© 2025 Journal of Pharmacy & Pharmacognosy Research, 13 (3), 919-924, 2025
ISSN 0719-4250
https://jppres.com

DOI: https://doi.org/10.56499/jppres24.2069_13.3.919

Original Article

Extract of *Holothuria scabra* exhibits synergistic effect with chemotherapeutic agents against breast cancer *in vitro*

[Extracto de *Holothuria scabra* muestra un efecto sinérgico con agentes quimioterapéuticos contra el cáncer de mama *in vitro*]

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Abstract

Context: Sea cucumbers have been identified as highly potential contenders in the fight against breast cancer.

Aims: To investigate the potential of *Holothuria scabra* extract for anticancer against breast cancer cells *in vitro* by investigating its synergistic effects when integrated with frequently used chemotherapeutic medications.

Methods: The Combenefit software was employed to assess the human breast cancer cell line T47D viability towards a synergistic combination of *H. scabra* extract and chemotherapeutic medications (cisplatin, doxorubicin, and neratinib). The investigation encompassed assessing the combination by utilizing a cell viability experiment employing a water-soluble tetrazolium-1 reagent. Subsequently, the selective cytotoxic effect was assessed by subjecting the highest cytotoxicity score to TIG-1-20 normal cells.

Results: The synergistic model was evaluated by comparing interaction analysis through Loewe, Bliss, and Highest Single Agent (HSA) models. Combinations of *H. scabra* and Cisplatin showed synergistic activity with similar T47D cell viability values. However, the Loewe synergistic model had a higher T47D cell viability value, while the combination of *H. scabra* and neratinib had a higher T47D cell viability value. Moreover, co-administration of *H. scabra* and doxorubicin demonstrated notable efficacy in inhibiting the proliferation of T47D cells while exhibiting no cytotoxic effects on TIG-1-20 cells.

Conclusions: Concurrent administration of *H. scabra* extract and doxorubicin exhibits promising synergistic properties in inhibiting the proliferation of T47D breast cancer cells while demonstrating no toxicity towards TIG-1-20 normal cells.

Keywords: chemotherapy; complementary medicine; natural products; sea cucumbers.

Resumen

Contexto: Los pepinos de mar han sido identificados como contendientes de alto potencial en la lucha contra el cáncer de mama.

Objetivos: Investigar el potencial del extracto de *Holothuria scabra* como anticancerígeno contra las células de cáncer de mama *in vitro* mediante la investigación de sus efectos sinérgicos cuando se integra con medicamentos quimioterapéuticos de uso frecuente.

Métodos: Se empleó el software Combenefit para evaluar la viabilidad de la línea celular de cáncer de mama humano T47D frente a una combinación sinérgica de extracto de *H. scabra* y medicamentos quimioterapéuticos (cisplatino, doxorrubicina y neratinib). La investigación abarcó la evaluación de la combinación mediante la utilización de un experimento de viabilidad celular empleando reactivo tetrazolio-1 soluble en agua. Posteriormente, se evaluó el efecto citotóxico selectivo sometiendo la puntuación más alta de citotoxicidad a células normales TIG-1-20.

Resultados: El modelo sinérgico se evaluó comparando el análisis de interacción a través de los modelos Loewe, Bliss y Highest Single Agent (HSA). Las combinaciones de *H. scabra* y cisplatino mostraron una actividad sinérgica con valores similares de viabilidad celular T47D. Sin embargo, el modelo sinérgico de Loewe tuvo un valor de viabilidad celular T47D más alto, mientras que la combinación de *H. scabra* y neratinib tuvo un valor de viabilidad celular T47D más alto. Además, la administración conjunta de *H. scabra* y doxorrubicina demostró una eficacia notable en la inhibición de la proliferación de células T47D, sin exhibir efectos citotóxicos sobre las células TIG-1-20.

