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Research article

Anti-migration, anti-proliferative, and pro-apoptotic effects on cancer cell line HCT116 using sponge extracts from Lugait, Misamis Oriental, Philippines

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ABSTRACT

Sponge samples coded KL-03P, KL-12P, KL-15P, KL-16P, and KL-18NP collected from Lugait, Misamis Oriental, Philippines were subjected to fractionation resulting in hexane (H), dichloromethane (D), and aqueous (A) fractions of each sponge. They were subsequently subjected to anti-migration, anti-proliferative, and pro-apoptotic activity testing using the Scratch Wound assay, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, and the Caspase Glo 3/7 assay, respectively. Fractions KL-03P (D), KL-12P (D), KL-16P H) and KL-18NP (D) showed anti-proliferative and pro-apoptotic effects against HCT116 at 30-ppm concentration. Cell shrinkage and noticeable fragmentations indicative of apoptosis were apparent among these fractions based on MTT final snaps. But among these active fractions, the unprecedented abilities of KL-18NP (D) were very notable across assays conducted. This fraction showcased the inhibition of cell migration, which gave a signal for potentially reduced oncogenicity or metastatic capability of HCT116. The negative percentage of wound healing exhibited by this fraction is a manifestation of its cytotoxicity effect. Very remarkably, KL-18NP (D) has a similar degree of responses across the positive controls, respectively. Hence, there is a significant negative relationship between the negative control versus KL-18NP (D) in terms of its anti-migratory and anti-proliferative properties, $p<0.05$. Additionally, there is a significant positive relationship between the negative control and KL-18NP (D) when it comes to its pro-apoptotic capability, $p<0.05$. These results make KL-18NP (D) a bioactive hit as an anti-cancer agent source. Confirmatory techniques, structure determination, and target identification methods will further ascertain its mechanism of action as well as the identification of its cellular targets.

Keywords: Anti-Migration; Anti-Proliferative; Pro-Apoptotic; Fold Change; Tiering Classification

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INTRODUCTION

Cancer is a world burden. Malignant tumors still claim about 8.8 million victims per year and are the cause of 1 out of 6 deaths, globally [1,2]. Therefore, there is an urgent need to address this ailment where the current treatment strategies are mainly radiation-based therapy and chemotherapy [3]. However, these strategies are associated with toxic adverse effects such as causing hair loss and others. And so, a quest for the discovery of novel anticancer compounds with selective or lesser adverse effects is desired [4,5]. Nowadays, the utilization of complementary and alternative medicines is more popular as a promising strategy for cancer therapy. Natural products have been studied in a variety of models of cancer where the results have been extremely promising [6]. The crude extracts from marine sources contain organic compounds showing peculiar molecular structures able to contribute to the discovery of novel drugs [7]. These marine sources are known to produce chemically mediated so-called secondary metabolites or natural products. Secondary metabolites are higher in chemical diversity and associated with limited phylogenetic distribution [8]. These structures were the results of their evolution caused by biodiversity, adaptability, and their symbiotic relationships with other organisms or the surrounding environment for survival. Among these marine sources, the potential medicinal benefit of the bioactive metabolites coming from marine sponges has brought much attention to researchers [9]. Sponges' remarkable properties are their ability to suffer damage through re-constitutive and regenerative capacity [10,11]. According to the literature, cell regeneration is associated with cell motility mechanisms involving cell-to-cell recognition, adhesion, and movement. Meanwhile, the re-constitutive ability of sponges follows after regeneration, wherein it involves cell division or proliferation. And so, because of these abilities, the interest to link these abilities to drug discovery would present a breakthrough in the field of medicine. And part of this endeavor is understanding the affinities of these

active metabolites from the complex source for optimum recovery and characterizing their effects, which in this study, evaluating anticancer potentials, namely, anti-migration, anti-proliferative and pro-apoptotic activities of the different extracts from various sponge samples against HCT116 or colon carcinoma cell line [12].

Experimental

Samples were initially collected from Lugait, Misamis Oriental, Philippines. These were freeze-dried and sample weights were 11.1500g KL-03, 161.7500g KL-12, 38.9570g KL-15, 22.9840g KL-16, and 60.1660g KL-18. Each freeze-dried sponge sample was chopped and steeped in a 1:1 ethyl acetate-methanol mixture for 3 days to obtain the nonpolar extract (NP). The sponge residues were then soaked in a 1:1 ethanol-water mixture for 3 days to obtain the polar extract (P). After solvent extraction, all the extracted samples obtained were concentrated in *vacuo* and/or freeze-dried to obtain the weights of the sponge extracts. The morphological identification analyses of the assigned samples were made by a marine biologist collaborator and these were classified as shown below. However, the KL-12 sample was not identified because it is not found in the sponge database on different websites. KL-18 characteristics are not also found in taxonomic keys utilized in morphological ID. These were the main reasons why sample codes were retained and constantly used during the study.

