, I., Lakshmi, B.S., Nurusshofa, Z., Sukarya, W., Indriyanti, L., 2023. Network Pharmacology Analysis Reveals Bioactive Compounds and Potential Targets of Sea cucumber for Cervical Cancer Therapy. F1000Research. 12, 1358. https://doi.org/10.12688/f1000research.138298.1¶

Ujianti,—I., Lakshmi,B.S., Nurusshofa,Z., Stujanna,E.N., 2023. Bioactive Compound of Holothoroidea. 1st ed. Sukarya, W.S. (Ed.). CV WIDINA MEDIA UTAMA, pp. 1–60.¶

Xiong, G., Wu, Z., Yi, J., Fu, L., Yang, Z., Hsieh, C., Cao, D. 2021. ADMETlab 2.0: an integrated online platform for accurate and comprehensive predictions of ADMET properties. Nucleic Acids Res. 49(W1), W5-W14. https://doi.org/10.1093/nar/gkab255 ¶

Yang, Y., Zhou, H., Liu, W., Wu, J., Yue, X., Wang, J., et al., 2018. Ganoderic acid A exerts antitumor activity against MDA-MB-231 human breast cancer cells by inhibiting the Janus kinase 2/signal transducer and activator of transcription 3 signaling pathway. Oncol Lett 16, 6515–6521. https://doi.org/10.3892/ol.2018.9475¶

Ye, T., Ge, Y., Jiang, X., Song, H., Peng, C., Liu, B., 2023. A review of anti-tumour effects of Ganoderma lucidum in gastrointestinal cancer ~~r.~~ Chin Med 18, 107. https://doi.org/10.1186/s13020-023-00811-y¶

Zhang, M., Zheng, J., Nussinov, R., Ma, B., 2017. Release of cytochrome C from Bax pores at the mitochondrial membrane. Sci Rep 7, 2635. https://doi.org/10.1038/s41598-017-02825-7¶

Zhao, X., Zhou, D., Liu, Y., Li, C., Zhao, X., Li, Y., et al., 2018. Ganoderma lucidum polysaccharide inhibits prostate cancer cell migration via the protein arginine methyltransferase 6 signaling pathway. Mol Med Rep 17, 147–157. https://doi.org/10.3892/mmr.2017.7904¶

~~Ahmad, M.F., Wahab, S., Ahmad, F.A., Ashraf, S.A., Abullais, S.S., Saad, H.H., 2022. Ganoderma lucidum: a potential pleiotropic approach of ganoderic acids in health reinforcement and factors influencing their production. Fungal Biol Rev 39, 100–125. https://doi.org/10.1016/j.fbr.2021.12.003¶~~

Akash, S., Bayıl, I., Hossain, M. S., et al., 2023. Novel computational and drug design strategies for inhibition of human papillomavirus-associated cervical cancer and DNA polymerase theta receptor by Apigenin derivatives. Sci Rep 13, 16565. https://doi.org/10.1038/s41598-023-43175-x ¶

¶

Bock, F.J., Tait, S.W.G., 2020. Mitochondria as multifaceted regulators of cell death. Nat Rev Mol Cell Biol 21, 85–100. https://doi.org/10.1038/s41580-019-0173-8¶

Canfell, K., Kim, J.J., Brisson, M., Keane, A., Simms, K.T., Caruana, M., et al., 2020. Mortality impact of achieving WHO cervical cancer elimination targets: a comparative modelling analysis in 78 low-income and lower-middle-income countries. Lancet 395, 591–603. https://doi.org/10.1016/S0140-6736(20)30157-4¶

Cheng, Y., & Xie, P. (2019). Ganoderic acid A holds promising cytotoxicity on human glioblastoma mediated by incurring apoptosis and autophagy and inactivating PI3K/AKT signaling pathway. Journal of Biochemical and Molecular Toxicology,33(11), e22392. https://doi.org/https://doi.org/10.1002/jbt.22392¶

Cui, C., Wang, P., Cui, N., Song, S., Liang, H., Ji, A., 2016. Stichopus japonicus Polysaccharide, Fucoidan, or Heparin Enhanced the SDF-1α/CXCR4 Axis and Promoted NSC Migration via Activation of the PI3K/Akt/FOXO3a Signaling Pathway. Cell Mol Neurobiol 36, 1311–1329. https://doi.org/10.1007/s10571-016-0329-4¶