Conclusiones: La administración simultánea de extracto de *H. scabra* y doxorrubicina exhibe prometedoras propiedades sinérgicas en la inhibición de la proliferación de células de cáncer de mama T47D, sin demostrar toxicidad hacia las células normales TIG-1-20.

Palabras Clave: medicina complementaria; pepinos de mar; productos naturales; quimioterapia.

ARTICLE INFO
Received: April 15, 2024.
Accepted: December 6, 2024.
Available Online: January 13, 2025.

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INTRODUCTION

The complex interplay of molecular mechanisms associated with breast cancer poses a significant challenge in the exploration of practical treatment approaches. Although radiotherapy and chemotherapy show potential, their use frequently results in unfavorable consequences that might substantially impact patient outcomes (Mitra and Dash, 2018). In order to address these issues, incorporating natural products as supplementary or synergistic treatments to enhance the efficacy of chemotherapeutic medicines in the treatment of breast cancer has become a new approach (Kan et al., 2023; Naja et al., 2015; Stöcker et al., 2023; Yen et al., 2022). In addition to improving the anticancer mechanisms of these medications (Kan et al., 2023; Naja et al., 2015; Yen et al., 2022), specific natural compounds also demonstrate favorable pharmacokinetic and pharmacodynamic characteristics, hence improving bioavailability and reducing the occurrence of side events (Chan et al., 2023; Kumar, 2021; Yen et al., 2022).

Marine-derived compounds have gained considerable interest in modern breast cancer treatment (De Sanctis et al., 2022). Among them, sea cucumbers have been identified as highly potential contenders in the fight against breast cancer (Wargasetia et al., 2022; Wargasetia and Widodo, 2017). The antibreast cancer effects of *Holothuria scabra*, a species of sea cucumber, have been the subject of intense investigation. Although it has been proven to cause cancer cell death effectively (Pranoto et al., 2023; Wargasetia et al., 2024; Wargasetia and Widodo, 2018), there is still a significant lack of research on its possible synergistic effects when used alongside traditional chemotherapeutic treatments. Hence, the primary aim of this research is to examine the potential of *H. scabra* extract to boost the elimination of breast cancer cells *in vitro* by exploring its complimentary or synergistic effects when combined with commonly used chemotherapeutic drugs.

MATERIAL AND METHODS

Sea cucumber extraction

Fresh samples of the sea cucumber *Holothuria scabra* were collected from Malang Sea, East Java, Indonesia (S8° 26'47.3346", E112° 39' 12.3444"). Sea cucumber identification was done based on the Food and Agriculture Organization of The United Nations Species Catalogue for Fishery Purposes (Purcell et al., 2012). Twenty grams of sea cucumber was ground to a powder and then extracted with 500 mL of absolute methanol using the maceration method for 48 hours. Macerate was filtered with Whatman filter paper No. 41. The filtration results were evaporated using a rotary evaporator (Buchi R-114) at a temperature of 50-60°C (Wargasetia et al., 2023).

Synergistic combination analysis

Synergistic combination analysis of *H. scabra* Jaeger (*Holothuriidae*) extracts and chemotherapy drugs (cisplatin from Dankos Farma, Jakarta, Indonesia), doxorubicin (Dankos Farma, Jakarta, Indonesia), and neratinib (AbMole, Abmole Bioscience Inc., Houston, USA) was performed by Combinations Benefit (Combenefit) software (SourceForge, San Diego, USA) (Di Veroli et al., 2016). Synergistic combination testing was conducted between *H. scabra* and cisplatin, *H. scabra* and doxorubicin, also *H. scabra* and neratinib. The graded concentration of the extract combination test was determined based on the IC_{50} value of each of the previously tested samples ($1/8IC_{50}$; $1/4IC_{50}$; $1/2IC_{50}$; IC_{50}; $2IC_{50}$; $4IC_{50}$; $8IC_{50}$). Determination of the IC_{50} value was done by determining the linear regression equation between the percentage of cell viability and the concentration of the extract (Widodo et al., 2022). The combination results were evaluated using a cell viability assay with a WST-1 reagent, and the results were inputted into the software to obtain the scoring of each combination and determine the optimal combination with the highest synergy score.