Table 1: Classification of Assigned Sponge Extracts

Sponge Extract	Scientific Name	Weights, g	HCT116, % Viability
KL-18NP	*Haliclona sp.*	1.8439	78.59 ± 14.18
KL-03P	*Callyspongia sp.*	1.4720	78.80 ± 10.49
KL-12P	unidentified	9.6869	80.05 ± 3.44
KL-16P	*Callyspongia sp.*	2.2836	80.29 ± 6.13
KL-15P	*Niphates sp.*	5.8630	81.43 ± 6.59

These crude extracts underwent sequential liquid-liquid extraction processes that involved partitioning low-polarity to high-polarity analytes for assays. For polar extraction, dissolution with 1:1 MeOH:H_2O was done, after which low polarity analytes were extracted by adding two volumes of n-hexane inside the separatory funnel. After shaking and separation of layers, the aqueous layer at the bottom underwent further partitioning using CH_2Cl_2, wherein, the obtained layers were separated, an aqueous layer at the top being the high polar fraction and the bottom CH_2Cl_2 layer as the medium polar fraction. On the other hand, nonpolar extraction involved dissolving the extract with MeOH followed by centrifugation within 3-5 minutes. The collected supernatant experienced fractionation using n-hexane and after separation, the resulting upper layer was assigned as the low polar fraction. The lower layer was then transferred to another separatory funnel and added with a certain amount of H_2O followed by adding two volumes of CH_2Cl_2. The resulting upper layer represented the high polar extract while the lower layer was the medium polar extract. Meanwhile, no presence of any residues was observed after centrifugation and rendered no EtOAc extraction. All the collected fractions were brought to dryness either air-drying or freeze-drying and weights were recorded to determine extraction recoveries.

Cell migration is a mechanism of cell dynamics. It involves the response of cells to a chemoattractant providing signals when recognized by the cell membrane receptors. Wherein, that signal conveyed through the receptor channel activates actin-myosin co-assembly thereby promoting cell migration. And in this study, sponge test fractions interference and effects on cell migration were assessed. That is, assessing the relative wound closure on the cell monolayer.

For the anti-proliferative assay, MTT is a soluble powder reagent that is added to cells and incubated with the cells, and this is very cheap and easy to obtain. So, this powder is added to the cells. It's incubated with the cells in regular media. Since any viable or proliferating cells will contain NADPH-dependent enzyme, particularly succinate dehydrogenase and this enzyme will naturally reduce MTT, and when they reduce MTT it will generate these insoluble crystals known as formazan and these are purple crystals formed at the bottom of your plate. So, now after the incubation period, once these crystals have formed, media will be removed from the cells carefully and these crystals will be dissolved in DMSO to solubilize them and get a purple solution. Its purpleness intensity will change based on how viable those cells are and how many crystals were formed and will be read using a UV-Vis spectrophotometer at 570 nm wavelength.

For the pro-apoptotic assay, the assay principle involved using DEVD, a caspase 3/7 specific sequence coupled with a DNA dye molecule. This substrate can freely diffuse across the cell membrane in live cells. Once inside apoptotic cells, the caspase 3/7 protein recognizes and cleaves the DEVD sequence and releases the DNA probe. When the probe enters the nucleus, it binds to the DNA producing a bright, fluorescent signal which will be captured and used to monitor cell morphology.

Extreme values were initially eliminated where retention is not warranted due to random errors like poor pipetting accuracy. This was governed by utilizing statistical tools like Q-test or Excel's quartile function applications to lower the data set's standard deviation, thus increasing the results' precision. Meanwhile, the T-test was used to evaluate how statistically significantly different the effects of test fractions are against HCT116 in comparison to the negative control thus, measuring the extent of changes made by these fractions on HCT116. Additionally, a normality test was done to also test how normally distributed at a 95% confidence interval the data are.

Data interpretation involved the use of tiering classification criteria. Tiering classification is a qualitative measurement made to categorize these fractions in terms of the likelihood of their assay effects on HCT116 and the likelihood of their occurrence. Effects were evaluated based on fold change. When this was associated with the probability that the parameter is normally distributed at a 95% confidence interval or its occurrence, which was determined using p-value, along with an assessment of how consistent the combinations are across the three trials, tiering classification was established. See Table 2 below.

Table 2: Legend for Evaluation of Fold Change versus p-values

Color	MTT	Caspase Glo 3/7	Scratch Wound
	Significant decrease in cell number compared to vehicle control **(fold change < 1, p < 0.05)**	Significant increase in cell number compared to vehicle control **(fold change > 1, p < 0.05)**	Significant decrease in wound area compared to vehicle control **(fold change < 1, p < 0.05)**
	Non-significant decrease in cell number compared to vehicle control **(fold change < 1, p ≥ 0.05)**	Non-significant increase in cell number compared to vehicle control **(fold change > 1, p ≥ 0.05)**	Non-significant decrease in wound area compared to vehicle control **(fold change < 1, p ≥ 0.05)**
	Non-significant increase in cell number compared to vehicle control **(fold change ≥ 1, p ≥ 0.05)**	Non-significant decrease in cell number compared to vehicle control **(fold change ≤ 1, p ≥ 0.05)**	Non-significant increase in wound area compared to vehicle control **(fold change ≥ 1, p ≥ 0.05)**
	Significant increase in cell number compared to vehicle control **(fold change ≥ 1, p < 0.05)**	Significant decrease in cell number compared to vehicle control **(fold change ≤ 1, p < 0.05)**	Significant increase in wound area compared to vehicle control **(fold change ≥ 1, p < 0.05)**
	Not tested	Not tested	Not tested

