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IN VITRO DETERMINATION OF CYTOTOXIC ACTIVITY OF FUCOIDAN EXTRACT OF *SARGASSUM SERRATIFOLIUM* C. AGARDH (ARAGAN) AGAINST MCF7 (HUMAN BREAST CANCER) CELL LINE

Jessica Joyce A. Duque[1,2,3,4*], Carmella V. Kapangyarihan[1,2,3,4], Charlene B. Lorenzo[1,2,3,4], Paulene A. Sernada[1,2,3,4], Keesha T. Vergara[1,2,3,4], Albeine Grace D.C. Viliran[1,2,3,4], Dr. Angelita A. Rodriguez[1,2,3,5], Juneve F. Tejada[1,2,3,5] and Teresa May B. Bandiola[1,2,3,5]

[1]College of Pharmacy; [2]Research Development and Innovation Center; [3]Our Lady of Fatima University, Quezon City, Philippines; [4]Undergraduate Thesis; [5]Research Adviser

ABSTRACT

Objectives: *This study aimed to determine the cytotoxic property of the fucoidan extract of Sargassum serratifolium C. Agardh using MTT assay, Allium cepa (Onion) genotoxicity test, and Artemia salina (Brine shrimp) lethality test.*

Methodology: *The ethanolic extract of Sargassum serratifolium C. Agardh (Aragan) was subjected to phytochemical screening prior to cytotoxicity determination. Then, the ethanolic extract was further extracted to produce a semi-purified fucoidan extract of Sargassum serratifolium C. Agardh (Aragan). The semi-purified fucoidan extract underwent Fourier Transform Infrared Spectroscopy (FT-IR) to determine the presence of sulfated polysaccharides which gives way to its cytotoxic activity. Determination of cytotoxic property of the fucoidan extract was done using MTT assay, Allium cepa (Onion) genotoxicity test, and Artemia salina (Brine shrimp) lethality test.*

Findings: *Preliminary phytochemical testing of the plant extract resulted positive to flavonoids, alkaloids, reducing sugars, and fixed oils. The MTT assay result showed that the fucoidan extract from S. serratifolium (Aragan) did not illicit any cytotoxic activity to MCF7 (Human Breast Cancer) cell line up to 100ug/mL concentration. Also, the Allium cepa (Onion) genotoxicity test showed that there is a minimal effect in the growth inhibition of the root tips of the onion. Lastly, the Artemia salina (Brine shrimp) lethality test showed that fucoidan extract has a significant difference with the negative control, 1% DMSO with artificial seawater but it is not comparable to the positive control, 2% ethyl alcohol.*

Conclusion: *Sargassum serratifolium C. Agardh (Aragan) does not possess any cytotoxic activity in MCF7 (Human Breast Cancer) cell line up to 100ug/mL.*

Keywords: Sargassum, Cytotoxic, MTT, Genotoxicity, Lethality.

INTRODUCTION

Cancer is a generic term for a large group of diseases that can affect any part of the body. One defining feature of cancer is the rapid creation of abnormal cells that grow beyond their usual boundaries, which can then invade adjoining parts of the body and spread to other organs.[2] It is among the leading causes of death worldwide, accounting for 8.2 million deaths in 2012.[1] In the Philippines, it is the third leading cause of morbidity and mortality, after communicable diseases and cardiovascular diseases according to the Department of Health (Department of Health–Health Intelligence Service or DOH–HIS, 1992, 1996).There are more than 200 types of cancer, each with different causes, symptoms, and treatments. One of which is breast cancer. It is a type of cancer that forms in tissues of the breast. Breast cancer occurs in both men and women, although male breast cancer is rare.[3] Worldwide, it is estimated that more than 1.68 million women were diagnosed with breast cancer in 2012, with incidence rates varying across the world.[4] Sargassum, common and abundant brown seaweed or algae (Ang 1985, Trono and Lluisma 1990, Montes 1993, Hurtado and Ragaza 1999) in the lower inter-and-subtidal zones of the Philippines, is a potential source of sodium alginate. Brown algae of the Sargassum genus belong to the family Sargassaceae of the order Fucales, and these algae are widely distributed in the coast of Europe, Asia, Africa, Australia, North America, and South America. Sargassum species are rich and abundant source of biologically active polysaccharides, including alginic acids, laminarans and fucoidans. Fucoidans isolated from these algae have various structural characteristics, ranging from sulfated homofucans to heteropolysaccharides (Menshova, et. al, 2013). These unique compounds are very interesting due to their wide spectrum of pharmacological properties and low toxicity in vivo. Fucoidans have a complex and non-regular structure, making a detailed structural analysis of these molecules difficult. The structural diversity of fucoidans is still not fully characterized.