Cell viability assay

The viability testing method with water-soluble tetrazolium-1 (WST-1) (Widodo et al., 2022) was performed on the human breast cancer cell line T47D cells (RRID: CVCL_0553, ATCC HTB-133™). 7,500 cells were cultured and incubated at 37°C, 5% CO_2 for 24 hours. The combination treatment of *H. scabra* and chemotherapeutic drugs was performed at graded concentrations for 24 hours, with sample concentrations as follows: 1) *H. scabra*: 19.75, 39.5, 79, 158, 316, 632, 1264 µg/mL; 2) cisplatin: 0.375, 0.75, 1.5, 3, 6, 12, 24 µg/mL; 3) doxorubicin: 0.15625, 0.3125, 0.625, 1.25, 2.5, 5, 10 µg/mL; 4) neratinib: 0.3125, 0.625, 1.25, 2.5, 5, 10, 20 µg/mL. The culture medium was replaced with a 5% WST-1 (Sigma-Aldrich, USA) medium and incubated for 30 minutes. The absorbance (optical density, OD) of the samples was measured at 450 nm wavelength using a microplate reader. The percentage of T47D cell viability was calculated using the equation [1].

$$\text{Cell viability (\%)} = \frac{[\text{OD Sample} - \text{OD Medium}]}{[\text{OD Cell Control} - \text{OD Medium}]} \times 100 \quad [1]$$

A comparison of synergistic combinations in each group with the highest score was analyzed, and then

the TIG-1-20 normal cells (RRID: CVCL_3181, JCRB - JCRB0501) were tested to determine the toxicity effect using the WST-1 assay. The toxicity assay was performed in three replications.

Statistical analysis

All data were presented as mean, with each value representing the average of four independent experiments. Combination analysis using Combenefit software showed the synergy/antagonism score for each combination and its statistical significance, indicated by an asterisk (*) on the matrix synergy plots. The significance values used were *p<0.05; **p<0.001; and ***p<0.0001.

RESULTS AND DISCUSSION

Ongoing studies and inquiries in the field of cancer treatment involve the integration of chemotherapy with natural compounds. This approach offers the potential for the sustainable advancement of cancer treatment. While there is potential for synergistic effects and improved outcomes, it is essential to approach this topic with caution and consider the bioactive compounds interactions (Huang et al., 2021; Mann, 2002). Natural ingredients, including sea cucumber *H. scabra*, contain bioactive compounds that may have complementary or synergistic effects with chemotherapy drugs. These compounds can target different pathways or mechanisms in cancer development, growth, and drug resistance (Wargasetia and Widodo, 2017). Combining them with chemotherapy drugs may enhance their efficacy and overcome resistance, potentially leading to improved treatment outcomes.

Pharmacological analysis of combination effects at the molecular level can be evaluated *in vitro*. The study used Combenefit software to analyze the interaction of drug and natural ingredient combinations. Combenefit applies a mathematical modeling approach by comparing *in vitro* experimental data to determine the form of synergistic or non-synergistic combinations (additive or independent) with three classic models, namely Loewe, Bliss, and Highest Single Agent (HSA) (Caesar and Cech, 2019). The combination of *H. scabra* and cisplatin showed the same comparison results of 316 µg/mL and 1.5 µg/mL with a T47D cell viability value of 3.14% in the three synergy models of Loewe, Bliss, and HSA with synergistic combination scores of 15 (p<0.001), 11 (p<0.001), and 19 (p<0.0001), respectively (Fig. 1A). The combination of *H. scabra* and doxorubicin showed the same comparison results of 39.5 µg/mL and 0.3125 µg/mL with a T47D cell viability value of 1.32% in the Bliss and HSA models with synergistic combination scores of 83 (p<0.0001) and 87 (p<0.0001). In contrast, different results were shown in the Loewe synergistic model, which had a combination score of 78 (p<0.0001) with *H. scabra* and doxorubicin comparison values of 19.75 µg/mL and 0.3125 µg/mL, and a T47D cell viability value of 5.34% (Fig. 1B). Different results were shown from the combination of *H. scabra* and neratinib with a ratio of 39.5 µg/mL and 0.3125 µg/mL and the highest T47D cell viability value of 50.56% from the three synergistic models of Loewe, Bliss, and HSA with synergistic combination scores of 21 (p<0.001), 23 (p<0.001) and 25 (p<0.0001) (Fig. 1C).