A statistically significant negative relationship between the negative control versus bioactive fraction in terms of its anti-migratory and anti-proliferative properties is said to be top tier. This holds when a statistically significant positive relationship between the negative control and the bioactive fraction is evident concerning its pro-apoptotic capability. Accordingly, if these relationships were observed yet results were found to be not statistically significant, *p>0.05*, activities of the fraction are considered 2nd tier. If only two out of the three trials were found to agree yet not significant and a fourth trial was conducted and found to be confirmatory in agreement, the fraction is categorized as 3rd tier. If the fourth trial result is not confirmatory, the evaluation of activity is classified as "inconclusive". Lastly, if treatment with a test sample rendered only one out of three trials to be active, then evaluation is classified as "flagged".

RESULTS AND DISCUSSION

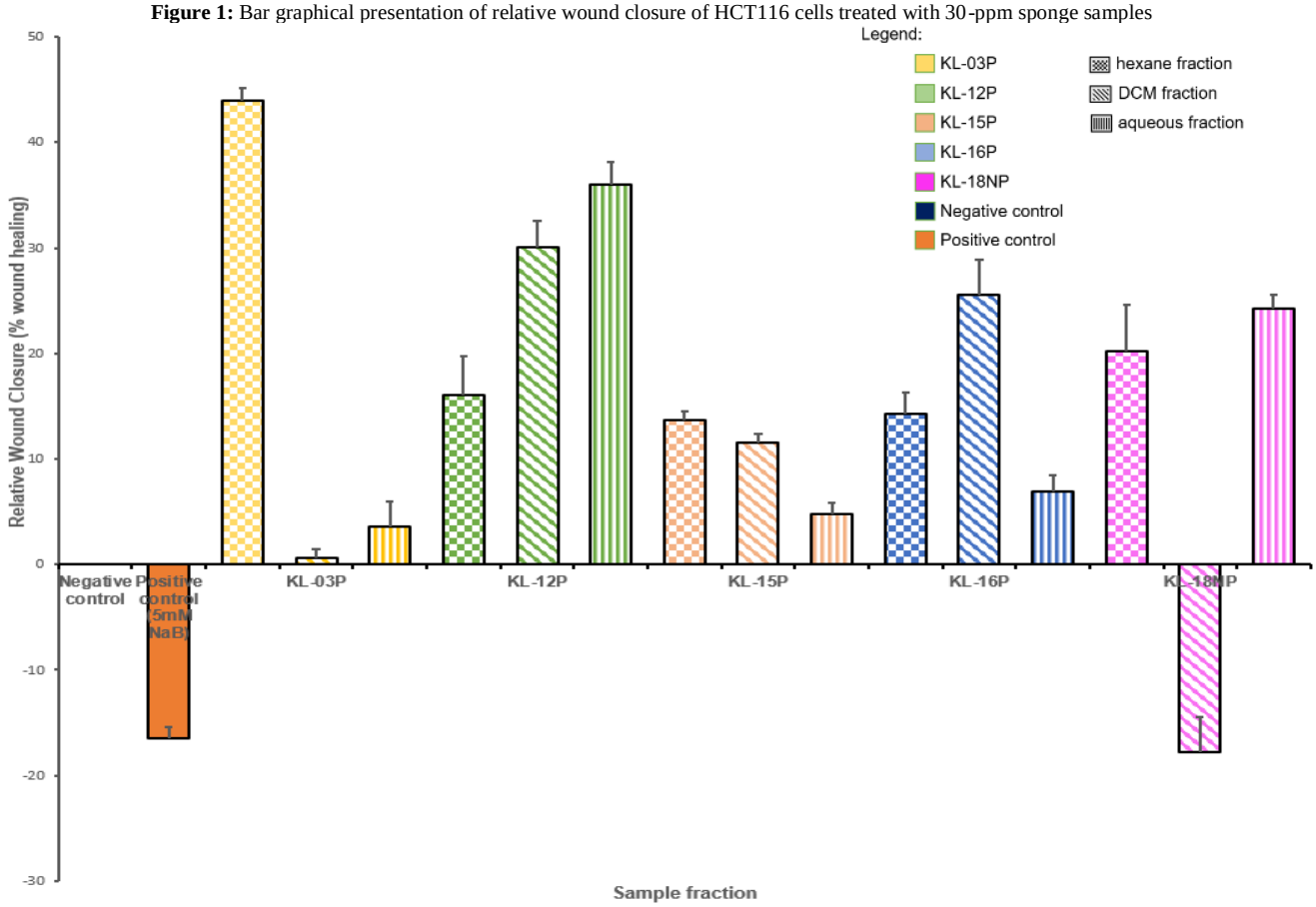
Anti-Migration Assay: (Scratch Wound Assay). Because of variabilities due to scratching, the elimination of extreme values was carried out through quartiles. Compared to other assays, more scratch wound assay data sets did not follow a normal distribution, *p>0.05*.

