Pharmacogn J. 2017; 9(1): 14-20
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com | www.journalonweb.com/pj | www.phcog.net

Original Article

# Cytotoxicity of Fucoidan from Three Tropical Brown Algae Against Breast and Colon Cancer Cell Lines

**Alim Isnansetyo[1], Fadilah Nor Laili Lutfia[2], Muhammad Nursid[3], Trijoko[4], Ratna Asmah Susidarti[5]**

**ABSTRACT**

**Introduction:** Fucoidan is a sulfated polysaccharide that has a wide range of bioactivities including anti-cancer. This polysaccharide commonly is extracted from marine brown seaweed. There is lack of information on the fucoidan extracted from tropical brown algae and its anti-cancer activity. **Objectives:** The objectives of this study were to purify fucoidan from *Sargassum* sp., *Turbinaria* sp. and *Padina* sp., and to evaluate their cytotoxicity against breast cancer (MCF-7) and colon cancer cells (WiDr). **Materials and Methods:** Fucoidan extraction was conducted by using acid extraction method. Purified fucoidans were obtained by DEAE cellulose column chromatography and confirmed by HPLC and FT-IR spectrometry. The cytotoxicity was evaluated by using the MTT (3-[4,5-dimethylthiazol-2-yl] -2,5- diphenyltetrazolium bromide) assay. **Results:** Fucoidan from *Sargassum* sp. and *Turbinaria* sp. showed low cytotoxicity with $IC_{50}$ ranging between 461-663 μg/mL. Higher cytotoxicity against MCF-7 and WiDr was showed by fucoidan from *Padina* sp. with $IC_{50}$ of 144 and 118 μg/mL, respectively. While its $IC_{50}$ against Vero cells was 501 μg/mL.Standard fucoidan from *Fucus vesiculosus* exhibited $IC_{50}$ of 60, 63 and 211 μg/mL against MCF-7, WiDr and Vero Cells, respectively. Although the $IC_{50}$ was higher than that of standard fucoidan, *Padina* sp. fucoidan showed cytotoxicity comparable with standard fucoidan at concentrations below 100 μg/mL. **Conclusion:** These results indicated that *Padina* sp.fucoidan showed potential selective cytotoxicity, and promising for the development of an anti-cancer compound.

**Key words:** Fucoidan, Breast cancer, Colon cancer, Phaeophyta, Cytotoxicity.

**Alim Isnansetyo[1], Fadilah Nor Laili Lutfia[2], Muhammad Nursid[3], Trijoko[4], Ratna Asmah Susidarti[5]**

*[1]Department of Fisheries, Faculty of Agriculture, GadjahMada University, Jl. Flora, Bulaksumur, Yogyakarta, INDONESIA.*
*[2]Study Program of Biotechnology, Post Graduate School, GadjahMada University, INDONESIA.*
*[3]Research and Development Center for Marine and Fisheries Product Processing and Biotechnology, Ministry of Fisheries and Marine Affair, INDONESIA.*
*[4]Faculty of Biology, GadjahMada University, Sekip Utara, Yogyakarta, INDONESIA.*
*[5]Faculty of Pharmacy, GadjahMada University, Sekip Utara, Yogyakarta, INDONESIA.*

**Correspondence**

Alim Isnansetyo, Departement of Fisheries, Faculty of Agriculture, Gadjah Mada University, Jl Flora, Bulaksumur, YOGYAKARTA 55281,

Phone: +62-274-551218,

E-mail: isnansetyo@yahoo.com; isnansetyo@ugm.ac.id

History
- Submission Date: 18-07-2016;
- Review completed: 22-08-2016;
- Accepted Date: 10-09-2016.

**DOI : 10.5530/pj.2017.1.3**

**Article Available online**
http://www.phcogj.com/v9/i1



## INTRODUCTION

Fucoidan is a polysaccharide of brown seaweed and marine invertebrates (such as sea urchin and sea cucumber) with a main component of L-fucose and sulfate ester group.[1] Chemical structure of fucoidan from brown seaweed is very complex and varies among seaweeds species.[2] The main difference is in the backbone structure of fucoidan.[3,4] Monosaccharides component of fucoidan affect their physiological and biological activities.[5] Fucoidan has a wide range of bioactivity among others as immunostimulan,[6-9] and antitumor/anticancer.[4,10,11]

Cancer is a disease that attacks the modern society globally, and as a major disease today and in the future. WHO have reported that there are 14 million new cases and 8.2 million cancer related death in 2012, and annual cancer cases will rise to 22 million within the next two decades.[12] Two cancers that cause the major deaths in men are lung cancer and colorectal cancer, whereas in women are breast cancer and lung cancer.[13] Girish *et al*[14] have found the potential activity of *Pavonia odorata* Willd extract against human breast and lung cancers. Breast cancer is also effectively inhibited by β-mangostin isolated from *Cratoxylum arborescens* either *in vitro* or *in vivo*.[15] How ever, research on tropical brown seaweeds as sources of fucoidan for anti-cancer agent is still limited.

