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Evaluation of the potential of *Stichopus Herrmanni* extract in inhibiting cervical cancer cell proliferation

Irena Ujianti [a,*], Bety Semara Lakhsmi [b], Zahra Nurusshofa [c], Wawang S Sukarya [d]

[a] Department of Medical Physiology, Faculty of Medicine, Universitas Muhammadiyah Prof.Dr. Hamka, Jakarta, Indonesia
[b] Department of Public Health, Faculty of Medicine, Universitas Muhammadiyah Prof.Dr. Hamka, Jakarta, Indonesia
[c] Department of Pathology Anatomy, Faculty of Medicine, Universitas Muhammadiyah Prof.Dr. Hamka, Jakarta, Indonesia
[d] Department of Obstetric Gynecology, Faculty of Medicine, Universitas Muhammadiyah Prof.Dr. Hamka, Jakarta, Indonesia

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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

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

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.

* Corresponding author.
E-mail address: irenaujianti@uhamka.ac.id (I. Ujianti).

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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, D.T., 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 (Zhao et al., 2018; 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 (I. 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; I. 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 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.

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 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, natural 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, 2019; Islam et al., 2022). *In Silico* study by Akash 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.

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 min. 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 exceeded 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 Fig. 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 (Fig. 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 (Fig. 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. Fig. 6 showed a compound-target-disease interaction network. This network reveals drug action mechanisms within breast cancer treatment and comprises

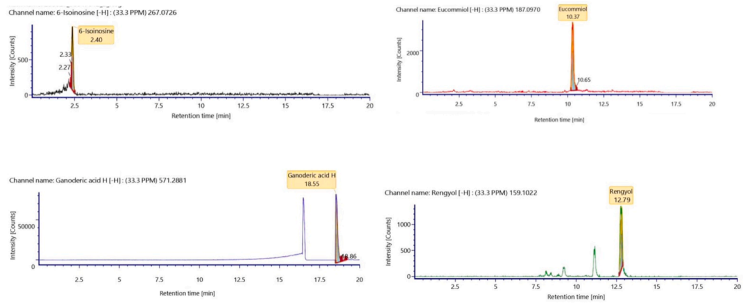

Fig. 1. Chromatogram for high performance liquid chromatography analysis of the sea cucumber extract, showing signal intensity in relation to the retention time for the compounds.

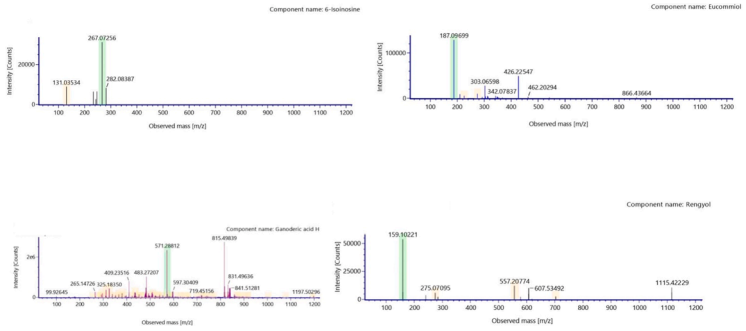

Fig. 2. Mass spectrum for the sea cucumber extract showing signal intensity in relation to molecular weight. The peaks in the graph represent the detected compounds.

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

Compound	Formula	RT (min)	Mass molecule (*m/z*)	Mass error (ppm)	RMS (ppm)	RMS intensity (%)	Total Fragments	Signal Intensity
Ganoderic acid	$C_{32}H_{44}O_9$	18.58	571.29	−5.3	3.16	8.17	144	56,921
6-Isoinosine	$C_{10}H_{12}N_4O_5$	2.41	267.07	−1.7	2.58	6.71	2	521
Eucommiol	$C_9H_{16}O_4$	10.42	187.09	1–1.9	4.32	4.90	1	2057
Rengyol	$C_8H_{16}O_3$	12.82	159.10	−3.5	3.45	7.67	1	817

Each compound was detected as an "H-" adduct indicating hydrogen loss.