Based on known data, fucoidans are members of a family of sulfated homo- and heteropolysaccharides that are mainly comprised of α-L-fucopyranose residues. In addition, fucoidans can contain small amounts of other monosaccharide residues, including galactose, xylose, mannose, ramnose and uronic acids. Acetyl groups are also known to be constituents of fucoidans. It has been established that fucoidans have multiple biological activities. These facts and the arising problem of anti-cancer treatment led the researchers to study and determine the cytotoxic activity of one of the Sargassum species which has been reported to be a genus of brown seaweeds present in the seas of the Philippines. The metabolic and physiological capabilities of marine organisms that allow them to survive in a complex habitat provide a tremendous potential for the production of unique metabolites that are not found in terrestrial environments. Thus, marine organisms have been recognized as an attractive source of potential pharmaceutical compounds (Faulkner, 2002). Hence, this study aimed in searching potential treatment for cancer and also in discovering one of the nature's gifts to mankind.

MATERIALS AND METHODS

Materials Used and Preparation of Treatments

Fresh samples of *Sargassum serratifolium* C. Agardh (Aragan) were collected from the seashore of Brgy. Namruangan, Cabugao, Ilocos Sur, Philippines. The plant samples were stored in a cooler with seawater during transport to Manila. The collected specimens were subjected for authentication at the National Museum of the Philippines. Plant samples were washed several times with distilled water to remove salts, sand, and epiphytes. The samples were air-dried under the shade and were grounded into fine particles using Wiley mill (osteorizer) and sieved in 2mm mesh. The powdered plant samples were kept in a clean, dried, well-sealed amber glass container to protect it from sunlight. MCF7 (Human breast cancer) cell line was selected to represent the dependent variable in determining in vitro cytotoxic activity of *Sargassum serratifolium* C. Agardh (Aragan) fucoidan extract. It was incubated, cultured, and stored at the University of the Philippines Diliman, where the experimentation and application of *Sargassum serratifolium* C. Agardh (Aragan) fucoidan extract were held. The whole study was done from June 2014 up to March 2015 at Our Lady of Fatima University, Quezon City, Philippines for the semi-purification procedure of the plant sample and phytochemical screening and at the University of the Philippines, Quezon City, Philippines for the biological testing.

Phytochemical Screening

Dried *Sargassum serratifolium* C. Agardh (Aragan) was weighed to find its moisture content. 100 g of the dried plant extract was placed in an amber bottle, then sufficient amount of 80% ethanol was added, enough to submerge the dried plant sample for 48 h. Then it was filtered and concentrated to yield the concentrated ethanolic *Sargassum serratifolium* C. Agardh (Aragan) extract (Miladiyah, 2011). It was subjected for organoleptic test (color, appearance and color) and pH determination. Then, it was tested to different phytochemical tests to identify the presence of different biomolecules using the standard procedures.

1. Test for flavonoids:

(a)-Shinoda's Test

One ml of concentrated ethanolic extract was mixed with 0.5 ml of hydrochloric acid and magnesium metal. A reddish color indicates the presence of flavonoids (Walform et al., 1990).

(b)-Alkaline Reagent Test

Five ml of 1% hydrochloric acid extract (1g of dried sample in 100 mL) was shaken with sodium hydroxide , appearance of yellow color indicates the presence of flavonoids.

2. Test for tannins:

The concentrated was tested for the presence of tannins using the Ferric chloride test (Trease and Evans, 1989). One drop of ferric chloride was added to 2 ml of the extract, the appearance of bluish or greenish black coloration indicates the presence of pyrogallol or catechol tannins, respectively.

3. Test for unsaturated sterols and or/ triterpenes:

For testing the presence of unsaturated sterols and triterpenes, 15mL concentrated ethanolic extract was evaporated to dryness. The residue was dissolved in 10 ml chloroform, filtered and the filtrate was divided into two equal portions for preceding the following tests (Wall et al, 1954).

(a)- Liebermann-Burchard test:

To the first portion of chloroform filtrate 1 ml of acetic acid anhydride was added, followed by 2 ml of sulfuric acid down the wall of the test tube. The appearance of a reddish violet color at the junction of the two layers and a bluish green color in the acetic acid layer indicates the presence of unsaturated sterols and or/triterpenes.

(b) - Salkowiski's test:

To the second portion of chloroform filtrate an equal volume of sulfuric acid was added. The appearance of a red color indicates the presence of unsaturated sterol and /or triterpenes.

4. Test for carbohydrates and or/glycosides:

Five g of the air-dried powder of plant was boiled with 20 ml distilled water. The aqueous solution was filtered and the filtrate was tested for the presence of carbohydrates and glycosides using the procedures described by Harper (1975) and Balbaa (1986).

(a) – Molisch's test:

Two ml of the prepared filtrate was mixed with 0.2 ml of alcoholic solution of α-naphthol 10% in addition to 2 ml of sulfuric acid; formation of bluish violet zone indicates the presence of carbohydrates and /or glycosides.

(b) – Fehling's test:

In a test tube 5 ml of the filtrate was treated with 5 ml Fehling's solutions (A & B) and will be heated; the appearance of a red precipitate indicates the presence of reducing sugars.

(c) – Benedict's test:

To 1 ml of the filtrate, 5 ml of Benedict's reagent was added. The mixture was heated; appearance of red precipitate indicates the presence of reducing sugars.

(d) – Iodine test:

Two mL of iodine solution was added to the concentrated ethanolic extract. Formation of dark blue or purple coloration indicates the presence of carbohydrates.