Cumashi *et al*[5] have found the potent anti-angiogenesis activity of fucoidan from *Laminaria saccharina, L. digitata, Fucus evanescens, F. serratus, F. distichus, F. spiralis, F. vesiculosus, Ascophyllum nodosum* and *Cladosiphon okamuranus*. Fucoidans from *L. saccharina, L. digitata, F. serratus, F. distichus*, and *F. vesiculosus* strongly blocks MDAMB-231 breast carcinoma cell adhesion to platelets. A commercial fucoidan from *F. vesiculosus* also active against HCT-15 colon carcinoma cells through apoptosis- inducing activity.[16] Senthilkumar *et al*[17] have reviewed the fucoidan from *F. vesiculosus*, *C. novae-caledoniae, C. okamuranus, S. japonica, U. pinnatifida* with cytotoxicity against cancer cells. Fucoidan from *Sargassum* sp. and *F. vesiculosus* also reduces viability of lung carcinoma and melanoma cells.[18] Fucoidan from *Eclonia cava, Sargassum hornery,* and *Costaria costata* from South Korea inhibits colony formation in human melanoma and colon cancer cells.[19] Murphy *et al*[20] have reviewed

**Cite this article :** Isnansetyo A, Laili Lutfia FN, Nursid M, Trijoko, Susidarti RA. Cytotoxicity of Fucoidan from Three Tropical Brown Algae Against Breast and Colon Cancer Cell Lines. Pharmacog J. 2017;9(1):14-20.

anticancer substance including fucoidan from brown seaweeds. Marudhupandi *et al*[21] reported the cytotoxicity of fucoidan from a tropical brown seaweed, *Turbinaria* sp. collected from Tamilnadu India against the human lung cancer A549 cell line. In the present study we purified fucoidan from *Sargassum* sp., *Turbinaria* sp. and *Padina* sp., and evaluated its anticancer activity against colon and breast cancer cell lines. To the best of our knowledge, the anticancer activities of fucoidan from these three brown seaweeds species against these two cancer cell lines have not been reported before.

## MATERIALS AND METHODS

### Sampling and Identification of Brown Seaweeds

Sampling was carried out in the intertidal zone during low tide. *Sargassum* sp., *Turbinaria* sp. and *Padina* sp. were collected and then put in a cool box and transported to the laboratory. Identification was carried out based on Dhargalkar,[22] Trono.[23]

### Extraction and Purification

The seaweed samples were washed with fresh water, then air dried in the laboratory to obtain the dry biomass, and stored in plastic bags. Extraction was carried out by the method of Kim *et al*[13] Purification was carried out by DEAE cellulose column chromatography (Sigma-Aldrich, St. Louis, MO, USA) eluted with distilled water and gradient 0.5 to 3 M NaCl [24] The total carbohydrate of the purified fucoidan in the fractions was determined by the phenol-sulfuric acid method according to Masuko *et al*[25] using L-fucose as a reference. The fucoidan fractions were evaporated, dialysed (MW cut-off 12,300 Da) for 48 hours, then freeze-dried and stored at 4°C until use.

The purified fucoidan was analyzed by HPLC using Agilent Hi-Plex columns Ligand-Exchange (Agilent Hi-Plex H for Carbohydrate) 7.7 × 100 mm, with a refractive index detector (RID-10A, Shimadzu, Japan) with a flow rate of 0.7 mL/min at 60°C. HPLC using the autosampler (SIL-10ADVP, Shimadzu, Japan) with injection volume of 20 µL. Fucoidan sample concentration was 5 µg/mL. The measurement of sulphate in the fucoidan was based on the barium sulphate ($BaSO_4$) determination using barium chloride ($BaCl_2$),[26] using $Na_2SO_4$ as a standard.

### FTIR spectra

FTIR spectra were recorded by a FTIR spectrometer in KBr with a wave number range of 4000-450 $cm^{-1}$. The ratio of fucoidan and KBr was approximately 1: 100 (2 mg sample + 200 mg KBr). The IR spectra of fucoidan from the seaweeds were compared with the IR spectrum of standard fucoidan (Sigma-Aldrich, St. Louis, MO, USA).

### Cytotoxicity Assay

Cell lines used in this assay were a colon cancer cells (human collon adenocarcinoma cells) (WiDr), breast cancer cells (human breast adenocarcinoma cells) (MCF-7) and a normal cells, an African green monkey kidney cells (vero cells). Each of these cells were cultured in RPMI or M199 medium (Sigma-Aldrich, St. Louis, MO, USA) with the addition of 10% FBS (Sigma-Aldrich, St. Louis, MO, USA), antibiotics Panstrep (Gibco, Grand Island, NY, USA) and Fungison (Gibco). The cells were cultured at 37°C in a $CO_2$ incubator until confluent. After incubation, the medium was replaced with a new medium, then the cells were harvested and re-cultured in 96-well microplate at a density of $10^4$ cells/100 µl/well using a medium containing 2% FBS. Then fucoidan with a serial dilution was added to wells. As a negative control cells were treated with sterile dimethyl sulfoxide (DMSO), whereas the positive control was treated with a standard fucoidan from *Fucus vesiculosus* (Sigma-Aldrich, St. Louis, MO, USA). Cells were then incubated in the same conditions as above. Cells proliferation was observed by MTT assay (3- [4,5-dimethylthiazol-2-yl] -2,5- diphenyltetrazolium bromide [Sigma-Aldrich, St. Louis, MO, USA]).[27] Growth was detected with a microplate reader at a wavelength of 570 nm. The growth rate of cells exposed to fucoidan compared to the positive and negative controls, and then the cells mortality expressed in percent (%) was calculated.