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

Name	Compound ID
Rengyol	363,707
Eucommiol	154,373
Ganoderic Acid	73,554,535
Isoinosine	15,333,826

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. Fig. 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

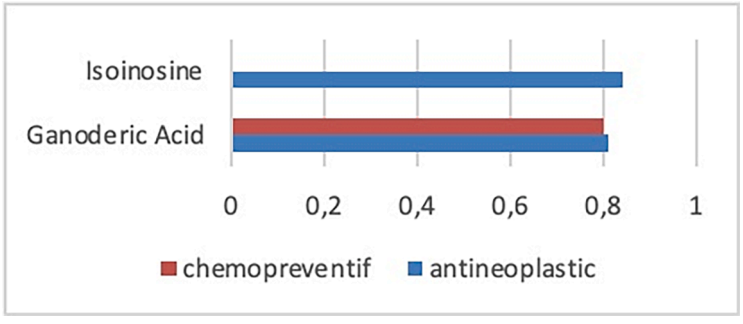

Fig. 3. SAR-based prediction for the potential of sea cucumber as an anticancer and chemopreventive agent.

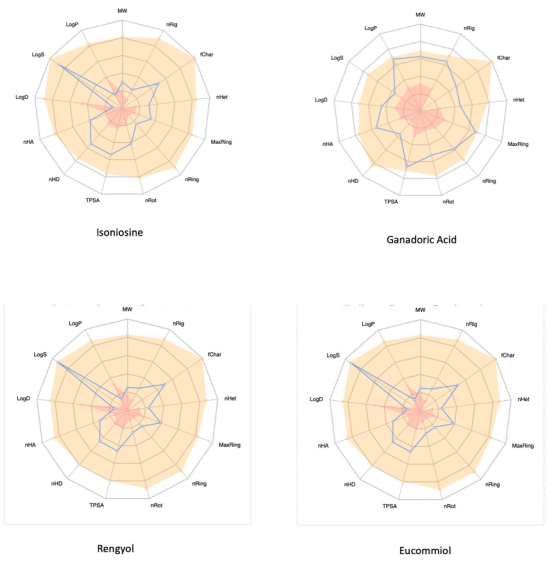

Fig. 4. Toxicity analysis of the sea cucumber extract.

Table 3
Important nodes with network analyzer result.

Names	Betweenness centrality	Closeness centrality	Clustering coefficient	Degree	Topological coefficient
AKT1	0.04	0.76	0,55	82	0.43
STAT3	0.03	0.72	0.58	74	0.44
TP53	0.22	0.92	0.36	110	0.36
EGFR	0.04	0.75	0.54	80	0.42
CASP8	0.03	0.68	0.59	64	0.46
MTOR	0.01	0.67	0.75	60	0.50
PTEN	0.03	0.75	0.56	82	0.44
BRCA1	0.02	0.72	0.60	76	0.45
MYC	0.04	0.77	0.54	84	0.42
CTNNB1	0.03	0.75	0.56	80	0.43

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 et al., 2020). Fig. 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 Fig. 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 h. 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 (Fig. 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 19,057 µg/mL and 2293 ng/mL, respectively.

Discussion

Modern pharmacology research strives to discover novel drugs and evaluate their efficacy against various diseases (I. Ujianti 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. Conversely, the E7 oncogene facilitates cell growth and proliferation by activating the PI3K/AKT/mTOR pathway, supporting the transformation of a cell into cancer.

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 (Ahmad et al., 2022; Galappaththi et al., 2023). Aberrations in RTK signaling can lead to cancer, indicating ganoderic acid's potential therapeutic benefits. 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

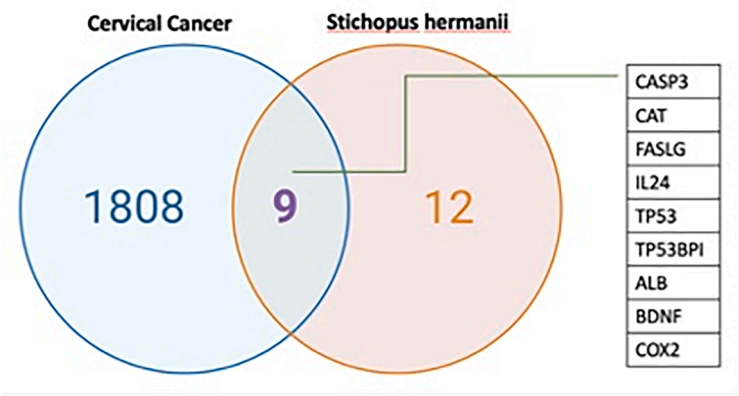

Fig. 5. Venn Diagram depicting the intersection of cervical cancer and *Stichopus* spp.