The effectiveness of the synergistic combination of *H. scabra* and chemotherapy drugs was also evaluated based on its effect on the viability of normal TIG-1-20 cells (Table 1). The results showed that the synergistic combination of *H. scabra* and doxorubicin with the ratio of 39.5 µg/mL and 0.3125 µg/mL did not give toxic effects on TIG-1-20 cells with a viability value of 94.21%. Combining *H. scabra* and cisplatin with a ratio of 39.5 µg/mL and 0.3125 µg/mL has a toxic effect on TIG-1-20 cells, which causes a decrease in cell viability of 7.73%. The combination of *H. scabra* and doxorubicin was found to be effective in suppressing the viability of T47D cells and was not toxic to TIG-1-20 cells.

Doxorubicin is a chemotherapeutic drug that belongs to the class of anthracyclines. It is known to intercalate between the base pairs of DNAs, inhibiting DNA synthesis and transcription. Doxorubicin's primary mechanism of action in killing the T47D breast cancer cell line involves intercalation and inhibition of DNA synthesis and transcription. In addition to its direct DNA-intercalating action, doxorubicin produces reactive oxygen species (ROS) that can cause oxidative stress, leading to DNA damage and cell death. This oxidative stress can also contribute to the cytotoxic effects of doxorubicin in T47D breast cancer cells (Pichot et al., 2009). The potential of combining doxorubicin with herbal ingredients in breast cancer therapy is an area of ongoing research. Several studies have explored the use of doxorubicin in combination with various herbal ingredients or drug delivery systems to enhance its efficacy and reduce side effects.

The previous study showed that the methanol extract of *H. scabra* exhibited cytotoxic activity by inhibiting the growth of T47D cancer cells in a dose-dependent manner, with an IC_{50} value of 152.98 µg/mL—the extract induced apoptosis in 99% of cancer cells (Wargasetia and Widodo, 2018). Chemotherapy drugs often have significant side effects due to their toxic nature.