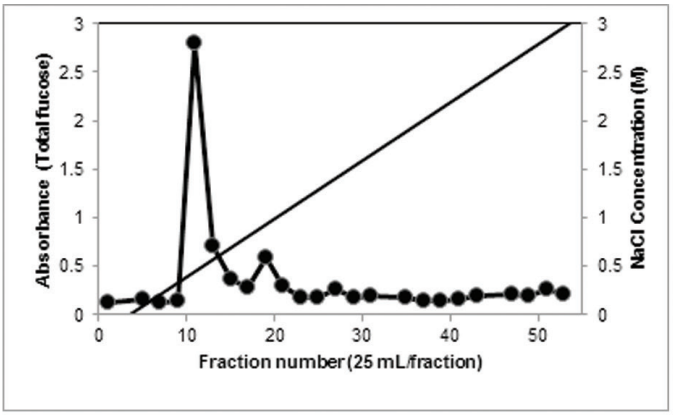
## RESULTS AND DISCUSSION

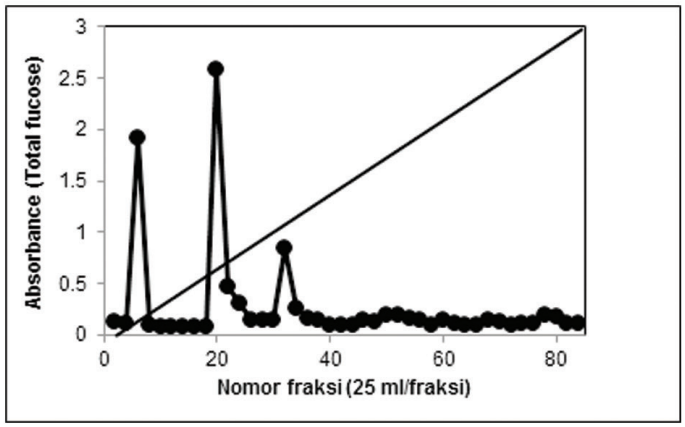
Crude fucoidan extracts were purified by DEAE cellulose eluted with distilled water followed by 0.5 to 3 M of NaCl. Each fraction was measured for total sugar content with fucose as a standard (Figure 1). Fractionation of *Sargassum* sp. crude fucoidan showed the separation of the dominant fraction and two minor fractions. The main fraction was used for subsequent study.The fractionation of *Turbinaria* sp. crude fucoidan, also found a similar pattern with that of crude fucoidan from *Sargassum* sp., but there was a medium peak eluted before the main peak. The similar pattern was also found in the fractionation of *Padina* sp. crude fucoidan where a major fraction was obtained when the crude fucoidan was eluted with 0.5 M NaCl. This DEAE cellulose column chromatogtaphy showed that a major peak was appeared whith 0.5 M NaCl elution either for *Sargassum* sp., *Turbinaria* sp.or *Padina* sp. or fucoidan crude extracts. Each major peak was pooled and used for the subsequent experiments.

Pure fucoidans obtained from the DEAE cellulose column chromatography were confirmed by using HPLC (Figure 2) and FTIR spectra (Figure 3). HPLC produced a single peak with the same retention time with standard fucoidan from *F. vesiculosus*. FTIR spectra also confirmed that the obtained compound were fucoidan.The spectra showed the typical bands of polysaccharide. The broad band centered at 3435 $cm^{-1}$ assigned to hydrogen bonded O-H stretching vibration.[28-30] The band at 2939 $cm^{-1}$ was attributed the C-H stretching pyranoid ring and C6 of fucose and/or galactose units.[13,30] The band at 1614 $cm^{-1}$ indicated the stretching of asymmetric carboxylate O-C-O vibration.[28,29] The band at 1424 $cm^{-1}$ suggested C-OH deformation vibration with contribution of O-C-O symmetric stretching vibration of carboxylate group.[29] The presence of O=S=O stretching vibration of sulphate esters was indicated by a band at 1258 cm-113,[28,29] The band at 1040 $cm^{-1}$ corresponded to stretching vibration of C-O-C/C-OH.[30] The band at 820 $cm^{-1}$ assigned the C-O-S bending vibration of sulfate group.[28-31] The band at 580 $cm^{-1}$ indicated the asymmetric and symmetric O=S=O deformation of sulfate.[31] These FT-IR spectra analysis proved that the extraction and purification processes used and purification process used in this study were effective to obtain fucoidan from the three tropical brown algae.

To evaluate the potential bioactivity of fucoidan as an anti-cancer, a cytotoxicity test against colon cancer cells (human collon adenocarcinoma cells) (WiDr), breast cancer cells (human breast adenocarcinoma cells) (MCF-7) and a normal cells of green monkey kidney cells (Vero cells) was conducted. The cytotoxicity was compared with a standard fucoidan from *F. vesiculosus*. Results showed that standard fucoidan exhibited high cytotoxic activity against colon cancer and killed almost all cells at a dose of 1000 µg/mL (Figures 4 and 5). Lower cytotoxicity activity was found for three fucoidans from brown algae examined in this study. Fucoidan from *Padina* sp. showed cytotoxicity comparable with standard fucoidan at concentrations below 100 µg/mL. However, increase in the concentrations above 100 µg/mL did not significantly increase in the cytotoxicity activity. The same pattern of cytotoxicity was obtained against breast cancer cells (MCF-7) (Figure 4).

The results of cytotoxicity test also indicated that the cytotoxicity of fucoidan varied depending on the seaweeds species as their sources, and cell lines used. Fucoidan from *Sargassum* sp. and *Turbinaria* sp. exhibited low cytotoxic activity with $IC_{50}$ values in the range of 461-663 µg/mL. The cytotoxic activities were lower comparing to the standard fucoidan



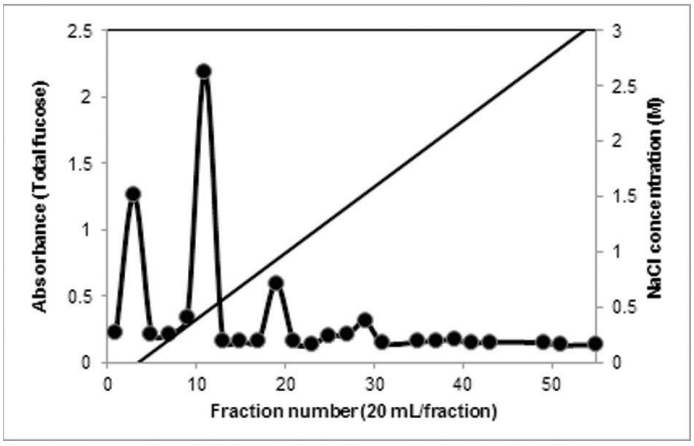




**Figure 1:** Fractionation of crude fucoidan extracts of *Sargassum sp.*(A),*Turbinaria* sp.(B) and *Padina* sp.(C) based on total sugar content with fucose standard on DEAE cellulose coloumn chromatography.