Table 3: Scratch Wound Tiering Classification of Fractions with Calculated % Wound Healing

SAMPLE FRACTION	Fold change			Classification	Average Relative Wound Closure (% wound healing)
	1	2	3		
Negative control	1.0	1.0	1.0		0.0
Positive control	-0.500	-0.418	-0.374		-16.43 ± 1.05
KL-03P (H)	1.187	1.059	1.039	Flagged	43.96 ± 1.23
KL-03P (D)	-0.005	0.020	0.034	2nd	0.67 ± 0.80
KL-03P (A)	0.087	0.155	0.040	2nd	3.60 ± 2.33
KL-12P (H)	0.358	0.410	0.480	2nd	16.11 ± 3.61
KL-12P (D)	0.884	0.808	0.672	2nd	30.10 ± 2.47
KL-12P (A)	0.995	0.880	0.944	2nd	36.04 ± 2.15
KL-15P (H)	0.414	0.351	0.316	2nd	13.74 ± 0.77
KL-15P (D)	0.356	0.264	0.287	2nd	11.54 ± 0.90
KL-15P (A)	0.159	0.132	0.086	2nd	4.77 ± 1.09
KL-16P (H)	0.477	0.341	0.311	2nd	14.26 ± 2.00
KL-16P (D)	0.816	0.663	0.536	2nd	25.54 ± 3.31
KL-16P (A)	0.232	0.189	0.122	2nd	6.86 ± 1.64
KL-18NP (H)	0.502	0.452	0.620	2nd	20.24 ± 4.39
KL-18NP (D)	-0.592	-0.470	-0.344	Top	-17.77 ± 3.34
KL-18NP (A)	0.659	0.626	0.614	2nd	24.28 ± 1.27

Meaning, the true population parameter involved in these fractions would fall in the range of less than 95% of the time. Table 3 shows all data in terms of mean fold change values across trials. The mean relative wound closure was based on the average of each trial.

Figure 1: Bar graphical presentation of relative wound closure of HCT116 cells treated with 30-ppm sponge samples



The average of these means was presented as mean ± standard deviation across the three trials showing the effect of test samples at 30-ppm against HCT116 cell motility. The KL-03P (H) fraction was classified as "flagged" with its unnoticeable anti-migratory effect by providing the highest fold change values (>1) across trials. KL-03P (D) exhibited strong inhibition of cell migration and was tapped as 2nd tier extract. Among these fractions, only KL-18NP (D) was categorized as top tier with negative fold change values across trials indicative of its cytotoxicity effect, each having $p<0.05$.

The relative wound closure expressed as percent wound healing is graphically presented by bar graphs showing and comparing anti-migratory responses of fractions at 30-ppm concentration against HCT116 cells.

Antiproliferative activity assay:

(MTT) Assay. For the MTT assay, some questionable values were eliminated using the Q-test, and narrowing the remaining values to a final data set of nine independent results was made using quartiles.

[a]Cytotoxicity index: high (0-10.99%), moderate (11.00-50.99%), mild (51.00-100%) Error bar presentation of these activities from various fractions at 30-ppm is shown in this graph.

Most of the fractions analyzed showed off normal distribution at more than 95% confidence interval by having p-values, $p<0.05$, except for KL-03P (A), KL-15P (A), and KL-16P (A). In most fractions, the true population parameter would fall in the range of more than 95% of the time.

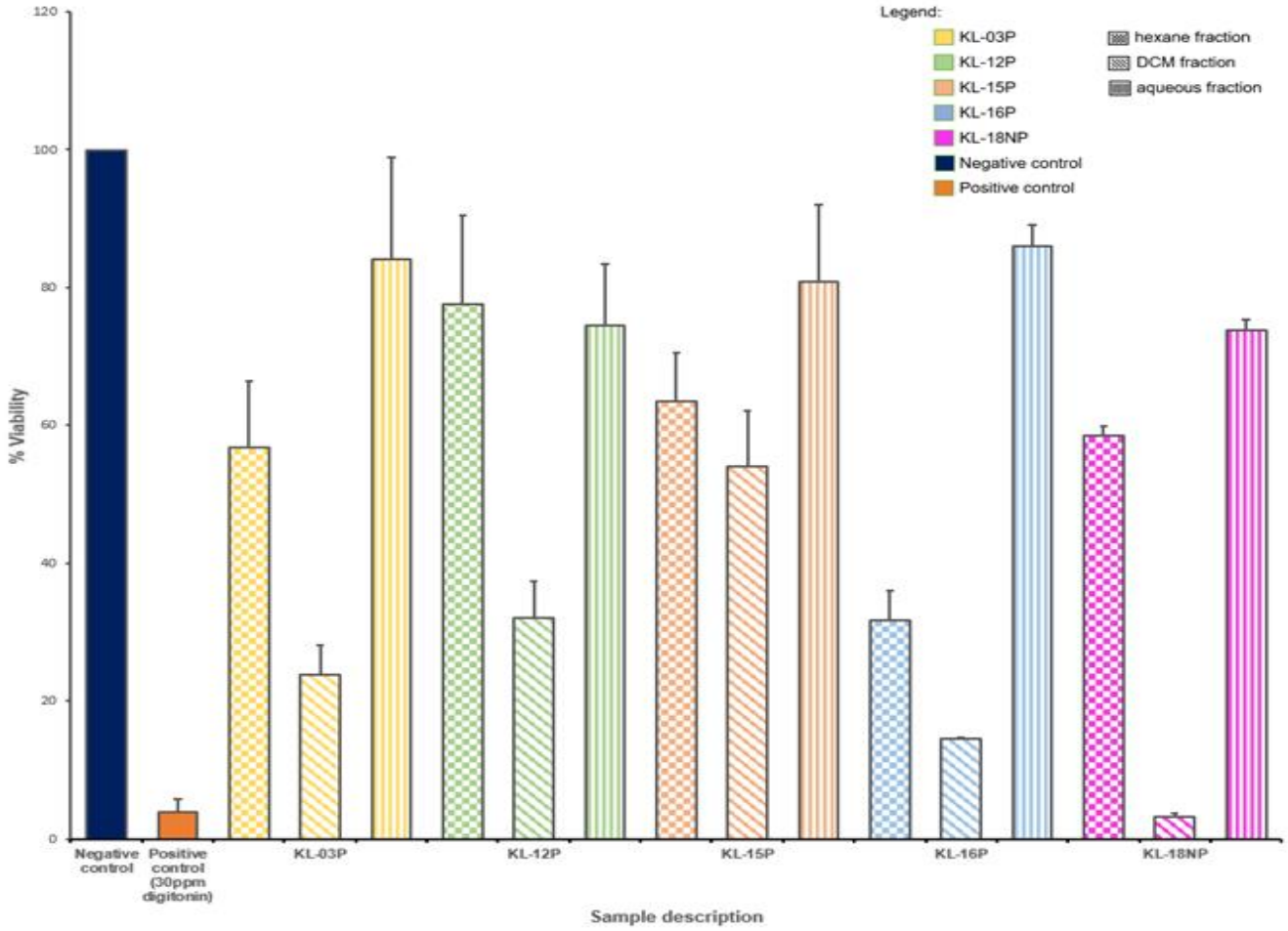
Table 4: MTT Tiering Classification of Fractions with % Viability