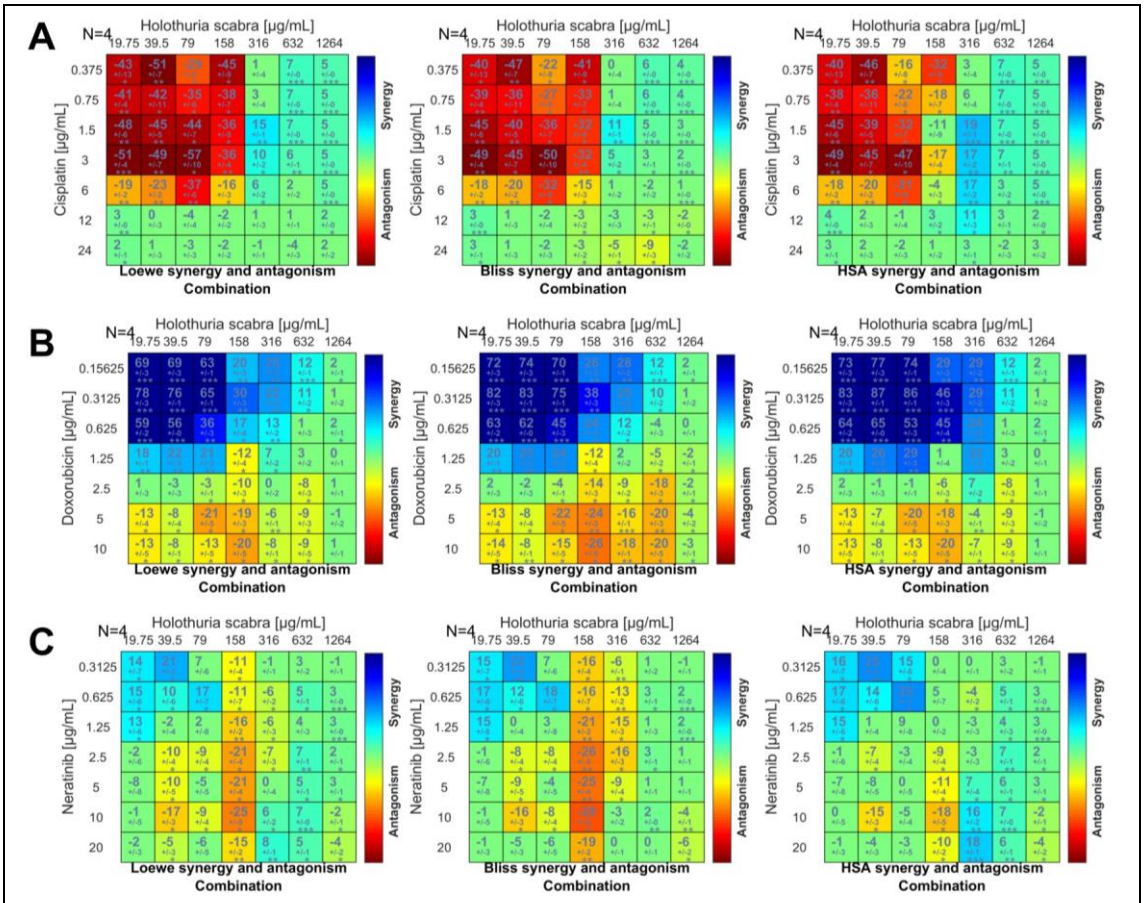

Figure 1. COMBENEFIT analysis results matrix of the combination of *H. scabra* and chemotherapeutic drugs with graded concentrations of 1/8IC_{50}, 1/4IC_{50}, 1/2IC_{50}, IC_{50}, 2IC_{50}, 4IC_{50}, 8IC_{50} for each sample in the classical synergy analysis models of Loewe, Bliss and HSA.

(A) Combination of *H. scabra* and cisplatin; **(B)** Combination of *H. scabra* and doxorubicin; **(C)** Combination of *H. scabra* and neratinib.

Table 1. Comparison of synergy scores of *H. scabra* and chemotherapeutic drugs in each of the Loewe, Bliss, and HSA classical synergy models, and their effects on T47D and TIG-1-20 cells.

Synergy model	Concentration (µg/mL)		Synergy score	T47D cell viability (%)	T1G-1 cell viability (%)
	H. scabra	Cisplatin			
Loewe	316	1.5	15	3.14 ± 1.29	7.73 ± 0.99
Bliss	316	1.5	11	3.14 ± 1.29	7.73 ± 0.99
HSA	316	1.5	19	3.14 ± 1.29	7.73 ± 0.99
		Doxorubicin			
Loewe	19.75	0.3125	78	5.34 ± 2.56	94.21 ± 2.38
Bliss	39.5	0.3125	83	1.32 ± 1.02	85.83 ± 3.95
HSA	39.5	0.3125	87	1.32 ± 1.02	85.83 ± 3.95
		Neratinib			
Loewe	39.5	0.3125	21	50.56 ± 3.49	96.42 ± 9.67
Bliss	39.5	0.3125	23	50.56 ± 3.49	96.42 ± 9.67
HSA	39.5	0.3125	25	50.56 ± 3.49	96.42 ± 9.67

HSA: Highest single agent.