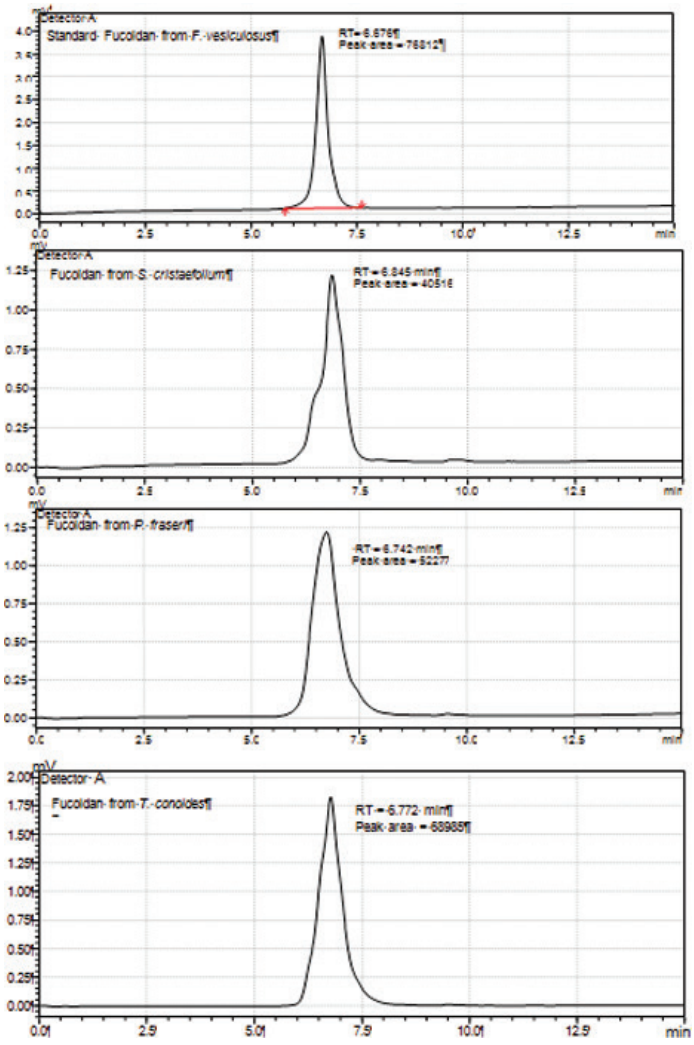



**Figure 2:** HPLC profile of standard fucoidan and purified fucoidan from *Sargassum sp.*, *Turbinaria* sp. and *Padina* sp.

from *F. vesiculosus*. Fucoidan from *Padina* sp. exhibited higher cytotoxic activity with $IC_{50}$ value of 144 and 118 µg/mL against breast and colon cancer cells, respectively (Table 1). Cytotoxicity of *Padina* sp. fucoidan was higher than that of standard fucoidan against a normal Vero cells with $IC_{50}$ 511 µg/mL and 211 µg/mL, respectively, suggested that the former one might be more selective cytotoxic than the later one.

Ale *et al*[18] have evaluated anti-cancer activity of fucoidan from *Sargassum* sp., against Lewis Lung Carcinoma cells (LLC) and melanoma B16 cells (MC) in low concentration of Fetal Bovine Serum (FBS) (2%) as used in this study. Results indicated that*Sargassum* sp. fucoidan reduces the viability of LLC cells up to 40 ± 7% at 1,000 µg/mL. The cell viability reduction is more signifancantly noted against MC cells, resulting in only 56 ± 5% viable cells upon addition of 100 and 200 µg/mL. Our study results confirmed that the different fucoidan sources and different cell lines used in cytotoxicity test resulted the variation of cytotoxicity activities. *Padina* sp. fucoidan used in our study has higher cytotoxicity comparing to that of *Sargassum* sp. and *Turbinaria* sp. Without considering the cell lines used in cytotoxicity test, fucoidan from *Sargassum* sp. against WiDr and MCF-7 exhibited the similar cytotoxicity level with *Sargassum* sp. used by Ale *et al*[18] against LLC cells.

Several studies have evaluated cytotoxicity of fucoidan against collon and breast cancer.[5,10,16,19] Cumashi *et al*[5] found that fucoidans from *L. saccharina*, *L. digitata*, *F. serratus*, *F. distichus* and *F. vesiculosus* at 100 µg/mL strongly blocked MDAMB-231 breast carcinoma cell adhesion to platelets, an effect which might have critical implications in tumor-metastasis. Hyun *et al*[16] reported that fucoidan from *F. vesiculosus* at 100 µg/mL demonstrates apoptotic activity against HCT-15 cells (human colon carcinoma cells). Fucoidans from *S. hornery*, *Eclonia cava* and *Costaria costata* have been reported non significant cytotoxic activity against human skin melanoma (SK-MEL-28) and colon cancer cells (DLD-1) after treatment during 24 h at concentration from 1 to 200 µg/mL.[19] Fukahori *et al*[32] also have proved by evaluating cytotoxicity of fucoidan against 15 cancer cell lines with the broad range $IC_{50}$ between

