BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 15, Number 2, October 2026 | Pages: 2247-2253 | DOI: 10.14421/biomedich.2026.152.2247-2253 ISSN 2540-9328 (online)

Cytotoxic Activity and GC-MS Profile of a Seaweed-Associated Fungal Extract against T47D Breast Cancer Cells

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Manuscript received: 21 August 2026. Revision accepted: 16 November 2026, Published: 20 November 2026.

Abstract

Marine-associated fungi are increasingly recognized as sources of chemically diverse metabolites with potential biological activity, although their cytotoxic properties vary considerably among individual isolates. This study evaluated the cytotoxic activity and GC-MS profile of an ethanol extract obtained from the seaweed-associated fungal isolate SRL2K3 against T47D breast cancer cells. The fungus was cultivated in Sabouraud Dextrose Broth for 14 days, and the culture filtrate was extracted with ethanol. Cytotoxicity was evaluated by MTT assay at 0.01–0.50% (v/v), with doxorubicin-HCl as a positive control. The extract produced 29.29 ± 5.87, 32.37 ± 7.10, 31.72 ± 5.03, 82.83 ± 1.59, and 95.70 ± 9.15% inhibition at concentration of 0.01, 0.02, 0.03, 0.25, and 0.50% (v/v), respectively. Four-parameter logistic analysis yielded an estimated IC50 of 0.10% (v/v). GC-MS analysis resolved 62 chromatographic peaks, with no individual peak exceeding 8% of the total corrected area. More confidently annotated constituents included 5-methyl-2-furancarboxaldehyde, a dihydroxy-methyl pyranone, tyrosol, ribitol, n-hexadecanoic acid, and C18:1 unsaturated fatty-acid-related compounds, whereas several of the largest peaks remained insufficiently resolved for confident structural assignment. The findings demonstrate measurable cytotoxic activity of the fungal ethanol extract against T47D cells and reveal a chemically heterogeneous profile. Further bioassay-guided fractionation, selectivity testing, and structural characterization are required to identify the constituents associated with the observed activity.

Keywords: breast cancer; cytotoxicity; fungal extract; GC-MS; marine fungi; T47D.

INTRODUCTION

Breast cancer remains a major global health burden despite substantial advances in early detection, molecular classification, and treatment. GLOBOCAN 2024 estimated approximately 20.6 million new cancer cases worldwide, with female breast cancer accounting for 11.8% of newly diagnosed cancers and remaining among the leading causes of cancer mortality in women (Sung et al., 2026). The burden is projected to increase further over the coming decades, particularly in regions undergoing demographic and socioeconomic transitions (Freihat et al., 2025). Breast cancer is also biologically heterogeneous, comprising molecular subtypes with distinct clinical behavior, prognosis, and responses to therapy (Łukasiewicz et al., 2021; Xiong et al., 2025). Current management includes surgery, radiotherapy, chemotherapy, endocrine therapy, targeted therapy, and immunotherapy according to disease stage and molecular characteristics (Roskoski, 2024). Despite these therapeutic advances, treatment-related toxicity, disease recurrence, and acquired resistance remain important clinical challenges and continue to encourage the exploration of new sources of bioactive compounds.

Fungi constitute an important reservoir of natural products because of their capacity to synthesize structurally diverse secondary metabolites. Marine- and mangrove-associated fungi are particularly attractive for bioprospecting because they inhabit environments characterized by salinity, nutrient fluctuations, tidal influence, and extensive biological interactions. Metabolites reported from these fungi include polyketides, alkaloids, terpenoids, peptides, phenolic compounds, steroids, and other structurally diverse molecules (Amaeze et al., 2026; Deshmukh et al., 2018). Fungi associated with marine macroalgae are of particular interest because this ecological association provides an additional source of chemically diverse fungal isolates that remain comparatively underexplored for their biological activities. Several macroalgae-associated fungi have produced metabolites with cytotoxic effects against cancer cell lines (Sahoo et al., 2021), while recent studies of marine- and mangrove-derived fungi have further demonstrated their potential as

sources of biologically active secondary metabolites (Grace et al., 2026; Zhang et al., 2025).