5. Test for alkaloids and /or nitrogenous bases:

About 10 g of the air- dried plant sample was extracted with 50 ml dil. hydrochloric acid. The acidic filtrate was rendered alkaline with ammonium hydroxide and extracted with three successive portions (each 15 ml

of chloroform). The chloroform extracts were evaporated to dryness and the residues was dissolved in 2 ml of dilute hydrochloric acid and was tested with Mayer's and modified Dragendorff's reagent (Fulton,1932) and Hager's reagent and Wagner's reagent (Trease and Evans, 1989).

(a) – Mayer's reagent:

When added to the residue solution, turbid or white precipitate is formed; this indicates the presence of alkaloids.

(b)- Dragendorff's reagent:

When added to the residue solution an orange precipitate is formed, this indicates the presence of alkaloids.

(c)- Hager's reagent:

When added to the residue solution a yellow precipitate is formed, this indicates the presence of alkaloids.

(d) - Wagner's reagent:

When added to the residue solution reddish brown precipitate is formed, this indicates the presence of alkaloids.

6. Test for saponins:

One g of the dried plant sample was boiled with 10 ml water for few minutes and was filtrated. The filtrate was vigorously shaken. The persistent froth (1 cm height) for 1 h indicates the presence of saponins (Wall et al.,1954).

7. Test for proteins and/or amino acids:

(a) – Xanthoproteic test:

Two mL of concentrated ethanolic extract was treated with a few drops of concentrated Nitric acid. Formation of yellow color indicates the presence of proteins.

8. Test for fixed oil and volatile oil

Small quantity of the concentrated ethanolic extract was dropped in a filter paper. Appearance of oil stains on the paper indicates the presence of fixed oil. And absence of oil stain indicates the presence of volatile oil.

9. Organoleptic Screening

(a) – Color
(b) – Odor
(c) – Appearance
(d) – pH

Extraction and Semi-purification of Plant Sample

The active compound, fucoidan extract, of *Sargassum serratifolium* C. Agardh (Aragan) was extracted according to Michailovna et al. and Gamal-Eldeen et al. with some modifications. The researchers only had done the extraction specific for fucoidan. The powdered plant sample was extracted with 80% Ethanol at room temperature for 48 hours to remove the lower molecular weight components. The resulting residue was extracted with boiling distilled water for 6 h twice. Then it was strained using a muslin cloth then filtered using a filter paper. The strained and filtered residue was subjected to further extraction using 0.1 M HCl for 10 h. The filtrate was adjusted to pH 6.0 by 1 M NaOH using a pH meter and was concentrated to one-fifth (1/5) of its original volume by sand bath, and was precipitated with 95% ethanol, and centrifuged for 20 min at 3000g to give the fucoidan extract.

Finally, the fucoidan extract was evaporated to dryness and was used for chemical analysis and tested for its cytotoxic properties.

Instrumentation Fourier Transform Infrared Spectroscopy (FT-IR)

Fourier Transform Infrared Spectroscopy (FT-IR) was used to determine the chemical composition of the dried plant sample, *Sargassum serratifolium* C. Agradh (Aragan) and it was also used to determine the presence of sulfated polysaccharide or fucoidan on the semi-purified extract of the plant sample.

Fourier Transform Infrared spectra were obtained from discs containing 10 mg dried sample in approximately 90 mg potassium bromide (KBr). All spectra were obtained using a Nicolet 6700 FT-IR Spectrometer equipped with a Smart Purge detector. Background (pure potassium bromide) was subtracted

using the OMNIC software. All spectra were collected by co-adding 128 scans at a resolution of 2 cm-2 and a gain of 1.0. Twelve replicates of each sample were analyzed and spectra for the replicated runs were averaged. Fourier self deconvolution was conducted on the average spectra for the amide I band, using a resolution enhancement factor of 1.8 and full height band width of 13 cm-1. The self deconvolution provided information on the number and location of components. The peaks were identified by software and assigned according to the literature values.

In Vitro Biological Assay and Data Gathering

1. MTT Assay

The MTT cytotoxicity assay performed in this study was adapted from Mosmann (1983). In detail, MCF7 cells were seeded at 6 x 104 cell/mL, in sterile 96-well microtiter plates. The plates were incubated overnight at 37°C and 5% CO_2. Following incubation, cells were treated with 10µL of each sample dilution. The initial concentration of the fucoidan extract was 100µg/mL which was serially diluted in complete medium with 3.16-fold dilutions to give four concentrations. Four concentrations were used for the four (4) test samples: 50 ug/mL, 25 ug/mL, 12.5 ug/mL and 6.25 ug/mL. Doxorubicin served as positive control while dimethyl sulfoxide (DMSO) served as negative control. The treated cells were again incubated for 72 hours at 37°C and 5% CO_2. After incubation, the media was removed and 20 uL 3-(4,5-dimethylethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) in 5mg/mL PBS was added. The cells were again incubated at 37°C and 5% CO2 for 4 hours. Then, 150 uL DMSO was added to each well. Absorbance was read at 570 nm. The inhibition concentration 50 (IC50) was computed using the software "icpin" which uses a linear interpolation of the graph of absorbance against concentration.

Three trials in three replicates per concentration were performed for each test samples.