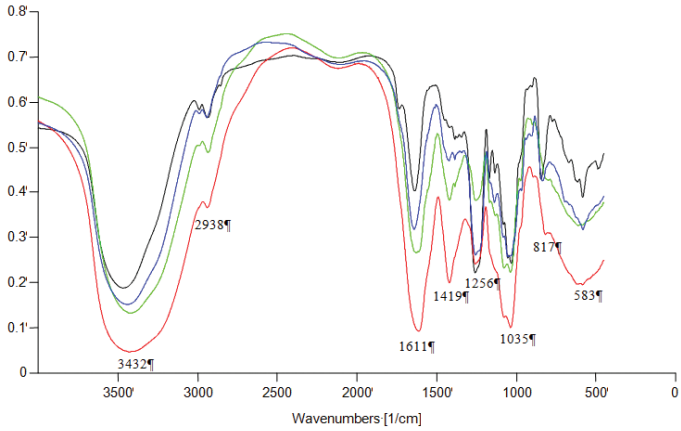



**Figure 3:** Overlaid FTIR spectra of standard fucoidan (black line) and purified fucoidan from *Sargassum* sp. (red line), *Turbinaria* sp.(green line) and *Padina* sp.(blue line).

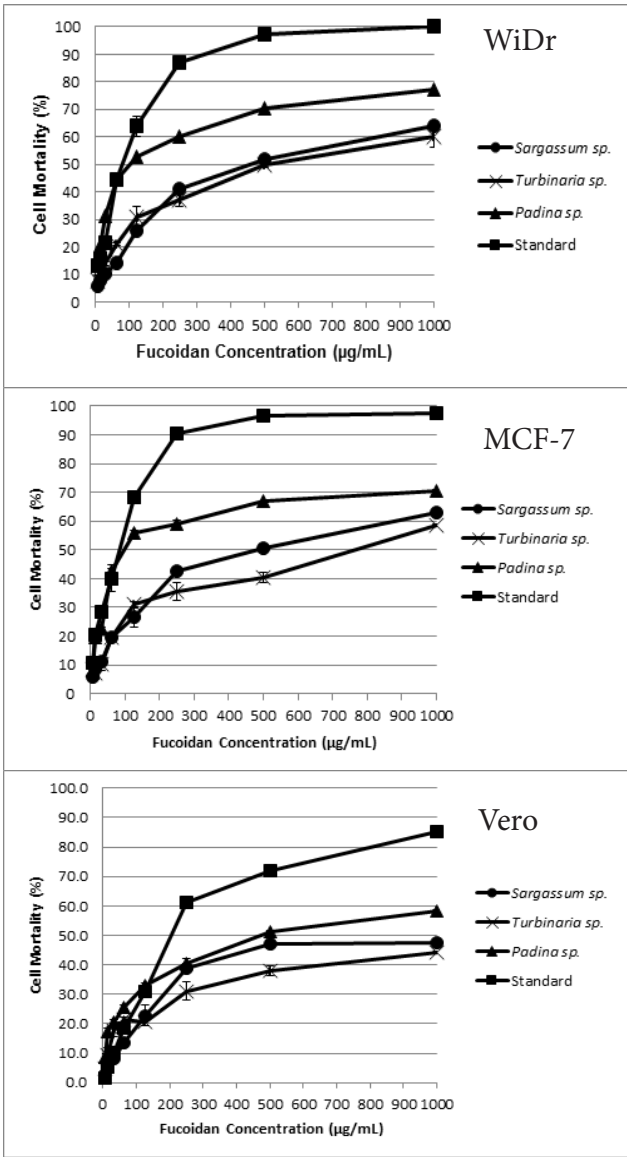



**Figure 4:** Cytotoxicity of fucoidan from *Sargassum* sp., *Turbinaria* sp., *Padina* sp. and standard fucoidan against human collon adenocarcinoma (WiDr) human breast adenocarcinoma (MCF-7) and kidney green monkey (Vero) cells.

**Tabel 1: $IC_{50}$ of fucoidan from *Sargassum* sp., *Turbinaria* sp., *Padina* sp. and fucoidan standard against human breast adenocarcinoma (MCF-7), human collon adenocarcinoma (WiDr) and kindey epithelial African green monkey (Vero) cells**

| Fucoidan Sample | $IC_{50}$ (µg/mL) | | |
|---|---|---|---|
| | MCF-7 | WiDr | Vero |
| *Sargassum* sp. | 461 | 476 | 757 |
| *Turbinaria* sp. | 663 | 547 | >1000 |
| *Padina* sp. | 144 | 118 | 501 |
| Standard Fucoidan | 60 | 63 | 211 |

18.71and 299.20 µg/mL. The above reports indicate that the cytotoxicities of fucoidan are greatly vary depending on the sources of fucoidan and cell lines used in cytotoxicity assay. Results of our study confirmed fucoidans from three tropical brown seaweeds exhibits cytotoxicity variation. Fucoidan from *Padina* sp. shows selective cytotoxicity with $IC_{50}$ 118 to 144 µg/mL against cancer cell lines and 501 µg/mL against a normal cell line suggested that this brown seaweed may a prospective source of fucoidan for developing cytotoxic agent in cancer therapy especially against breast and collon cancer. The further studies are needed to investigate the variability of fucoidan related to the season, sampling location and extraction method, and also evaluation of the cytotoxicity against more broad range cancer cell lines.

## ACKNOWLEDGEMENTS

This research was financially supported by a grant from Directorate of Higher Education, Ministry of Education and Culture, the Republic of Indonesia under the program of Hibah Kompetensi (Competency Research Grant) 2013/2014.