D'arcy, M.S., 2019. Cell death: a review of the major forms of apoptosis, necrosis and autophagy. Cell Biol Int 43, 582–592. https://doi.org/10.1002/cbin.11137¶

Debela, D.T., Muzazu, S.G.Y., Heraro, K.D., Ndalama, M.T., Mesele, B.W., Haile, D.C., et al., 2021. New approaches and procedures for cancer treatment: Current perspectives. SAGE Open Med. 9, 20503121211034366. https://doi.org/10.1177/20503121211034366¶

Denny, L., de Sanjose, S., Mutebi, M., Anderson, B.O., Kim, J., Jeronimo, J., et al., 2017. Interventions to close the divide for women with breast and cervical cancer between low-income and middle-income countries and high-income countries. Lancet. 389, 861–870. https://doi.org/10.1016/S0140-6736(16)31795-0¶

Derks, M., van Lonkhuijzen, L.R.C.W., Bakker, R.M., Stiggelbout, A.M., de Kroon, C.D., Westerveld, H., et al., 2017. Long-term morbidity and quality of life in cervical cancer survivors: a multicenter comparison between surgery and radiotherapy as primary treatment. Int J Gynecol Cancer 27, 350–356. https://doi.org/10.1097/IGC.0000000000000880¶

Druzhilovskiy, D.S., Rudik, A.V., Filimonov, D.A., Gloriozova, T.A., Lagunin, A.A., Dmitriev, A.V., Poroikov, V., 2017. Computational platform Way2Drug: from the prediction of biological activity to drug repurposing. Russian Chemical Bulletin. 66, 1832-1841. https://doi.org/10.1007/s11172-017-1954-x¶

Emran, T. B., Shahriar, A., Mahmud, A. R., Rahman, T., Abir, M. H., Siddiquee, M., Hassan, M. M. (2022). Multidrug resistance in cancer: Understanding molecular mechanisms, immunoprevention, and therapeutic approaches. Frontiers in Oncology. 12, 891652. https://doi.org/10.3389/fonc.2022.891652¶

Fagbohun, O.F., Joseph, J.S., Oriyomi, O.V., Rupasinghe, H.V., et .al., 2023. Saponins of North Atlantic Sea Cucumber: Chemistry, Health Benefits, and Future Prospectives. Marine Drugs, 21(5), 262. https://doi.org/10.3390/md21050262¶

Fujiwara, A., Nishi, M., Yoshida, S., Hasegawa, M., Yasuma, C., Ryo, A., & Suzuki, Y. 2016. Eucommicin A, a β-truxinate lignan from Eucommia ulmoides, is a selective inhibitor of cancer stem cells. Phytochemistry, 122, 139–145 https://doi.org/https://doi.org/10.1016/j.phytochem.2015.11.017¶

Galappaththi, M.C.A., Patabendige, N.M., Premarathne, B.M., Hapuarachchi, K.K., Tibpromma, S., Dai, D. Q., et al., 2023. A Review of Ganoderma Triterpenoids and Their Bioactivities. Biomolecules 13, 1–68. https://doi.org/10.3390/biom13010024¶

Gill, B. S., Kumar, S., Navgeet, 2016. Triterpenes in cancer: significance and their influence. Mol Biol Rep 43, 881–896. Islam, M.R., Islam, F., Nafady, M.H., Akter, M., Mitra, S., Das, R., Urmee, H., Shohag, S., Akter, A.; Chidambaram, K., 2022. Natural small molecules in breast cancer treatment: Understandings from a therapeutic viewpoint. Molecules, 27(7), 2165. ¶

Janakiram, N.B., Mohammed, A., Rao, C.V., 2015. Sea cucumbers metabolites as potent anti-cancer agents. Mar Drugs 13, 2909–2923. https://doi.org/10.3390/md13052909¶

Liang, C., Tian, D., Liu, Y., Li, H., Zhu, J., Li, M., et al., 2019. Review of the molecular mechanisms of Ganoderma lucidum triterpenoids: Ganoderic acids A, C2, D, F, DM, X and Y. Eur J Med Chem 174, 130–141. https://doi.org/10.1016/j.ejmech.2019.04.039¶