2. *Allium cepa* Genotoxicity Test

The onion genotoxicity test provides an easy screening of chemicals or samples with genotoxic effects, especially to plants; usually root growth test is applied to find the EC50 value for root elongation.

One set of onions for the macroscopic and microscopic analyses was prepared to make it easier for the roots to grow. Then, the outer skin and the old roots of onion bulbs were removed. For the macroscopic analysis, 24 onion bulbs were used. The onion bulbs were initially exposed to distilled water in a vial to let the roots grow. The base of the onion must reach the surface of the water. After 48 hours of exposure to distilled water, 8 sets composed of 3 onions each were divided and prepared. The first six (6) sets of onions were exposed to different concentrations of Sargassum serratifolium C. Agardh (Aragan) fucoidan extract: 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, and the other two (2) sets of onions were exposed to the positive control, Maleic hydrazide and negative control, Dimethyl sulfoxide (DMSO). The fucoidan extract from Sargassum serratifolium C. Agardh (Aragan) used for the test was prepared by dissolving it to Dimethyl sulfoxide (DMSO). After 48 hours, the lengths of the roots were measured using vernier caliper. In macroscopic analysis, the mean of the different length of the roots were taken and listed down. The results were plotted against the different concentration in order to determine the EC50. Three replicates per concentration of the Sargassum serratifolium C. Agardh (Aragan), the positive control, maleic hydrazide and negative control, dimethyl sulfoxide (DMSO) were performed. The roots were cut and stored in vials containing a farmer's solution (3 parts of ethyl alcohol; 1 part glacial acetic acid) prior to sectioning and slide preparation. The sectioning of root tips was done by the Philippine Kidney Dialysis Foundation, a recommended agency for slide preparation of specimens. The root tips were placed on a slide and the last 1–2 mm of the tip were cut off, the rest were discarded. The growing tip is slightly tapered and slightly whiter in color than the rest of the root. The tips were covered with a few drops of toluidine blue to stain the cells. After two minutes, the stains were carefully blotted away by touching a paper towel to the slide. The root tips were covered with one or two drops of water and cover slips were gently lowered over the root tips. The slides were covered with a paper towel and it was pressed firmly on the cover slip using the thumb to create a single layer of cells. In microscopic analysis, the slides were subjected under the light microscope to examine the cells. One-thousand (1000) random dividing cells were observed per slide. The percentages of dividing cells per phase were tallied and the numbers of aberrant cells per phase were recorded. Aberrations observed were chromosomal break, chromosomal loss and chromosomal bridges and some abnormalities in cell division.

3. *Artemia salina* (Brine shrimp) Lethality Test

The brine shrimp assay is a rapid, reliable and inexpensive general bioassay tool for active plant extracts. The procedure allows the determination of LC50 values in µg/mL of active constituents in the brine medium. The eggs of brine shrimp, Artemia salina leach, are readily available in dry state, as food for tropical fish. When placed in the brine solution, the eggs hatch within 48 hours, providing large numbers of larvae (nauplii). The researchers put the brine shrimp eggs mixed with artificial salt water in a glass tank for them to be hatched. After 48 hours, the larvae or nauplii were collected using micropipette (Pisutshanan et al, 2004). Then, six concentrations of fucoidan extract of Sargassum serratifolium C. Agardh (Aragan) were prepared by dissolving it in artificial salt water: 100µg/mL, 50µg/mL, 25µg/mL, 12.5µg/mL, 6.25µg/mL and 3.125µg/mL. Two (2%) ethyl alcohol and 1% Dimethyl sulfoxide with artificial salt water was used as the positive and negative control, respectively. The different concentrations and the controls were placed in a 24-well culture plates, in four replicates. Ten nauplii were placed in each well submerging, and then incubated at room temperature for 24 hours. After that, the number of mobile nauplii was counted which is considered alive, while immobile ones were considered dead. The percentage mortality was then plotted against the logarithm of concentration and then the LC_{50} was determined from the graph.

Statistical Tool

One-way analysis of variance (ANOVA) was used to test the null hypothesis, to identify the equality of the means at one time by using variances. The mean of the samples were calculated within each group, and then the variances among the means were compared with the average variance within each group. The test statistic is the ratio of the variances among means divided by the average variance within groups. The ANOVA utilized F-statistics (f-test) which is used to ratio the variance calculated among the means to the variance within the samples.

RESULTS

The results are tabulated below:

CONSTITUENTS	TEST	THEORETICAL RESULT	EXPERIMENTAL RESULT	REMARKS
Flavonoids	*Addition of Mg metal & HCl*	Reddish color	Reddish color	Positive (+)
	Addition of NaOH	Yellow color	Yellow color	Positive (+)
Tannins	*Addition of Ferric Chloride*	Bluish or greenish black coloration	No change in color	Negative (-)
Unsaturated Sterols o Triterpenes	*Libermann-Burchard Test*	Reddish violet color at the junction and a bluish green color in acetic acid layer	Formation of junction and brownish-yellow in acetic acid layer	Negative (-)
	Salkowski's Test	Red color	Green-colored solution	Negative (-)
Carbohydrates and/o glycosides	*Molisch's Test*	Bluish violet zone	--	Negative (-)
	Fehlings Test	Brick red precipitate	--	Negative (-)
	Benedict's Test	Red precipitate	++	Positive (+)
	Iodine Test	Dark blue or purple coloration	--	Negative (-)
Test for alkaloids and/or nitrogenous bases:	*Mayers' reagent*	Turbid or white precipitate	++	Positive (+)
	Dragendorff's Reagent	Orange precipitate	++	Positive (+)
	Hager's Reagent	Yellow precipitate	++	Positive (+)
	Wagner's Reagent	Reddish brown precipitate	++	Positive (+)
Saponin	*Froth Test*	Presence of persistent froth	No froth formation	Negative (-)
Proteins and/or amin acid	*Xanthoproteic Test*	Yellow color	Formation of blue solution	Negative (-)
Fixed Oil	*Stain Test*	Presence of stain in filter paper	Oil like stain	Positive (+)
Volatile Oil	*Stain Test*	Absence of stain in filter paper	--	Negative (-)

Figure: 1 Phytochemical Screening Results

Figure 1 show that the ethanolic extract of *Sargassum serratifolium* C. Agardh (Aragan) were positive for flavonoids, alkaloids, reducing sugars, and fixed oil.

Table 1. Organoleptic Result of Ethanolic extract of *Sargassum serratifolium* C. Agardh (Aragan)

Organoleptic Screening	**Result**
Color	Dark brown
Odor	Smelly, spongy, slimy scourge
Appearance	Dark brown flakes
pH	6 (slightly acidic)

Table 1 shows that the extract is dark brown with smelly, spongy, and slimy scourge, and it has a flaky-appearance. It is slightly acidic with a pH of six (6) which is a characteristic of a fucoidan.

Table 2. Fourier Transform Infrared Spectrometry (FTIR) Results of *Sargassum serratifolium* C. Agardh (Aragan)

Functional Group	**Type of Bond**	**Range (cm^{-1})**	**FTIR Result**	**Interpretation**
Alcohols, Phenols	O-H	3500-3200	3423.35	Strong broad
Alkanes	C-H (stretch)	3000-2850	2926.32	Medium
Alkanes	C-H (stretch)	3000-2850	2853.42	Medium
Primary Amines	N-H (bend)	1650-1580	1632.77	Medium
Aromatics	C-C (stretch)	1500-1400	1422.07	Medium
Aromatic Amines	C-N (stretch)	1335-1250	1256.75	Strong
Alcohols, Carboxylic acids, Esters, Ethers	C-O (stretch)	1320-1000	1164.38	Strong
Aliphatic Amines	C-N (stretch)	1250-1020	1063.29	Medium
Aliphatic Amines	C-N (stretch)	1250-1020	1034.65	Medium
Primary / Secondary Amines	N-H (wag)	910-665	905.48	Strong broad
Aromatics	C-H	900-670	820.32	Strong
Alkynes	C-H (bend)	700-610	676.16	Strong broad
Alkynes	C-H (bend)	700-610	617.35	Strong broad

Table 2 shows that the seaweed contains a strong broad concentration for alcohols, phenols, primary/secondary amines and alkynes. It showed a strong concentration of aromatic amines, alcohols, carboxylic acids, esters, ethers and aromatics. And it has also a medium concentration of alkanes, primary amines, aromatics, and aliphatic amines. The strong and broad band 3423.35 cm-1 common to all polysaccharides represents O-H stretching of hydroxyls and bound water which overlaps in part with the C-H stretching between 2850-3000 cm-1 (Freitas, 2010). The bands found between 1000-1320cm-1 represent C-O vibrations indicating the acidic nature of polysaccharides (Mao, 2008). Moreover, the strong band between 1350-1450 cm-1 corresponds to sulfate groups while the band between 700-900 cm-1 in the plant sample represents S-OR esters. The band between 1050-1200 cm-1 see in Sargassum serratifolium polysaccharides but not in fucoidan may be attributed to the C=S thiocarbonyl. The band between 950-1250 cm-1 represents weak P-H phosphine, P-H bending, which may account for the characteristic odor of the plant sample.

Table 3. The Inhibitory Concentration in 50% of the Population (IC_{50}) of *Sargassum serratifolium* C. Agardh (Aragan) Fucoidan Extract using MTT Assay

FUCOIDAN EXTRACT	**RESULT**	**INTERPRETATION**
Trial 1	No LI	No absorbance; didn't inhibit cell proliferation
Trial 2	No LI	No absorbance; didn't inhibit cell proliferation
Trial 3	No LI	No absorbance; didn't inhibit cell proliferation

***No Li =** No Linear Interpolation

Table 3 shows that *Sargassum serratifolium* C. Agardh (Aragan) fucoidan extract did not inhibit the cell proliferation of the cancer cells in all of the concentration used. No linear interpolation was calculated to all of the trials since the absorbance reading of the fucoidan extract was higher or equal to the negative control, dimethyl sulfoxide. As seen in Table 3.0, the macroscopic average length of the roots of *Allium cepa* (Onion) is used in determining the effective concentration in 50% of the population which gives the greatest genotoxicity effect to the growth of the *Allium cepa* (Onion) root tips. The length in every concentration with

three replicates were summed and averaged. It shows that the shortest average root length in all of its replicate is the 100% fucoidan extract which is 23.99mm, thus this means that 100% concentration gives the greatest effectiveness among others. In the table below, it also shows that 50% fucoidan extract has a significant effect on the onion roots, having a 28.43mm average root length. It shows also that 100% concentration is far more effective than the positive control, Maleic hydrazide (36.25mm).