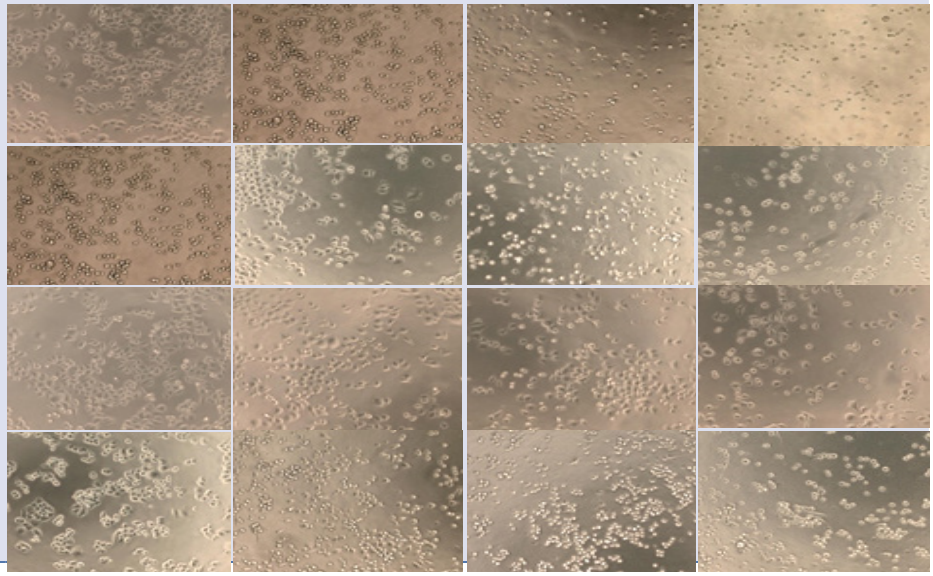
## CONFLICT OF INTEREST

We do not have any conflict of interest.

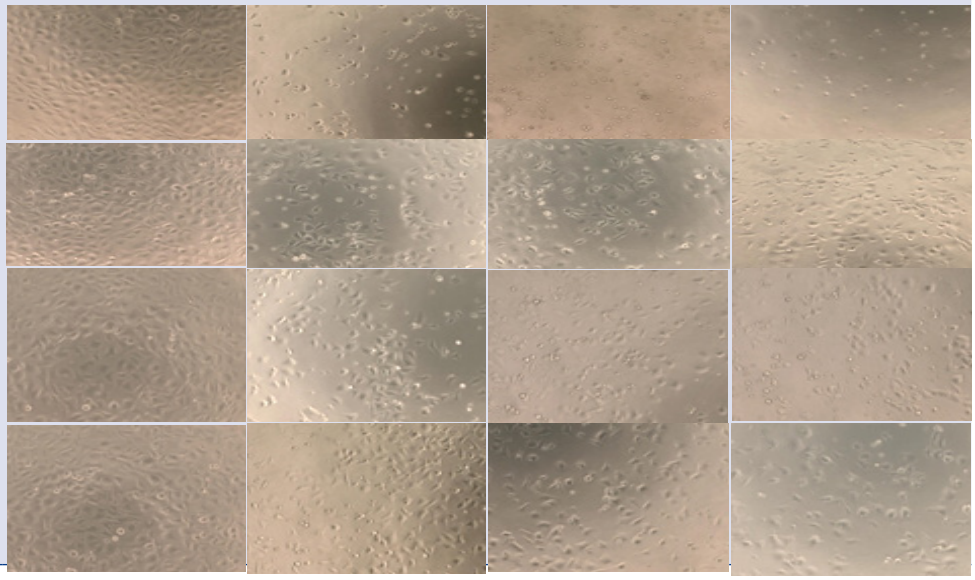
## REFERENCES

1. Chizhov AO, Dell A, Morris HR, Haslam SM, McDowell RA, Shashkov AS, *et al.* A study of fucoidan from the brown seaweed Chorda filum. Carbohydr Res. 1999;320(1):108-19. http://dx.doi.org/10.1016/S0008-6215(99)00148-2.
2. Pereira MS, Mulloy B, Mourão PA. Structure and anticoagulant activityof sulfated fucans. Comparison between the regular, repetitive, and linearfucans from echinoderms with the more heterogeneous and branched polymers-from brown algae. J Biol Chem. 1999;274(12):765-67. http://dx.doi.org/10.1074/jbc.274.12.7656.
3. Li B, Lu F, Wei X, Zhao R. Fucoidan: structure and bioactivity. Molecules. 2008;13:1671-95. http://dx.doi.org/10.3390/molecules13081671.
4. Synytsya A, KimW-J, Kim S-M, PohlR, Synytsya A, Kvasnicka F, Copikova J, ParkYI. Structure and antitumour activity of fucoidan isolated from sporophyll of Korean brown seaweed Undaria pinnatifida. Carbohydr Polym. 2010;81(1):41-8. http://dx.doi.org/10.1016/j.carbpol.2010.01.052.
5. Cumashi A, Ushakova NA, Preobrazhenskaya ME, D'InceccoA, PiccoliA, Totani L, *et al.* A comparative study of the anti-inflammatory, anticoagulant, antiangiogenic, andanti adhesive activities of nine different fucoidans from brown seaweeds. Glycobiology. 2007;17(5):541-52. http://dx.doi.org/10.1093/glycob/cwm014.
6. Yoo Y-C, Kim W-J, Kim S-Y, Kim S-M, Park Y-I. Immunomodulating activity of a fucoidan isolated from Korean Undaria pinnatifid as porophyll. Algae. 2007;22(4):333-8. http://dx.doi.org/10.4490/ALGAE.2007.22.4.333.
7. Teruya T, Takeda S, Tamaki Y, Tako M. Fucoidan isolated from *Laminaria angustata* var longissima induced macrophage activation. Biosci Biotechnol Biochem. 2010;74(9):1960-2. http://dx.doi.org/10.1271/bbb.100294.
8. Ramberg JE, Nelson ED, Sinnott RA. Immunomodulatory dietary polysaccharides: a systematic review of the literature. Nutrition J. 2010;9(1):54. http://www.nutritionj.com/content/9/1/54. http://dx.doi.org/10.1186/1475-2891-9-54.
9. Isnansetyo A, Fikriyah A, Kasanah N, Murwantoko. Non specific immune potentiating activity of fucoidan from a tropical brown algae (Phaeophyceae),