Cytotoxic activity among marine-associated fungal extracts is nevertheless highly variable and cannot be predicted solely from ecological origin. A previous study reported substantial differences among extracts from fungi associated with the marine sponge *Haliclona fascigera* when tested against T47D and other human cell lines (Handayani et al., 2018). A previous study similarly observed variable biological activity among fungal isolates obtained from *Dactylospongia* sp.,(Sandrawati et al., 2020). Whereas, Sandrawati et al., 2023 reported cytotoxic activity among endophytic fungi isolated from the mangrove plant *Acanthus ilicifolius*. Variable cytotoxic responses have also been reported among fungi associated with *Rhizophora* species (Zhou et al., 2022). More recently, a study demonstrated that biological screening can distinguish more promising marine fungal strains for subsequent chemical and metabolomic characterization (Puspitasari et al., 2026). Taken together, these studies indicate that biological activity is strongly isolate-dependent and support the need to evaluate individual fungal resources rather than infer activity from their marine origin alone.

T47D cells provide a useful model for preliminary screening against hormone-responsive breast cancer. The cell line represents a luminal A phenotype and retains characteristic estrogen- and progesterone-related signaling, with particularly pronounced responsiveness to progesterone (Yu et al., 2017). This makes T47D a relevant model for initial evaluation of compounds or extracts with potential activity against hormone-responsive breast cancer cells. Fungal metabolites may influence cancer cells through several biological processes. For example, averufanin produced by *Aspergillus carneus* has been reported to induce DNA damage, cell-cycle arrest, and apoptosis (Demirel et al., 2023). However, such mechanisms cannot be inferred from cytotoxicity screening alone. The MTT assay primarily reflects alterations in cellular metabolic activity and should not be interpreted as direct evidence of a particular mode of cell death (Ghasemi et al., 2021).

The present study investigated an ethanol extract obtained from the seaweed-associated fungal isolate SRL2K3. The study aimed to evaluate its cytotoxic response against T47D breast cancer cells and to characterize its chemical profile by GC-MS. The GC-MS data were interpreted by considering both chromatographic abundance and spectral-library match quality, thereby avoiding definitive compound assignment based solely on peak size. This combined biological and chemical assessment was designed to determine whether the SRL2K3 extract warrants further investigation through bioassay-guided fractionation, selectivity testing, and structural characterization of its active constituents.

MATERIALS AND METHODS

Fungal isolate

The fungal isolate SRL2K3, previously obtained from a seaweed-associated sample, was used in this study. The isolate was maintained as a pure culture and refreshed on agar medium prior to fermentation. Macroscopic colony morphology was documented before fermentation and is presented in Figure 1.

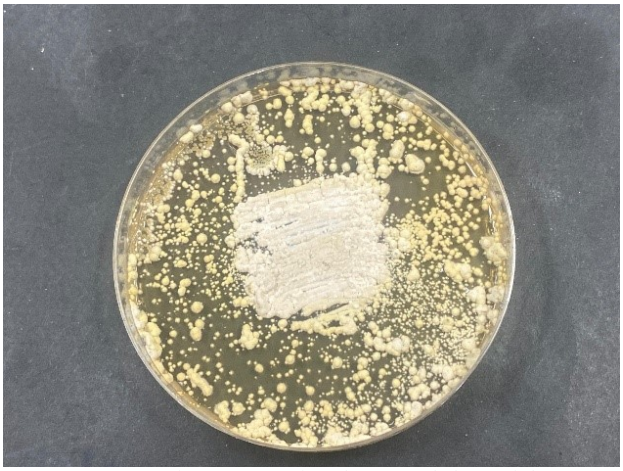


Figure 1. Representative macroscopic colony morphology of the seaweed-associated fungal isolate SRL2K3 prior to fermentation.

Fermentation and preparation of the ethanol extract

A 1 × 1 cm agar block was excised from an actively growing SRL2K3 culture and aseptically transferred into 200 mL of sterile Sabouraud Dextrose Broth. The culture was incubated for 14 days at room temperature with agitation at 150 rpm. Following fermentation, the fungal biomass was separated from the culture filtrate by filtration. The resulting culture filtrate was extracted with ethanol at a filtrate-to-solvent ratio of 1:1 (v/v). The extraction procedure was performed twice, after which the ethanol-containing extract was concentrated at 40°C to obtain the crude extract. The resulting crude extract was used for subsequent cytotoxicity and GC-MS analyses.