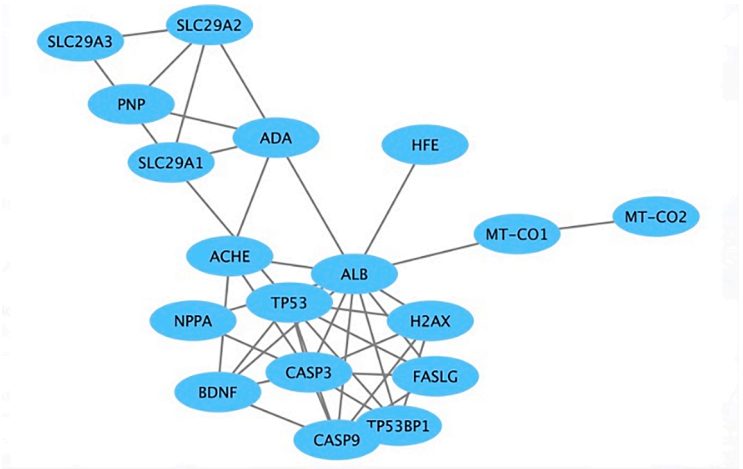

Fig. 6. Target pathway network of the sea cucumber extract for treating cervical cancer.

Fig. 7. Gene ontology and KEGG pathway enrichment analysis.

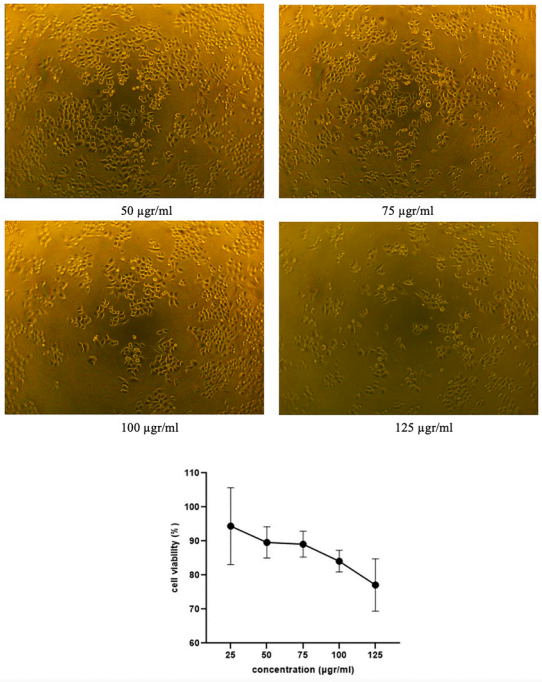

Fig. 8. Cell viability analysis using the MTT assay.

acid specifically enhances Bax and Caspase-3 protein expression, 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, 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 et al. conducted a study showing 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.

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. Further empirical studies are necessary to validate these findings and understand the mechanisms by which these compounds may exert their effects on cancer cells.

Ethical approval

The in vitro study protocol was approved by the Health Research Ethics Committee-Faculty of Medicine Universitas Muhammadiyah Prof. Dr.Hamka with number: KEPKK/FK/009/02/2023

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Consent for publication

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Author agreement statement

We the undersigned declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We understand that the Corresponding Author is the sole contact for the Editorial process. He/she is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs

CRediT authorship contribution statement

Irena Ujianti: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Bety Semara Lakhsmi:** Visualization, Data curation. **Zahra Nurusshofa:** Formal analysis, Data curation. **Wawang S Sukarya:** Formal analysis, Data curation.

Declaration of competing interest

The authors declare no competing interests

Data availability

The results/data/figures in this manuscript have not been published elsewhere, nor are they under consideration by another publisher. All of the material is owned by the authors, no permissions are required.

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Supplementary materials

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