Human colon adenocarcinoma (WiDr)

| Fucoidan | Control | 125µg/mL | 500 µg/mL | 1000 µg/mL |
|---|---|---|---|---|
| Standard fucoidan |  | | | |
| *Sargassum* sp. | | | | |
| *Turbinaria* sp. | | | | |
| *Padina* sp. | | | | |

Human breast adenocarcinoma (MCF-7) )

| Fucoidan | Control | 125 µg/mL | 500 µg/mL | 1000 µg/mL |
|---|---|---|---|---|
| Standard fucoidan |  | | | |
| *Sargassum* sp. | | | | |
| *Turbinaria* sp. | | | | |
| *Padina* sp. | | | | |

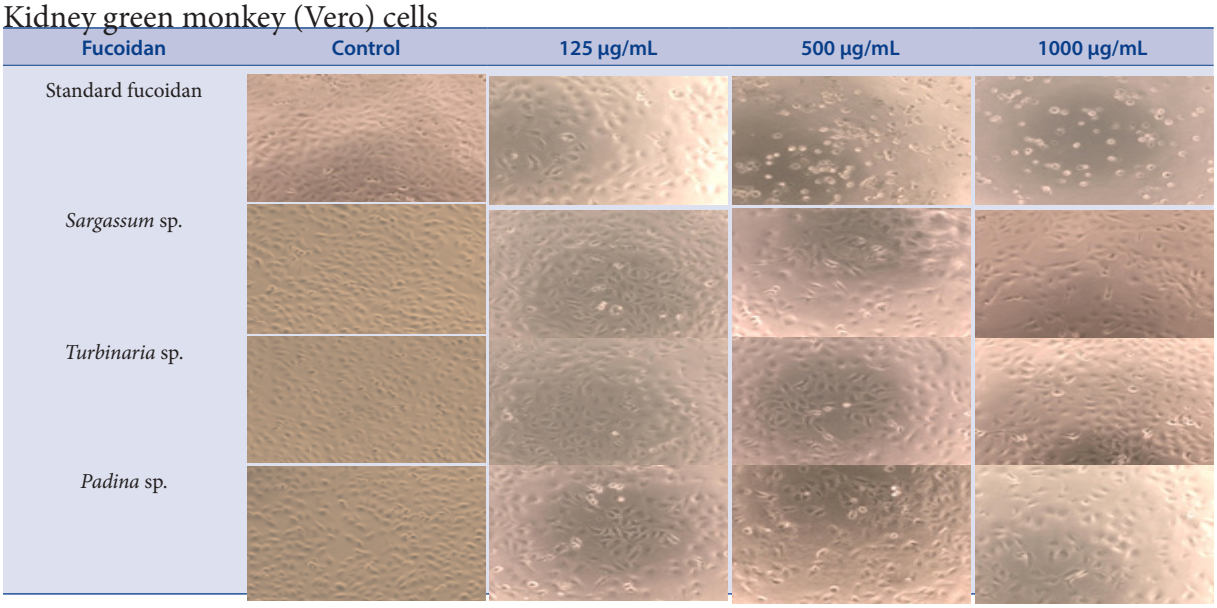



**Figure 5:** Morphology and density changes of human collon adenocarcinoma (WiDr) human breast adenocarcinoma (MCF-7) and kidney green monkey (Vero) cells treated with standard fucoidan and fucoidan from Sargassum sp., Turbinaria sp., Padina sp. at various concentrations

*Sargassum cristaefolium* in tilapia (Oreochromis niloticus). Aquacult. Int In Press. 2016;24(2):465-477. http://dx.doi.org/10.1007/s10499-015-9938-z.