SAMPLE FRACTION	TRIAL #			Classification	% Viability [a]
	1	2	3		
Negative control	**1.0**	**1.0**	**1.0**		**100.0**
Positive control	0.020	0.040	0.041		3.92 ± 1.84
KL-03P (H)	0.464	0.650	0.595	Top	56.85 ± 9.55
KL-03P (D)	0.192	0.269	0.259	Top	23.93 ± 4.17
KL-03P (A)	0.673	0.942	0.915	2nd	84.17 ± 14.70
KL-12P (H)	0.630	0.861	0.843	2nd	77.66 ± 12.74
KL-12P (D)	0.267	0.372	0.326	Top	32.11 ± 5.25
KL-12P (A)	0.684	0.705	0.847	2nd	74.52 ± 8.86
KL-15P (H)	0.574	0.616	0.714	2nd	63.46 ± 7.17
KL-15P (D)	0.482	0.509	0.632	2nd	54.11 ± 7.96
KL-15P (A)	0.730	0.760	0.936	2nd	80.90 ± 11.12
KL-16P (H)	0.288	0.297	0.367	Top	31.76 ± 4.28
KL-16P (D)	0.148	0.144	0.145	Top	14.54 ± 0.20
KL-16P (A)	0.892	0.861	0.830	2nd	86.08 ± 3.10
KL-18NP (H)	0.601	0.576	0.580	2nd	58.58 ± 1.37
KL-18NP (D)	0.038	0.029	0.029	Top	3.22 ± 0.50
KL-18NP (A)	0.755	0.290	0.724	2nd	73.89 ± 1.55

Activities were evaluated in terms of each fraction's relative fold change and the corresponding p-value. Table 4 shows the classification of fractions in terms of their anti-proliferative activities and the calculated % viability presented as mean ± standard deviation. It can be interpreted that there were significant decreases in the absorbance of all samples across three trials. However, not all

these responses are statistically significant. Based on fold change and p-values, at least one fraction representative of each sponge sample shows off as classified top tier, except for those KL-15P sponge sample extracts which were categorized as 2nd tier fractions. It is also notable that KL-18NP (D) exhibited viability with a cytotoxicity index categorized as high. Additionally, KL-03P (D), KL-12P (D), KL-16P (H), and (D) exhibited top-tier activities yet gave moderate cytotoxicity.

Figure 2: Bar graphical presentation for % viability of HCT116 cells treated with 30-ppm sponge samples



Among samples, the dichloromethane extracts displayed the least % viability followed by hexane fractions and then the aqueous ones. Interestingly, the KL-18NP (D) fraction at 30-ppm concentration, exhibited relative % viability of 3.22 ± 0.50 which is very comparable to that of positive control spiked with 30-ppm digitonin. In contrast, KL-16P (A) showed relatively high % viability at 86.08 ± 3.10%.