Table 4. The Effective Concentration in 50% of the Population (EC_{50}) of *Sargassum serratifolium* C. Agardh (Aragan) Fucoidan Extract using *Allium cepa* (Onion) Genotoxicity Test

REPLICATE	1	2	3	Average in 3 replicates
CONCENTRATION	**Root Length (mm)**			
100%	20.2955	17.3448	34.3336	23.9913
50%	23.7727	38.0964	23.4333	28.4340
25%	34.7751	38.9192	58.7250	44.1398
12.5%	56.0045	40.1000	39.8630	45.3225
6.25%	50.0409	29.6663	39.9857	39.8976
3.125%	32.7619	34.0014	45.8615	37.5416

Table 5. The Lethal Concentration in 50% of the Population (LC_{50}) of *Sargassum serratifolium* C. Agardh (Aragan) Fucoidan Extract using *Artemia salina* (Brine shrimp) Lethality Test

Concentration	Trial	Average	Overall Average	Interpretation
100ug/mL	1	0.53	0.53	Has a 0.53 survival ratio
	2	0.73		
	3	0.47		
	4	0.37		
50 ug/mL	1	0.63	0.53	Has a 0.53 survival ratio, the same with the 100ug/mL concentration
	2	0.77		
	3	0.40		
	4	0.30		
25 ug/mL	1	0.77	0.65	Has a 0.65 survival ratio
	2	0.67		
	3	0.77		
	4	0.40		
12.5 ug/mL	1	0.83	0.77	Has a 0.77 survival ratio
	2	0.83		
	3	0.77		
	4	0.63		
6.25 ug/mL	1	0.67	0.69	Has a 0.69 survival ratio
	2	0.70		
	3	0.60		
	4	0.77		
3.125 ug/mL	1	0.70	0.68	Has a 0.6825 survival ratio
	2	0.53		
	3	0.67		
	4	0.83		
Positive Control (2% Ethyl Alcohol)	1	0.53	0.53	Has a 0.53 survival ratio, similar with the 100ug/mL and 50ug/mL concentration
	2	0.73		
	3	0.47		
	4	0.37		
Negative Control (1% DMSO + Artificial Seawater)	1	0.93	0.86	Has a 0.86 survival ratio
	2	0.70		
	3	0.90		
	4	0.90		

Table 5 shows the average survival ratio of *Artemia salina* (Brine shrimp) nauplii against the different concentration of the fucoidan extract, positive control, 2% ethyl alcohol and negative control, 1% dimethyl sulfoxide with artificial seawater. It shows the ratio of the number of mobile nauplii (alive) and the number of immobile nauplii (dead) in four trials with three replicates. The results of each trial were averaged overall. It shows that 100ug/mL and 50ug/mL concentration of the fucoidan extract has the least number of survival of brine shrimp nauplii, thus 100ug/mL and 50ug/mL concentration exhibit the greatest lethal effect. The table also shows that 100ug/mL and 50ug/mL concentration has a comparable effect with the positive control, 2% ethyl alcohol.

Table 6. The Result Comparison of the Inhibitory Concentration in 50% of the Population (IC_{50}) of Experimental Control, *S. serratifolium* Fucoidan Extract and the Positive Control, Doxorubicin using MTT Assay

Groups	Concentra-tion	Mean	Variance	F-test	P-value	F-test Critical	Decision	Interpreta-tion
Doxo-rubicin	25 ug/mL	0.145556	0.001551	51.16276	9.02E-10	2.6571966	Reject the Null Hypothesis (Ho)	There is a significant difference between the fucoidan and Doxorubicin.
	12.5 ug/mL	0.128	0.000651					
	6.25 ug/mL	0.127444	0.000711					
	3.125 ug/mL	0.120778	0.000555					
Fucoidan	50 ug/mL	0.633333	0.002352					
	25 ug/mL	0.751444	0.021383					
	12.5 ug/mL	0.687222	0.00747					
	6.25 ug/mL	0.620556	0.005537					

Table 6 shows the comparison of the inhibitory concentration in 50% of the population (IC_{50}) of the fucoidan extract to the positive control, Doxorubicin. The different concentrations of the two groups were differentiated by getting the mean and the variance. The f-test was computed at 0.05 level of significance and decision was made by comparing the computed f-test value to the critical value. The null hypothesis was rejected because the computed f-value, 51.16 is greater than the critical value, 2.66.