10. Kawamoto H, Miki Y, Kimura T, Tanaka K, Nakagawa T. Effects of fucoidan from mozuku on human stomach cell lines. Food Sci Technol Res. 2006;12(3):218-22. http://dx.doi.org/10.3136/fstr.12.218.
11. Kim EJ, Park SY, Lee J-Y, ParkJHY. Fucoidan present in brown algae induces apoptosis of human colon cancer cells. BMC Gastroenterol. 2010;10(1):96. http://www.biomedcentral.com/1471-230X/10/96. http://dx.doi.org/10.1186/1471-230X-10-96.
12. World Health Organization.org. Geneva: World Health Organization. [updated 2015, February; cited 2015 November 29]. Available from http://www.who.int/mediacentre/factsheets/fs297/en/index.html.
13. World Health Organization.org. Geneva: World Health Organization. (updated 2013, January; cited 2015 November 29). Available from http://www.who.int/features/factfiles/cancer/en/.
14. Girish HV, Vinod AB, Dhananjaya BL, Kumar SD, Duraisamy S. The anticancer potential of *pavonia odorata* willd. extract on human breast (MD-MB-231), prostate (PC-3) and lung (Calu-6) cancer cell lines. Pharmacogn J. 2016;8(1):28-30.
15. Syam S, Bustamam A, Abdullah R, Sukari MA, Hashim NM, Yahayu M, *et al.* Cytotoxicity and oral acute toxicity studies of b-mangostin isolated from *Cratoxylum arborescens*. Pharmacogn J. 2014;6(1):47-56. http://dx.doi.org/10.5530/pj.2014.1.8.
16. Hyun JH, Kim SC, Kang JL, Kim MK, Boo HJ, Kwon JM, *et al.* Apoptosis inducing activity of fucoidan in HCT-15 colon carcinomacells. Biol Pharm Bull. 2009;32(10):1760-4. http://dx.doi.org/10.1248/bpb.32.1760.
17. Senthilkumar K, Manivasagan P, Venkatesan J, Kim S-K. Brown seaweed fucoidan: Biological activity and apoptosis, growth signaling mechanism in cancer. Int J Biol Macromol. 2013;60:366-74. http://dx.doi.org/10.1016/j.ijbiomac.2013.06.030.
18. Ale MT, Maruyama H, Tamauchi H, Mikkelsen JD, Meyer AS. Fucoidan from Sargassum sp. and Fucus vesiculosus reduces cell viability of lungcarcinoma and melanoma cells in vitro and activates natural killer cells in mice *in vivo*. Int J Biol Macromol. 2011;49(3):331-6. http://dx.doi.org/10.1016/j.ijbiomac.2011.05.009.
19. Ermakova S, Sokolova R, Kim SM, Um BH, Isakov V, Zvyagintseva T. Fucoidans from brown seaweeds *Sargassum hornery, Eclonia cava, Costaria costata*: structural characteristics and anticancer activity. Appl Biochem Biotechnol. 2011;164(6):841-50. http://dx.doi.org/10.1007/s12010-011-9178-2
20. Murphy C, Hotchkiss S, Worthington J, McKeown SR. The potential of seaweed as a source of drugsfor use in cancer chemotherapy. J App Phycol. 2014;26(5):2211-64. http://dx.doi.org/10.1007/s10811-014-0245-2.
21. Marudhupandi T, Kumar TTA, senthil SL, Suja G, Vinothkumar T. *In vitro* anticancer activity of fucoidan from *Turbinaria conoides* against A549 cell lines. Int J Biol Macromol. 2015;72:919-23. http://dx.doi.org/10.1016/j.ijbiomac.2014.10.005.
22. Dhargalkar VK, Kavlekar D. Seaweeds: a field manual. 2004; National Institute of Oceanography, Dona Paula.36 p.
23. Trono Jr GC.Seaweeds In: FAO Species Identification Guide For Fishery PurposesThe Living Marine Resources of The Western Central Pacific Vol. 1 Seaweeds, corals, bivalves and gastropods. Carpenterand KE, NiemVH (Eds.), 1998;FAO.Rome. 1-100.
24. Rioux D, Colliec-Jouault S, Pinczon D, Bosch S, Siavoshian S, LeBert V, *et al.* Antitumor and antiproliferative effects of a fucan extracted from *Ascophyllum nodosum* against a non-small-cell bronchopulmonary carcinoma line. Anticancer Res. 1996;16(13):1213-8.
25. Masuko T, Minami A, Iwasaki N, Majima T, Nishimura SI, Lee YC. Carbohydrate-analysis by a phenol–sulfuric acid method in microplate format. Anal Biochem. 2005;339(1):69-72. http://dx.doi.org/10.1016/j.ab.2004.12.001.
26. Dodgson KS, Price RG. A note on the determination of the ester sulphate content of sulphated polysaccharides. Biochem J. 1962;84(1):106-10. http://dx.doi.org/10.1042/bj0840106.
27. Isnansetyo A, Kamei Y. MC21-A, a bactericidal antibiotic produced by a new marine bacterium, *Pseudoalteromonas phenolica* sp. nov., against methicillin-resistant Staphylococcus aureus (MRSA). Antimicrob Agents Chemother. 2003;47(2):480-8. http://dx.doi.org/10.1128/AAC.47.2.480-488.2003
28. Silva TMA, Alves LG, de Queiroz KCS, Santos MGL, Marques CT, Leite EL. Partial characterization and anticoagulant activity of a heterofucan from the brown seaweed Padina gymnospora. Braz J Med Biol Res. 2005;38(4):523-33. http://dx.doi.org/10.1590/S0100-879X2005000400005
29. Immanuel G, velmurugan MS, Marudhupandi T, Radhakrishnan S, Palavesam A. The effect of fucoidan from brown seaweed *Sargassum wightii* on WSSV resistance and immune activity in shrimp *Penaeus monodon* (Fab). Fish & Shellfish Immunol. 2012;32(4):551-64. http://dx.doi.org/10.1016/j.fsi.2012.01.003.
30. Wang Q, Song Y, He Y, Ren D, Kow F, Qiao Z, *et al.* Structural characterisation of algae *Costaria costata* fucoidan and its effects on CCl4-induced liver injury. Carbohydr Polym. 2014;107:247-54. http://dx.doi.org/10.1016/j.carbpol.2014.02.071.
31. Pielesz A, Binias W. Cellulose acetate membrane electrophoresis and FTIR spectroscopy as methods of identifying a fucoidan in *Fucus vesiculosus* Linnaeus, Carbohydr Res. 2010;345(18):2676-82. http://dx.doi.org/10.1016/j.carres.2010.09.027.

32. Fukahori S, Yano H, Akiba J, Ogasawara S, Momosaki S, Kojiro M. Fucoidan. A major component of brown seaweed prohibits the growth of human cancer cell lines *in vitro*. Mol Med Rep. 2008;1(4):537-54. http://dx.doi.org/10.3892/mmr.1.4.537.

**Cite this article :** Isnansetyo A, Laili Lutfia FN, Nursid M, Trijoko, Susidarti RA. Cytotoxicity of Fucoidan from Three Tropical Brown Algae Against Breast and Colon Cancer Cell Lines. Pharmacog J. 2017;9(1):14-20.