Pro-apoptotic activity:

(Caspase Glo 3/7 assay). For Caspase Glo 3/7 assay, retention of all values across the replicates based on Q-test and rejection cannot be warranted. However, the quartile application has identified those extreme values that affect the standard deviation narrowing the number of independent values enough for three trials, each consisting of three replicate readings. All of the fractions analyzed showed off normal distribution at more than 95% confidence interval by having p-values, $p<0.05$. Meaning, in all fractions, the true population parameter would fall in the range of more than 95% of the time. Table 5 shows all the data in terms of the mean fold change values across three replicates per trial and the average of these means was presented as mean ± standard deviation across trials. This shows how the effects of test samples at 30 ppm affected the luminescence intensities after incubation as compared to those responses of the negative control. Relative to the negative control, all samples except for KL-16P (D), show a significant increase in luminescence intensities having fold change values greater than 1. All trials of KL-18NP were categorized as top tier characterized by a significant increase in luminescence intensities with a statistically significant difference against the negative control, $p<0.05$. Additionally, all the dichloromethane fractions of other assigned samples were also classified top tier except for that of KL-16P which was tagged as "flagged" by consistently showing fold change values much lower than 1. This treatment with the test sample did not result in an increase in caspase-3/7 activity in all three trials. Meanwhile, aqueous fractions were qualified as 2nd tier except those of KL-16P and KL-18NP samples. KL-03P (D) and KL-18NP (D) at 30-ppm concentration showed off the highest fold

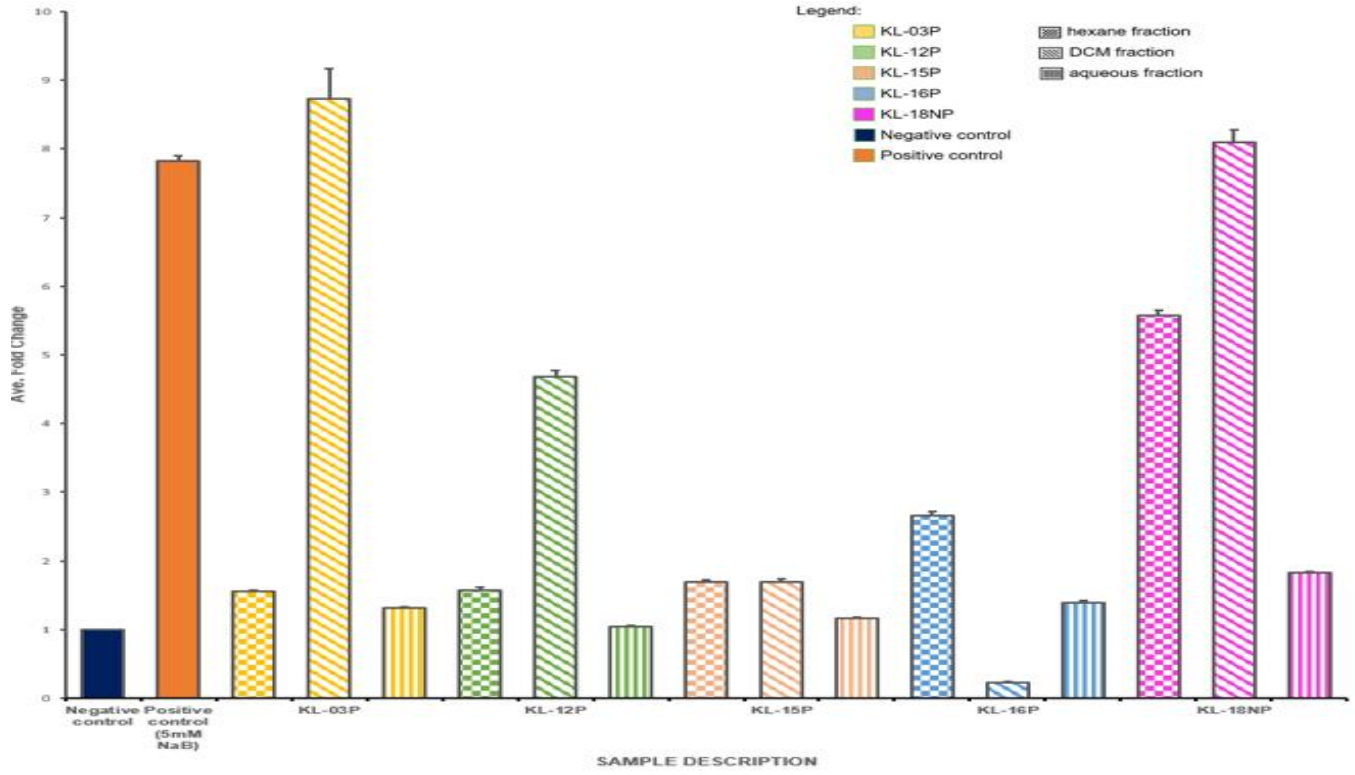
change values very closely comparable to those of the positive control spiked with 5mM sodium butyrate (NaB).