The bioactive compound of *H. scabra* may possess properties that can mitigate these side effects, and it has unique chemical structures and properties. These compounds may target specific molecular pathways or signaling cascades involved in cancer development or progression that current chemotherapy drugs do not address effectively. By combining them with chemotherapy, it may be possible to target these novel pathways and improve treatment efficacy.

CONCLUSION

The use of *H. scabra* extract combined with doxorubicin can produce synergistic effects in suppressing the viability of T47D breast cancer cells and is not toxic to normal TIG-1-20 cells. The results still require further research to confirm the synergistic mechanism of *H. scabra* and doxorubicin on breast cancer cells and its selectivity on normal cells.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

The work was supported by the Institute of Research and Community Service, Maranatha Christian University under the Rector's Decree number: 024/SK/ADD/UKM/VII/2022.

REFERENCES

Caesar LK, Cech NB (2019) Synergy and antagonism in natural product extracts: When 1 + 1 does not equal 2. Nat Prod Rep 36: 869–888. https://doi.org/10.1039/c9np00011a

Chan WJ, Adiwidjaja J, McLachlan AJ, Boddy AV, Harnett JE (2023) Interactions between natural products and cancer treatments: Underlying mechanisms and clinical importance. Cancer Chemother Pharmacol 91(2): 103–119. https://doi.org/10.1007/s00280-023-04504-z

De Sanctis R, Jacobs F, Benvenuti C, Gaudio M, Franceschini R, Tancredi R, Pedrazzoli P, Santoro A, Zambelli A (2022) From seaside to bedside: Current evidence and future perspectives in the treatment of breast cancer using marine compounds. Front Pharmacol 13: 909566. https://doi.org/10.3389/fphar.2022.909566

Di Veroli GY, Fornari C, Wang D, Mollard S, Bramhall JL, Richards FM, Jodrell DI (2016) Combenefit: An interactive platform for the analysis and visualization of drug combinations. Bioinform 32(18): 2866–2868. https://doi.org/10.1093/bioinformatics/btw230

Huang M, Lu J, Ding J (2021) Natural products in cancer therapy: Past, present and future. Nat Prod Bioprospect 11: 5–13. https://doi.org/10.1007/s13659-020-00293-7

Kan LL-Y, Chan BC-L, Leung P-C, Wong C-K (2023) Natural-product-derived adjunctive treatments to conventional therapy and their immunoregulatory activities in triple-negative breast cancer. Molecules 28(15): 5804. https://doi.org/10.3390/molecules28155804

Kumar NB (2021) The promise of nutrient-derived bioactive compounds and dietary components to ameliorate symptoms of chemotherapy-related cognitive impairment in breast cancer survivors. Curr Treat Options Oncol 22: 67. https://doi.org/10.1007/s11864-021-00865-w

Mann J (2002) Natural products in cancer chemotherapy: Past, present and future. Nat Rev Cancer 2: 143–148. https://doi.org/10.1038/nrc723

Mitra S, Dash R (2018) Natural products for the management and prevention of breast cancer. Evid Based Complement Altern Med 2018: 8324696. https://doi.org/10.1155/2018/8324696

Naja F, Fadel RA, Alameddine M, Aridi Y, Zarif A, Hariri D, Mugharbel A, Khalil M, Nahleh Z, Tfayli A (2015) Complementary and alternative medicine use and its association with quality of life among Lebanese breast cancer patients: A cross-sectional study. BMC Complement Altern Med 15: 444. https://doi.org/10.1186/s12906-015-0969-9

Pichot CS, Hartig SM, Xia L, Arvanitis C, Monisvais D, Lee FY, Frost JA, Corey SJ (2009) Dasatinib synergizes with doxorubicin to block growth, migration, and invasion of breast cancer cells. Br J Cancer 101: 38–47. https://doi.org/10.1038/sj.bjc.6605101