Liby, K.T., Yore, M.M., Sporn, M.B., 2007. Triterpenoids and rexinoids as multifunctional agents for the prevention and treatment of cancer. Nat Rev Cancer 7, 357–369. https://doi.org/10.1038/nrc2129¶

Liu, J., Zhang, C., Hu, W., Feng, Z., 2019. Tumor suppressor p53 and metabolism. J Mol Cell Biol 11, 284–292. https://doi.org/10.1093/jmcb/mjy070¶

Liu, F., Xu, J., Yang, R., Liu, S., Hu, S., Yan, M., et al., 2023. New light on treatment of cervical cancer: Chinese medicine monomers can be effective for cervical cancer by inhibiting the PI3K/Akt signaling pathway. Biomed Pharmacother 157, 114084. https://doi.org/10.1016/j.biopha.2022.114084¶

Mackenzie, M., O'Loughlin, P.M., Griffiths, H., Van, D.P., 2021. Sea cucumbers (Echinodermata, Holothuroidea) from the JR275 expedition to the eastern Weddell Sea, Antarctica. Zookeys 1054, 155–172. https://doi.org/10.3897/zookeys.1054.59584¶

Manta-Vogli, P.D., Schulpis, K.H., Loukas, Y.L., Dotsikas, Y., 2020. Perinatal free carnitine and short chain acylcarnitine blood concentrations in 12,000 full-term breastfed newborns in relation to their birth weight. Pediatr Neonatol 61, 620–628. https://doi.org/10.1016/j.pedneo.2020.07.015¶

Mbaveng, A.T., Fotso, G.W., Ngnintedo, D., Kuete, V., Ngadjui, B.T., Keumedjio, F., et al., 2018. Cytotoxicity of epunctanone and four other phytochemicals isolated from the medicinal plants Garcinia epunctata and Ptycholobium contortum towards multi-factorial drug-resistant cancer cells. Phytomedicine 48, 112–119. https://doi.org/10.1016/j.phymed.2017.12.016¶

Mercier, A., Hamel, J. F., 2013. Sea cucumber aquaculture: hatchery production, juvenile growth and industry challenges. In: Advances in aquaculture hatchery technology. Woodhead Publishing. pp. 431–454.¶

Mortazavie, F., Taheri, S., Tandel, P., Zare, F., Tamaddon, G., 2022. The effect of Ganoderic Acid A on miR-17-5p and miR-181b expression level and apoptosis induction in human leukemia Nalm-6 cells. Iran J Pediatr Hematol Oncol 12, 152–163. https://doi.org/10.18502/ijpho.v12i3.10058¶

Napitupulu, L., Tanaya, S.S., Ayostina, I., Andesta, I., Fitriana, R., Ayunda, D., et al., 2022. Trends in Marine Resources and Fisheries Management in Indonesia: A Review. World Res Inst.¶

Oh, G. W., Ko, S. C., Lee, D.H., Heo, S. J., Jung, W. K., 2017. Biological activities and biomedical potential of sea cucumber (Stichopus japonicus): a review. Fish Aquat Sci. 20, 1–17. https://doi.org/10.1186/s41240-017-0071-y¶

Pratomo, A., Bengen, D.G., Zamani, N.P., Madduppa, H., 2022. Environmental DNA Metabarcoding Reveals the Eukaryotes Diversity in Marine Protected Area of Lombok Island, Indonesia. Omni-Akuatika. 18, 137–152. http://dx.doi.org/10.20884/1.oa.2022.18.2.1009¶

Rauf, A., Abu-Izneid, T., Khalil, A. A., Imran, M., Shah, Z. A., Emran, T. B., Mitra, S., Khan, Z., Alhumaydhi, F. A., & Aljohani, A. S. M. 2021. Berberine as a potential anticancer agent: A comprehensive review. Molecules, 26, 7368. https://doi.org/10.3390/molecules26237368¶

Ru, R., Chen, G., Liang, X., Cao, X., Yuan, L., & Meng, M. (2023). Sea Cucumber Derived Triterpenoid Glycoside Frondoside A: A Potential Anti-Bladder Cancer Drug. Nutrients, 15(2), 378. https://doi.org/10.1186/ 10.3390/nu15020378 ¶