T47D cell culture

T47D human breast cancer cells were maintained in Dulbecco's Modified Eagle Medium supplemented with fetal bovine serum and antibiotics. Cells were cultured at 37°C in a humidified atmosphere containing 5% CO_2 and were used for cytotoxicity experiments during logarithmic growth.

MTT cytotoxicity assay

The cytotoxic activity of the ethanol extract was evaluated against T47D breast cancer cells using the MTT assay. Cells were seeded into 96-well plates and allowed to attach before treatment. The extract was tested at final concentrations of 0.01, 0.02, 0.03, 0.25, and 0.50% (v/v), while doxorubicin-HCl was included as a positive control. Cells were exposed to the test extract for

24 h. MTT reagent was subsequently added, and the resulting formazan product was dissolved in DMSO before absorbance was measured using a microplate reader. Untreated-cell and medium controls were included, and each treatment concentration was evaluated in triplicate. Cytotoxic activity was expressed as percentage inhibition relative to the untreated T47D cell control according to the following equation:

$$\text{Inhibition (\%)} = \left[1 - \frac{\text{Net absorbance of treated cells}}{\text{Net absorbance of untreated cell control}}\right] \times 100$$

Concentration-response data were fitted using a four-parameter logistic model to estimate the concentration producing 50% inhibition (IC50). Data are presented as mean ± standard deviation (SD). The positive control produced an estimated IC50 of 40.10 ppm, whereas the ethanol extract produced an estimated IC50 of 0.10% (v/v).

GC-MS analysis

Chemical profiling of the ethanol extract was performed by gas chromatography-mass spectrometry (GC-MS) using an Agilent 7890B gas chromatograph coupled to a 5977B Mass Selective Detector. Separation was carried out on an HP-5ms Ultra Inert column (30 m × 250 µm × 0.25 µm), with helium as the carrier gas at a constant flow rate of 2.9 mL/min. The oven temperature was initially maintained at 70°C for 5 min, increased to 290°C at a rate of 10°C/min, and held at 290°C for 3 min. The injector temperature was set at 290°C, and 1 µL of sample was introduced in splitless mode. The mass spectrometer was operated in electron ionization mode at 70 eV, with ion-source and quadrupole temperatures of 230 and 150°C, respectively. Mass spectra were acquired over an *m/z* range of 50–550. These analytical conditions were consistent with those described by Indraningrat et al., 2026. Chromatographic integration was performed using ChemStation, and the resulting mass spectra were searched independently against the NIST14 and Wiley 9th Edition spectral libraries. Tentative compound assignments were interpreted by considering both spectral-library match quality and agreement between the NIST and Wiley results. Greater confidence was assigned to peaks showing concordant matches and higher quality scores in both libraries. Peaks with low-quality or conflicting assignments were classified as unresolved rather than assigned definitively to a specific compound. Relative abundance was calculated as the percentage contribution of each integrated peak area to the total corrected chromatographic area.

RESULTS AND DISCUSSION

Cytotoxic activity against T47D cells

The fungal ethanol extract reduced the metabolic activity of T47D cells across the tested concentration range. At the three lowest concentrations, inhibition remained relatively similar, with values of 29.29 ± 5.87% at 0.01% (v/v), 32.37 ± 7.10% at 0.02% (v/v), and 31.72 ± 5.03% at 0.03% (v/v). A marked increase in inhibition was observed at the two higher concentrations, reaching 82.83 ± 1.59% at 0.25% (v/v) and 95.70 ± 9.15% at 0.50% (v/v) (Table 1). Four-parameter logistic analysis produced an estimated IC50 of 0.10% (v/v) (Figure 2).

Table 1. Cytotoxic activity of the SRL2K3 ethanol extract against T47D breast cancer cells. Values are expressed as mean ± SD (n = 3).