Table 5: Caspase Glo 3/7 Assay Tiering Classification of Fractions

SAMPLE DESCRIPTION	TRIAL #			Classification	Ave. Fold Change
	1	2	3		
Negative control	1.0	1.0	1.0		1.0 ± 0.00
Positive control	7.747	7.892	7.840		7.83 ± 0.07
KL-03P (H)	1.537	1.548	1.574	2nd	1.55 ± 0.02
KL-03P (D)	8.232	8.946	9.011	Top	8.73 ± 0.43
KL-03P (A)	1.305	1.312	1.330	2nd	1.32 ± 0.01
KL-12P (H)	1.547	1.624	1.543	2nd	1.57 ± 0.05
KL-12P (D)	4.579	4.742	4.734	Top	4.68 ± 0.09
KL-12P (A)	1.064	1.036	1.044	2nd	1.05 ± 0.01
KL-15P (H)	1.718	1.696	1.667	Top	1.69 ± 0.03
KL-15P (D)	1.736	1.696	1.640	Top	1.69 ± 0.05
KL-15P (A)	1.182	1.152	1.149	2nd	1.16 ± 0.02
KL-16P (H)	2.718	2.661	2.596	Top	2.66 ± 0.06
KL-16P (D)	0.230	0.233	0.233	Flagged	0.23 ± 0.00
KL-16P (A)	1.365	1.397	1.425	Top	1.40 ± 0.03
KL-18NP (H)	5.554	5.521	5.658	Top	5.58 ± 0.07
KL-18NP (D)	7.892	8.219	8.192	Top	8.10 ± 0.18
KL-18NP (A)	1.811	1.849	1.822	Top	1.83 ± 0.02

Relative activities were plotted using a bar graph.

Figure 3: The bar graph on average fold change of HCT116 cells treated with 30-ppm sponge samples.



Summary of Results. After solvent partitioning and drying processes, the exact weights of the different extracts were measured using an analytical balance. Based on the actual weights of the recovered residues per treatment, the total percentage recoveries of all the components ranging from low to medium to high polarity extracts were calculated.

Summarizing all results showing the tiering classifications of all fractions on respective assays including the relative wound closure expressed as % wound healing as well as the % viability of the different fractions provides a conclusive narrative to a certain degree.

Table 6: Total Percent Recoveries of Sample Extracts

Sponge Extract	Initial Test Weights, g	Total % Recovery
KL-18NP	1.0424	88.56
KL-03P	1.0016	58.97
KL-12P	1.0194	81.94
KL-16P	1.0054	103.81
KL-15P	1.0030	107.33

Fractions KL-03P (D), KL-12P (D), KL-16P (H), and KL-18NP (D) at 30-ppm concentration showed off very active anti-

proliferative, from moderate to high cytotoxicity effects and pro-apoptotic effects against HCT116 cell line.

Cell shrinkage and fragmentation indicative of apoptosis were very evident among these fractions based on MTT final snapshots (Figure 4). But among these active fractions, very remarkable inhibition of cell migration giving a signal for potentially reduced oncogenicity or metastatic capability of HCT116 was showcased by KL-18NP (D) fraction. It can be noticed that assays being done on KL-18NP (D) fraction have a similar degree of responses (anti-migratory, anti-proliferative and pro-apoptotic effects) as the positive controls, respectively. KL-16P (D) exhibited the least apoptotic effects against HCT116. Treatment with the test sample did not result in an increase in caspase-3/7 activity in all three trials. Also, it is worth mentioning that KL-03P (D) exhibited the highest pro-apoptotic effect among fractions. The anti-migratory evaluation for KL-03P (H) resulted in a decrease in wound area in none out of the three trials. The rest of the fractions were mostly classified 2nd tier for all assays performed. It is also worthwhile to mention that these potent activities were in agreement with existing studies conducted on the respective species of these sponges.

Table 7: Summary of Results

SAMPLE FRACTION	Assay tiering classification			Relative Wound Closure (% wound healing)	% Viability [a]
	Scratch Wound	Caspase Glo 3/7	MTT		
KL-03P (H)	Flagged	2nd	Top	43.96 ± 1.23	56.85 ± 9.55
KL-03P (D)	2nd	Top	Top	0.67 ± 0.80	23.93 ± 4.17
KL-03P (A)	2nd	2nd	2nd	3.60 ± 2.33	84.17 ± 14.70
KL-12P (H)	2nd	2nd	2nd	16.11 ± 3.61	77.66 ± 12.74
KL-12P (D)	2nd	Top	Top	30.10 ± 2.47	32.11 ± 5.25
KL-12P (A)	2nd	2nd	2nd	36.04 ± 2.15	74.52 ± 8.86
KL-15P (H)	2nd	Top	2nd	13.74 ± 0.77	63.46 ± 7.17
KL-15P (D)	2nd	Top	2nd	11.54 ± 0.90	54.11 ± 7.96
KL-15P (A)	2nd	2nd	2nd	4.77 ± 1.09	80.90 ± 11.12
KL-16P (H)	2nd	Top	Top	14.26 ± 2.00	31.76 ± 4.28
KL-16P (D)	2nd	Flagged	Top	25.54 ± 3.31	14.54 ± 0.20
KL-16P (A)	2nd	Top	2nd	6.86 ± 1.64	86.08 ± 3.10
KL-18NP (H)	2nd	Top	2nd	20.24 ± 4.39	58.58 ± 1.37
KL-18NP (D)	Top	Top	Top	-17.77 ± 3.34	3.22 ± 0.50
KL-18NP (A)	2nd	Top	2nd	24.28 ± 1.27	73.89 ± 1.55

[a] Cytotoxicity index: high (0-10.99%), moderate (11.00-50.99%), mild (51.00-100%)

CONCLUSION

Sponges are known to possess remarkable reconstitutive and regenerative abilities ranging from wound healing or body part regeneration to the impressive re-building of a functional body from dissociated cells. This has made them important models in studies aimed at understanding the evolutionary history of animal regeneration mechanisms, including relationships between regeneration and development (reconstitutive ability).