Ru, R., Guo, Y., Mao, J., Yu, Z., Huang, W., Cao, X., et al., 2022. Cancer Cell Inhibiting Sea Cucumber (Holothuria leucospilota) Protein as a Novel Anti-Cancer Drug. Nutrients 14, 1–18. https://doi.org/10.3390/nu14040786¶

Sangpairoj, K., Chaithirayanon, K., Vivithanaporn, P., et .al,. 2016. Extract of the sea cucumber, Holothuria scabra, induces apoptosis in human glioblastoma cell lines. *Funct. Foods Health Dis*, *6*, 452-468. https://doi.org/10.31989/ffhd.v6i7.264¶

Sangwan, K., Sharma, V., Goyal, K. P., 2023. Pharmacological Profile of Novel Anti-cancer Drugs approved by USFDA in 2022: A Review. Curr Mol Med https://doi.org/10.2174/1566524023666230622151034 ¶

Ujianti, I., Lakshmi, B.S., Nurusshofa, Z., Sukarya, W., Indriyanti, L., 2023. Network Pharmacology Analysis Reveals Bioactive Compounds and Potential Targets of Sea cucumber for Cervical Cancer Therapy. F1000Research. 12, 1358. https://doi.org/10.12688/f1000research.138298.1¶

Ujianti, I., Lakshmi,B.S., Nurusshofa,Z., Stujanna,E.N., 2023. Bioactive Compound of Holothoroidea. 1st ed. Sukarya, W.S. (Ed.). CV WIDINA MEDIA UTAMA, pp. 1–60.¶

Xiong, G., Wu, Z., Yi, J., Fu, L., Yang, Z., Hsieh, C., Cao, D. 2021. ADMETlab 2.0: an integrated online platform for accurate and comprehensive predictions of ADMET properties. Nucleic Acids Res. 49(W1), W5-W14. https://doi.org/10.1093/nar/gkab255 ¶

Yang, Y., Zhou, H., Liu, W., Wu, J., Yue, X., Wang, J., et al., 2018. Ganoderic acid A exerts antitumor activity against MDA-MB-231 human breast cancer cells by inhibiting the Janus kinase 2/signal transducer and activator of transcription 3 signaling pathway. Oncol Lett 16, 6515–6521. https://doi.org/10.3892/ol.2018.9475¶

Ye, T., Ge, Y., Jiang, X., Song, H., Peng, C., Liu, B., 2023. A review of anti-tumour effects of Ganoderma lucidum in gastrointestinal cancer. Chin Med 18, 107. https://doi.org/10.1186/s13020-023-00811-y¶

Zhang, M., Zheng, J., Nussinov, R., Ma, B., 2017. Release of cytochrome C from Bax pores at the mitochondrial membrane. Sci Rep 7, 2635. https://doi.org/10.1038/s41598-017-02825-7¶

~~Zhao, X., Zhou, D., Liu, Y., Li, C., Zhao, X., Li, Y., et al., 2018. Ganoderma lucidum polysaccharide inhibits prostate cancer cell migration via the protein arginine methyltransferase 6 signaling pathway. Mol Med Rep 17, 147–157. https://doi.org/10.3892/mmr.2017.7904~~

TABLE LEGENDS

Table 1. Liquid chromatography-mass spectrometry analysis of the sea cucumber extract

Table 2. Profile of the bioactive compounds in the sea cucumber extract

Table 3. Important nodes with network analyzer result

FIGURE LEGENDS

Figure 1. Chromatogram for high performance liquid chromatography analysis of the sea cucumber extract, showing signal intensity in relation to the retention time for the compounds.

Figure 2. Mass spectrum for the sea cucumber extract showing signal intensity in relation to molecular weight. The peaks in the graph represent the detected compounds.

Figure 3. SAR-based prediction for the potential of sea cucumber as an anticancer and chemopreventive agent

Figure 4. Toxicity analysis of the sea cucumber extract

Figure 5. Venn Diagram depicting the intersection of cervical cancer and *Stichopus* spp.

Figure 6. Target pathway network of the sea cucumber extract for treating cervical cancer

Figure 7. Gene ontology and KEGG pathway enrichment analysis

Figure 8. Cell viability analysis using the MTT assay