Extract concentration (% v/v)	T47D inhibition (%)
0.01	29.29 ± 5.87
0.02	32.37 ± 7.10
0.03	31.72 ± 5.03
0.25	82.83 ± 1.59
0.50	95.70 ± 9.15

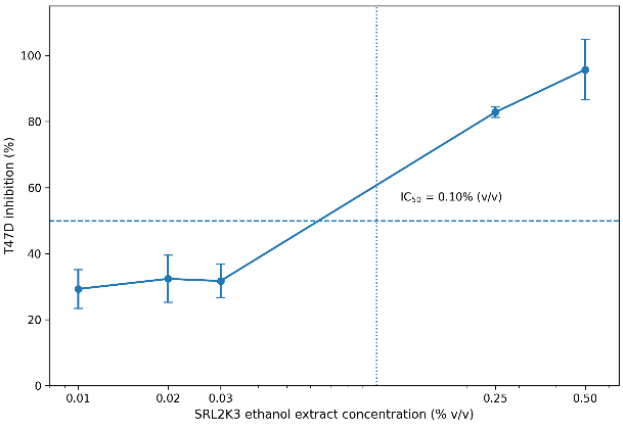


Figure 2. Cytotoxic response of the SRL2K3 ethanol extract against T47D breast cancer cells at different concentrations. Values are expressed as mean ± SD (n = 3). The horizontal dashed line indicates 50% inhibition, while the vertical dotted line indicates the estimated IC50 of 0.10% (v/v) obtained from four-parameter logistic analysis.

The cytotoxic response was not linear across the tested concentration range. Inhibition changed only slightly between 0.01 and 0.03% (v/v), followed by a pronounced increase between 0.03 and 0.25% (v/v). This pattern suggests that the effect of the crude extract became substantially greater once the concentration exceeded the lower tested range. Because relatively few concentrations were evaluated between 0.03 and 0.25% (v/v), however, the estimated IC50 of 0.10% (v/v) should be regarded as a model-derived estimate rather than a precisely resolved biological threshold. Additional concentrations within this interval would allow the steep portion of the concentration-response curve to be characterized more accurately.

Doxorubicin-HCl, used as the positive control, produced an IC50 of 40.10 ppm. Because doxorubicin and the fungal extract were expressed using different concentration units, their IC50 values cannot be directly compared as measures of relative potency. In the present

study, doxorubicin therefore served primarily as a reference confirming the responsiveness of the T47D assay system.

The cytotoxic response observed for SRL2K3 is consistent with previous reports showing substantial variation in the biological activity of marine-associated fungal extracts. Handayani et al. (2018) reported differences in cytotoxicity among marine sponge-derived fungal extracts tested against T47D and other human cell lines. Sandrawati et al. (2020) similarly observed variable biological activity among fungal isolates associated with *Dactylospongia* sp., whereas Sandrawati et al. (2023) reported cytotoxic activity among endophytic fungi obtained from the mangrove plant *Acanthus ilicifolius*. Variable cytotoxic responses have also been reported among fungi associated with Rhizophora species (Zhou et al., 2022). Together, these findings indicate that cytotoxic activity is highly isolate-dependent and cannot be inferred solely from the marine origin of a fungal strain.

A recent study showed that cytotoxic screening of Indonesian marine fungi could distinguish more active strains and provide a basis for subsequent metabolomic investigation (Puspitasari et al., 2026). The present findings extend this observation to a seaweed-associated fungal resource and identify SRL2K3 as an isolate whose ethanol extract warrants further chemical and biological investigation.

Direct numerical comparison of the present IC50 with values reported for other fungal extracts should nevertheless be approached cautiously. Previous studies commonly express fungal extract concentrations in mass-based units such as µg/mL, whereas the present experiment used percentage volume/volume. Differences in fermentation conditions, extraction procedures, exposure duration, cell density, and chemical composition further limit direct comparison between studies. The relevance of the present findings therefore lies primarily in establishing a measurable cytotoxic response within the experimental system used, rather than in ranking the potency of SRL2K3 against previously reported fungal extracts.

GC-MS profile of the fungal ethanol extract

The total ion chromatogram of the SRL2K3 ethanol extract showed a chemically complex profile containing 62 integrated chromatographic peaks over a retention-time range of approximately 3.23–28.06 min (Figure 3). Individual peaks represented from less than 0.01% to 7.62% of the total corrected chromatographic area, and no single peak accounted for more than 8% of the overall profile. The extract was therefore characterized by multiple chromatographic constituents rather than by one overwhelmingly dominant GC-MS-detectable component.