Pranoto D, Ratnawati H, Wargasetia TL, Octavallen A, Rachmat MAA (2023) Sea cucumber reduces breast cancer tumor size through inhibition of MMP9 dependent HER2 expression in breast cancer mice model. Trop J Nat Prod Res 7(9): 4010–4015. https://tjnpr.org/index.php/home/article/view/2652

Purcell SW, Lovatelli A, González-Wangüemert M, Solís-Marín FA, Samyn Y, Conand C (2012) Commercially important sea cucumbers of the world. Rome: Food and Agriculture Organization of the United Nations, 256 p. https://openknowledge.fao.org/handle/20.500.14283/cc5230en

Stöcker A, Mehnert-Theuerkauf A, Hinz A, Ernst J (2023) Utilization of complementary and alternative medicine (CAM) by women with breast cancer or gynecological cancer. PloS One 18(5): e0285718. https://doi.org/10.1371/journal.pone.0285718

Wargasetia TL, Ratnawati H, Liana LK, Larissa L, Imannuel EV (2024) *Holothuria scabra* extract improves histopathological features and inhibits cancer growth through IL-6 and NF-κB signaling pathways in breast cancer mice model. Res J Pharmacogn 11(2): 1–9. https://doi.org/10.22127/rjp.2023.417833.2233

Wargasetia TL, Ratnawati H, Widodo N (2022) Sea cucumber compounds targeting NF-κB in cancer treatment. Bioinform Biol Insights 16: 11779322221091740. https://doi.org/10.1177/11779322221091740

Wargasetia TL, Ratnawati H, Widodo N, Widyananda MH (2023) Antioxidant and anti-inflammatory activity of sea cucumber (*Holothuria scabra*) active compounds against KEAP1 and iNOS protein. Bioinform Biol Insights 17: 11779322221149613. https://doi.org/10.1177/11779322221149613

Wargasetia TL, Widodo (2017) Mechanisms of cancer cell killing by sea cucumber-derived compounds. Invest New Drugs 35: 820–826. https://doi.org/10.1007/s10637-017-0505-5

Wargasetia TL, Widodo N (2018) Methanol extract of Holothuria scabra inhibits cell growth and induces apoptosis in T47D breast cancer cells. ESMO Open 3(suppl. 2): A182–A183. https://doi.org/10.1136/esmoopen-2018-EACR25.434

Widodo N, Puspitarini S, Widyananda MH, Alamsyah A, Wicaksono ST, Masruri M, Jatmiko YD (2022) Anti-cancer activity of *Caesalpinia sappan* by downregulating mitochondrial genes in A549 lung cancer cell line. F1000Res 11: 169. https://doi.org/10.12688/f1000research.76187.2

Yen C, Zhao F, Yu Z, Zhu X, Li CG (2022) Interactions between natural products and tamoxifen in breast cancer: A

comprehensive literature review. Front Pharmacol 13: 847113. https://doi.org/10.3389/fphar.2022.847113

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Contribution	Wargasetia TL	Liana LK	Widodo N	Annisa Y	Hermanto FE
Concepts or ideas			x		
Design	x	x	x		
Definition of intellectual content	x	x	x	x	x
Literature search				x	x
Experimental studies				x	x
Data acquisition				x	
Data analysis	x		x	x	
Statistical analysis			x	x	
Manuscript preparation				x	x
Manuscript editing	x				
Manuscript review	x	x	x	x	x

Citation Format: Wargasetia TL, Liana LK, Widodo N, Annisa Y, Hermanto FE (2025) Extract of *Holothuria scabra* exhibits synergistic effect with chemotherapeutic agents against breast cancer *in vitro*. J Pharm Pharmacogn Res 13(3): 919–924. https://doi.org/10.56499/jppres24.2069_13.3.919

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