Table 7. The Result Comparison of the Inhibitory Concentration in 50% of the Population (IC_{50}) of Experimental Control, *S. serratifolium* Fucoidan Extract and the Negative Control, Dimethyl sulfoxide using MTT Assay

Groups	Concentra-tion	Mean	Variance	F-test	P-value	F-test Critical	Decision	Interpreta-tion
DMSO	0	0.623556	0.006786	1.089496	0.412593	3.478049691	Accept Null Hypothesis (Ho)	There is a significant difference between the fucoidan and DMSO.
Fucoidan	50 ug/mL	0.633333	0.002352					
	25 ug/mL	0.751444	0.021383					
	12.5 ug/mL	0.687222	0.00747					
	6.25 ug/mL	0.620556	0.005537					

Table 7 shows the comparison of the inhibitory concentration in 50% of the population (IC_{50}) of the fucoidan extract to the negative control, dimethyl sulfoxide. The different concentrations of the two groups were differentiated by getting the mean and the variance. The f-test was computed at 0.05 level of significance and decision was made by comparing the computed f-test value to the critical value. The null hypothesis was accepted because the computed f-value, 1.09 is less than the critical value, 3.48.

Table 8 shows the comparison of the effective concentration in 50% of the population (IC_{50}) of the fucoidan extract to positive control, maleic hydrazide. The different concentrations of the two groups were differentiated by getting the mean and the variance. The f-test was computed at 0.05 level of significance and

decision was made by comparing the computed f-test value to the critical value. The null hypothesis was accepted because the computed f-value, 2.31 is less than the critical value, 2.85. Table 9 shows the comparison of the effective concentration in 50% of the population (IC_{50}) of the fucoidan extract to negative control, dimethyl sulfoxide. The different concentrations of the two groups were differentiated by getting the mean and the variance. The f-test was computed at 0.05 level of significance and decision was made by comparing the computed f-test value to the critical value. The null hypothesis was accepted because the computed f-value, 2.27 is less than the critical value, 2.85.

Table 8. The Result Comparison of the Effective Concentration in 50% of the Population (EC_{50}) of Experimental Control, *S. serratifolium* Fucoidan Extract and the Positive Control, Maleic hydrazide using *Allium cepa* (Onion) Genotoxicity Test

Groups	Concentra-tion	Mean	Variance	F-test	P-value	F- test Critical	Decision	Interpreta-tion
Fucoidan	100%	23.98666667	82.2737333	2.30557002	0.092824836	2.847725996	Accept the Null Hypothesis (Ho)	There is no significant difference between the fucoidan and maleic hydrazide.
	50%	28.43333333	70.1122333					
	25%	44.14333333	163.863033					
	12.5%	45.32	85.5612					
	6.25%	39.9	103.7403					
	3.125%	37.54	52.3012					
Maleic Hydrazide	5mg/L	36.24666667	4.70083333					

Table 9. The Result Comparison of the Effective Concentration in 50% of the Population (EC_{50}) of Experimental Control, *S. serratifolium* Fucoidan Extract and Negative Control, Dimethyl sulfoxide using *Allium cepa* (Onion) Genotoxicity Test

Groups	Concentra-tion	Mean	Variance	F-test	P-value	F-test Critical	Decision	Interpreta-tion
Fucoidan	100%	23.98667	82.27373	2.267787639	0.09705689	2.847726	Accept the Null Hypothesis (Ho)	There is on significant difference between the fucoidan and DMSO.
	50%	28.43333	70.11223					
	25%	44.14333	163.863					
	12.5%	45.32	85.5612					
	6.25%	39.9	103.7403					
	3.125%	37.54	52.3012					
DMSO	1%	36.70667	13.97923					

Table 10. The Result Comparison of the Lethal Concentration in 50% of the Population (LC_{50}) of Experimental Control, *S. serratifolium* Fucoidan Extract and Positive Control, 2% Ethyl Alcohol using *Artemia salina* (Brine shrimp) Lethality Test

Groups	Concent ra-tion	*Average*	*Variance*	F-test	P-value	F-test Critical	Decision	Interpreta -tion
Fucoidan	100 ug/mL	0.525	0.0365909	3.2957763	0.00612	2.218816738	Reject the Null Hypothesis (Ho)	There is a significant difference between the fucoidan and 2% ethyl alcohol.
	50 ug/mL	0.525	0.0493182					
	25 ug/mL	0.65	0.0445455					
	12.5 ug/mL	0.766667	0.0187879					
	6.25 ug/mL	0.683333	0.0215152					
	3.125 ug/mL	0.683333	0.0178788					
2% Ethyl Alcohol		0.7	0.0218182					

Table 10 shows the comparison of the lethal concentration in 50% of the population (LC_{50}) of the fucoidan extract to positive control, 2% ethyl alcohol. The different concentrations of the two groups were differentiated by getting the average and the variance. The f-test was computed at 0.05 level of significance and decision was made by comparing the computed f-test value to the critical value. The null hypothesis was rejected because the computed f-value, 3.30 is greater than the critical value, 2.22.