Importantly, relative chromatographic abundance did not necessarily correspond to confidence of compound annotation (Table 2). The largest peak, observed at RT 8.594 min and representing 7.62% of the total area, produced hexanoic acid anhydride as the leading assignment in both the NIST and Wiley libraries, but with a relatively low match-quality score of 45. The peak at RT 11.200 min represented 6.77% of the total area but produced different leading assignments between the two libraries: NIST suggested 1,2-ethanediol, 1-(2-furanyl)- with a quality score of 59, whereas Wiley suggested 2,5-di(hydroxymethyl)-furan with a score of 72. Similarly, the peak at RT 14.730 min, representing 6.16% of the total area, produced only low-quality library matches. These peaks were therefore classified as unresolved rather than assigned to specific compounds.

In contrast, several peaks produced substantially stronger and more consistent spectral assignments. The peak at RT 4.362 min (4.83%) was tentatively assigned as 5-methyl-2-furancarboxaldehyde, with match-quality scores of 91 in NIST and 94 in Wiley. The peak at RT 9.235 min (2.61%) showed concordant matches to 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, with scores of approximately 90–91. A peak at RT 10.944 min representing 5.15% of the total area was tentatively assigned as 5-hydroxymethylfurfural, with NIST and Wiley scores of 87 and 80, respectively.

Additional well-supported annotations included 4-hydroxyphenethyl alcohol (tyrosol) at RT 14.045 min (3.58%), ribitol at RT 17.459 min (6.08%), and n-hexadecanoic acid at RT 19.521 min (2.14%). Tyrosol and ribitol each produced concordant match-quality scores of 90 in the two spectral libraries, while n-hexadecanoic acid produced scores of 99. Later-eluting peaks were also associated with C18 unsaturated fatty-acid-related constituents. At RT 21.192 min, both libraries produced very high-quality matches to C18:1 fatty acids, although the exact positional or geometric isomer could not be resolved from the library search alone. A later peak at RT 27.731 min showed a strong match to oleic acid.