Fractionation using different polarity solvents is a powerful and very encompassing technique offering impending solutions to cleverly exploit studies of sponges' characteristics. Applications of Scratch Wound, MTT, and Caspase Glo 3/7 assays became very efficient tools to assess the remarkable productivity of sponges and describe the effects of any potent materials present affecting mechanisms involved in the process of the cancer cell cycle.

Assigned sponge samples coded KL-03P, KL-12P, KL-15P, KL-16P, and KL-18NP were initially collected from Lugait, Misamis Oriental., Philippines. Crude samples were fractionated using different polarity solvents like hexane, dichloromethane, and aqueous solvent mixtures. These were dried via air-drying or freeze-drying methods. Dried fractions were subjected to Scratch Wound, MTT, and Caspase Glo 3/7 assays.

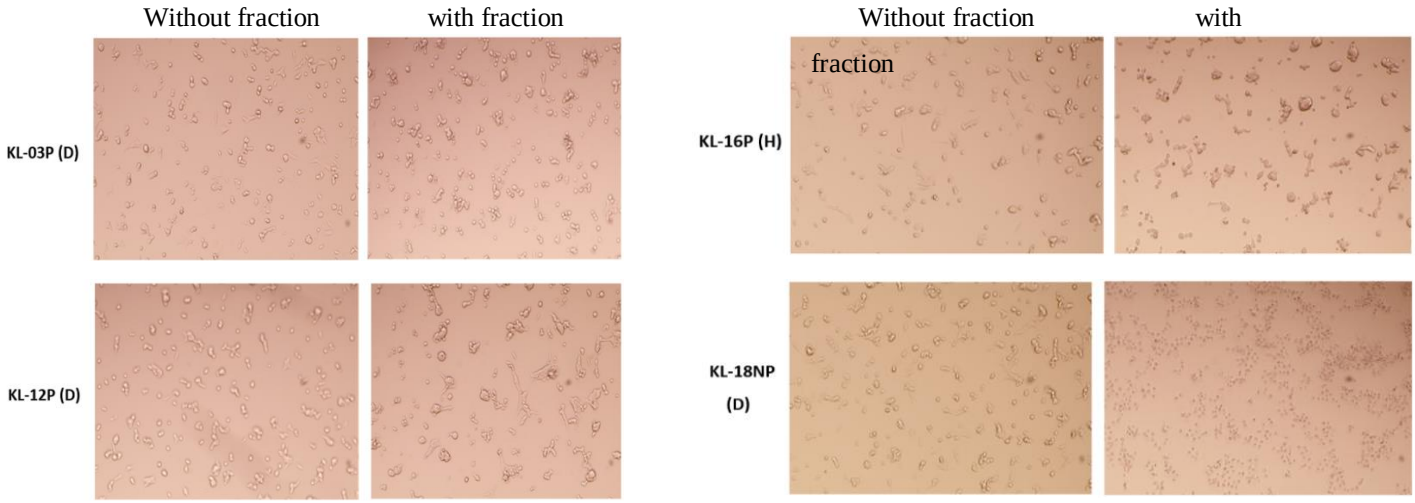
Fractions KL-03P (D), KL-12P (D), KL-16P (H), and KL-18NP (D) showed anti-proliferative and pro-apoptotic effects against HCT116 cell line at 30-ppm concentration. Cell shrinkage and formation of fragmented bodies indicative of potential apoptosis were very evident among these fractions based on MTT final snaps. But among these active fractions, the unprecedented abilities of KL-18NP (D) fraction were very notable across assays used. Inhibition of cell migration giving a signal for potentially reduced oncogenicity or metastatic capability of HCT116 was showcased by KL-18NP (D) fraction. The negative percentage of wound healing exhibited by this fraction is a manifestation of its high cytotoxicity effect. It can be noticed that assays being done on KL-18NP (D) fraction have a similar degree of responses (anti-migratory, anti-proliferative and

pro-apoptotic effects) as the positive controls, respectively. Hence, there is a significant negative relationship between the negative control versus KL-18NP (D) fraction in terms of its anti-migratory and anti-proliferative properties, *p<0.05.* Additionally, there is a significant positive relationship between the negative control and KL-18NP (D) fraction when it comes to its pro-apoptotic capability, *p<0.05.* These results make KL-18NP (D) a bioactive hit as an anti-cancer agent source and provide the idea of the significance of using confirmatory techniques or performing structure elucidation of the inherent bioactive components utilizing highly sensitive and high-throughput technologies available to further ascertain its mechanisms of action as well as identification of its cellular targets.

Interestingly, although some fractions may have exhibited less anti-migratory properties but very significant anti-proliferative and pro-apoptotic effects, these are nevertheless good prospects as potential chemopreventive agents targeting other hallmarks of cancer.

Figure 4: Images (10X magnification) of fractions, (all left) without fraction and (all right) with fraction, that exhibited moderate to high cytotoxicity effects on HCT116 at 30-ppm concentration

Supporting information



Conflicts of interest

The authors declare that there are no conflicts of interest exist.

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