Table 11. The Result Comparison of the Lethal Concentration in 50% of the Population (LC_{50}) of Experimental Control, *S. serratifolium* Fucoidan Extract and Negative Control, 1% DMSO with Artificial Seawater

Groups	Concentration	*Average*	*Variance*	F-test	P-value	F-test Critical	Decision	Interpretation
Fucoidan	100 ug/mL	0.525	0.0365909	5.856058	4.55E-05	2.218817	Reject the Null Hypothesis (Ho)	There is a significant difference between fucoidan and 1% DMSO with artificial seawater.
	50 ug/mL	0.525	0.0493182					
	25 ug/mL	0.65	0.0445455					
	12.5 ug/mL	0.766667	0.0187879					
	6.25 ug/mL	0.683333	0.0215152					
	3.125 ug/mL	0.683333	0.0178788					
1% DMSO with Artificial Seawater		0.7	0.0218182					

Table 11 shows the comparison of the lethal concentration in 50% of the population (LC_{50}) of the fucoidan extract to negative control, 1% DMSO with artificial seawater. The different concentrations of the two groups were differentiated by getting the average and the variance. The f-test was computed at 0.05 level of significance and decision was made by comparing the computed f-test value to the critical value. The null hypothesis was rejected because the computed f-value, 5.86 is greater than the critical value, 2.22.

DISCUSSION AND CONCLUSION

Based on the experimental procedures conducted and findings evaluated by the researchers, the following are concluded: The phytochemical constituents present in *Sargassum serratifolium* C. Agardh (Aragan) are flavonoids, alkaloids, reducing sugars, and fixed oil. There was no concentration of the fucoidan extract of *Sargassum serratifolium* C. Agardh (Aragan) that exerted or produced any inhibitory of effect concentration in 50% of the population (IC_{50}) in testing the cytotoxic activity using MTT Assay. The 100% concentration of the fucoidan extract of *Sargassum serratifolium* C. Agardh (Aragan) exerted or produced its greatest effectiveness in 50% of population (EC_{50}) in testing the genotoxicity using *Allium cepa* (Onion) Test. It shows that it has the shortest average root length in all of its replicate. The 100ug/mL and 50ug/mL of the fucoidan extract of *Sargassum serratifolium* C. Agardh (Aragan) exerted or produced its greatest lethal effecy in 50% of population (LC_{50}) in testing the lethality using Artemia salina (Brine shrimp) Test. It shows that it has the least survival ratio which is 0.53. There is a significant difference between the experimental group, *Sargassum serratifolium* C. Agardh (Aragan) fucoidan extract and the positive control, Doxorubicin in inhibiting the cell proliferation of the MCF7 (Human Breast Cancer) cell line. In a level of significance of 0.05, the computed f-value of 51.16276 is greater than the critical value, 2.6571966, thus, the null hypothesis is rejected. There is no significant difference between the experimental group, *Sargassum serratifolium* C. Agardh (Aragan) fucoidan extract and the negative control, Dimethyl sulfoxide in inhibiting the cell proliferation of the MCF7 (Human Breast Cancer) cell line. In a level of significance of 0.05, the computed f-value of 1.09 is less than the critical value, 3.48, thus, the null hypothesis is accepted. There is no significant difference between the experimental group, *Sargassum serratifolium* C. Agardh (Aragan) fucoidan extract and the positive control, maleic hydrazide in inhibiting the mitotic cell division of the *Allium cepa* (Onion) Root tips. In a level of significance of 0.05, the computed f-value of 2.31 is less than the critical value, 2.85, thus, the null hypothesis is accepted. There is no significant difference between the experimental group, *Sargassum serratifolium* C.

Agardh (Aragan) fucoidan extract and the negative control, dimethyl sulfoxide in inhibiting the mitotic cell division of the *Allium cepa* (Onion) Root tips. In a level of significance of 0.05, the computed f-value of 2.27 is less than the critical value, 2.85, thus, the null hypothesis is accepted. There is a significant difference between the experimental group, *Sargassum serratifolium* C. Agardh (Aragan) fucoidan extract and the positive control, 2% ethyl alcohol in killing *Artemia salina* (Brine shrimp) naupliis. In a level of significance of 0.05, the computed f-value of 3.30 is greater than that the critical value, 2.22, thus, the null hypothesis is rejected. There is a significant difference between the experimental group, *Sargassum serratifolium* C. Agardh (Aragan) fucoidan extract and the negative control, 1% DMSO with artificial seawater in killing *Artemia salina* (Brine shrimp) naupliis. In a level of significance of 0.05, the computed f-value of 5.86 is greater than that the critical value, 2.22, thus, the null hypothesis is rejected. After careful observation, the researchers then concluded that S*argassum serratifolium* C. Agardh (Aragan) fucoidan extract illicit no cytotoxic activity against MCF7 (Human breast cancer) cell line using MTT assay. The fucoidan extract from *Sargassum serratifolium* C. Agardh (Aragan) has an effect to the *Allium cepa* (Onion) root tips but is non-comparable to the positive control, maleic hydrazide using *Allium cepa* (Onion) genotoxicity test. Also, in the *Artemia salina* (Brine shrimp) test, the fucoidan extract has a minimal effect on brine shrimp naupliis compared to the positive control, 2% ethyl alcohol. The researchers further concluded that in "In Vitro Determination of Cytotoxic Activity of Fucoidan Extract of *Sargassum serratifolium* C. Agardh (Aragan) Against MCF7 (Human Breast Cancer) Cell Line," the fucoidan extract does not have an in vitro cytotoxic activity up to 100ug/mL concentration.

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