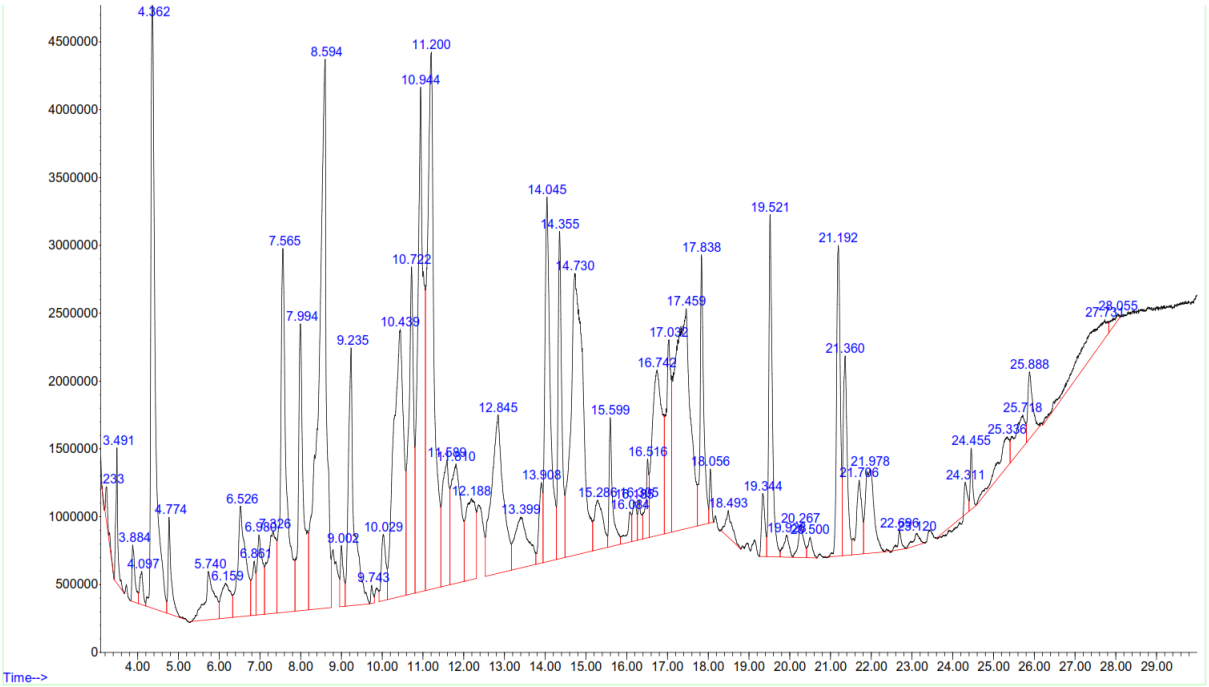


Figure 3. Total ion chromatogram of the SRL2K3 ethanol extract. Selected peaks with relatively well-supported spectral-library assignments are indicated by retention time. Major peaks at RT 8.594, 11.200, and 14.730 min produced low or inconsistent library matches and were therefore not assigned definitively.

Table 2. Selected GC-MS peaks and tentative compound annotations in the SRL2K3 ethanol extract.

RT (min)	Relative area (%)	Tentative annotation	NIST quality	Wiley quality
4.362	4.83	5-Methyl-2-furancarboxaldehyde	91	94
8.594	7.62	Unresolved[1]	45	45
9.235	2.61	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	91	90
10.944	5.15	5-Hydroxymethylfurfural	87	80
11.200	6.77	Unresolved[2]	59	72
14.045	3.58	4-Hydroxyphenethyl alcohol (tyrosol)	90	90
14.730	6.16	Unresolved	20	38
17.459	6.08	Ribitol	90	90
19.521	2.14	n-Hexadecanoic acid	99	99
21.192	2.11	C18:1 unsaturated fatty acid[3]	99	99
27.731	1.39	Oleic acid	93	93

[1] Best library match was hexanoic acid anhydride; however, the low match-quality score did not support confident annotation.
[2] NIST and Wiley produced different leading assignments, and the level of agreement was insufficient for confident structural assignment.
[3] High-quality library matches supported a C18:1 fatty acid, although the exact positional or geometric isomer could not be resolved.

The complete GC-MS profile highlights an important distinction between chromatographic abundance and confidence of chemical annotation. Several of the largest peaks were among those with the least secure spectral assignments, whereas some moderately abundant peaks showed much stronger agreement between the NIST and Wiley libraries. Accordingly, assigning chemical identities solely on the basis of relative peak area would overstate the analytical evidence.

Among the better-supported tentative assignments, the chemical profile included furan- and pyranone-related compounds, polyols, a phenolic alcohol, and saturated and unsaturated fatty-acid-related constituents. Such chemical diversity is consistent with reports describing marine- and mangrove-associated fungi as metabolically diverse natural-product resources (Amaeze et al., 2026; Deshmukh et al., 2018; Sahoo et al., 2021; Zhang et al., 2025). Nevertheless, detection of these constituents does not establish their contribution to the cytotoxic response observed against T47D cells.

This distinction is particularly important because chromatographic abundance cannot be equated with biological potency. A relatively minor constituent may contribute substantially to biological activity, whereas

highly abundant compounds may have little or no cytotoxic effect at the concentrations present in the crude extract. In addition, the response of a crude extract may reflect the combined effects of several constituents. However, additive or synergistic interactions cannot be inferred from the present data. The GC-MS results should therefore be interpreted as a preliminary chemical fingerprint rather than as evidence identifying the compound or compounds responsible for cytotoxicity.

The combination of biological screening and chemical profiling nevertheless provides a useful basis for subsequent investigation. Puspitasari et al. (2026) similarly combined cytotoxicity screening with metabolomic characterization to prioritize biologically active marine fungal resources. For SRL2K3, bioassay-guided fractionation followed by re-evaluation of individual fractions against T47D cells would allow the cytotoxic response to be associated more directly with a restricted chemical fraction. Subsequent structural characterization could then focus on metabolites present in the active fraction rather than simply on the most abundant constituents of the crude extract.

Biological interpretation and limitations

The MTT assay provides a convenient initial measure of cellular response to potentially cytotoxic substances, but its interpretation has important limitations. MTT reduction depends on the metabolic activity of viable cells and may be influenced by cell number, cellular metabolic state, experimental conditions, and interactions between the assay system and the test material. A reduction in MTT-derived signal therefore indicates decreased cellular metabolic activity but does not, by itself, establish a particular mode of cell death(Ghasemi et al., 2021).

The present findings consequently demonstrate cytotoxic activity of the SRL2K3 ethanol extract against T47D cells but do not establish whether the response involved apoptosis, necrosis, cell-cycle arrest, or another cellular process. Averufanin from *Aspergillus carneus*, for example, has been shown to induce DNA damage, cell-cycle arrest, and apoptotic cell death (Demirel et al., 2023). Although this illustrates the range of biological responses that fungal metabolites can produce, such mechanisms cannot be extrapolated to SRL2K3 without direct experimental evidence.

Another limitation is the absence of a non-cancerous breast cell comparator. Cytotoxicity against T47D cells alone does not demonstrate preferential activity toward malignant cells. The present extract should therefore be described as cytotoxic against T47D cells rather than as a selective anticancer agent. Testing against an appropriate non-cancerous cell model would permit assessment of selectivity and provide a stronger basis for evaluating biological relevance.

The use of a single breast cancer cell line also limits the extent to which the results can be generalized. T47D cells represent a hormone-responsive luminal A model with distinct estrogen- and progesterone-related characteristics (Yu et al., 2017). Breast cancer comprises multiple molecular subtypes with different biological properties and therapeutic responses (Łukasiewicz et al., 2021; Roskoski, 2024; Xiong et al., 2025). The present findings therefore describe the response of the T47D model specifically and should not be extrapolated to other breast cancer subtypes without additional experimental evidence.

Despite these limitations, the biological and chemical findings provide complementary evidence for prioritizing SRL2K3 for further investigation. The ethanol extract produced a marked concentration-related response in T47D cells, while GC-MS revealed a chemically heterogeneous profile containing several well-supported tentative assignments together with major unresolved peaks. Rather than attributing the activity to a particular GC-MS-detected compound at this stage, subsequent work should first localize the cytotoxic activity through bioassay-guided fractionation. Structural confirmation of metabolites in active fractions, evaluation against non-cancerous cells, and mechanistic studies would then provide a stronger basis for determining the biological relevance of this fungal resource.

CONCLUSIONS

The ethanol extract of the seaweed-associated fungal isolate SRL2K3 exhibited a concentration-related cytotoxic response against T47D breast cancer cells, reaching 95.70 ± 9.15% inhibition at 0.50% (v/v), with an estimated IC50 of 0.10% (v/v). GC-MS analysis resolved 62 chromatographic peaks, none of which represented more than 8% of the total corrected area, indicating a chemically heterogeneous extract. Several peaks produced strong and concordant NIST and Wiley library matches and were tentatively assigned to compounds including 5-methyl-2-furancarboxaldehyde, a dihydroxy-methyl pyranone, tyrosol, ribitol, n-hexadecanoic acid, and unsaturated C18 fatty-acid-related constituents. In contrast, several of the most abundant peaks remained unresolved because of low or inconsistent library-match quality. These findings demonstrate cytotoxic activity of the SRL2K3 ethanol extract against T47D cells but do not identify the constituent or constituents responsible for the observed response or establish selectivity toward cancer cells. Further bioassay-guided fractionation, structural confirmation of active metabolites, evaluation against non-cancerous cells, and mechanistic studies are required to clarify the biological relevance of the extract.

Acknowledgements: We would like to thank the Faculty of Medicine and Health Sciences and the Faculty of

Agriculture, Warmadewa University, for providing access to laboratory facilities used in this study.

Authors' Contributions: Desak Putu Oki Lestari and Anak Agung Gede Indraningrat designed the study. Desak Putu Oki Lestari, Anak Agung Gede Indraningrat, and Made Dharmesti Wijaya carried out the experimental work. Anak Agung Gede Indraningrat and Made Dharmesti Wijaya analyzed and interpreted the data. Desak Putu Oki Lestari prepared the initial manuscript. Anak Agung Gede Indraningrat contributed to study supervision and critical revision of the manuscript All authors read and approved the final version of the manuscript.

Competing Interests: The authors declare that there are no competing interests.

Funding: This research was supported by the Research and Community Service Unit, Faculty of Medicine and Health Sciences, Warmadewa University, under grant number 1023/Unwar/FKIK/PD-13/IX/2024.

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