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In vitro Cytotoxic Potential of Yacon (*Smallanthus sonchifolius*) Against HT-29, MCF-7 and HDFn Cell Lines

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Abstract: The objective of this study was to assess the cytotoxicity potential of hexane, methanol and DCM extracts of yacon (*Smallanthus sonchifolius*) leaves against breast cancer (MCF-7), colon cancer (HT-29) and normal Human Dermal Fibroblast (HDFn) cell lines by using AlamarBlue® assay. The finely ground leaves of *Smallanthus sonchifolius* was exhaustively extracted with hexane, followed by Dichloromethane (DCM) and lastly with methanol. All three extracts were concentrated using rotavapor and were prepared into 100 μg/mL using 0.2% Dimethyl Sulfoxide (DMSO) in complete Dulbecco's Modified Eagle Medium (DMEM) as solvent. This was then serially diluted (two-fold) during treatment on the different cell lines placed in a 96-well plate. Methotrexate, colchicine, tamoxifen and 5-fluorouracil were used as standard drug controls. AlamarBlue® was aseptically added to each well and maximum absorbance was measured at 570 nm using a microplate reader. Results showed significant reduction in cellular viability of MCF-7 cell lines caused by hexane, methanol and DCM extracts in a dose dependent manner with DCM being the most potent. The DCM extract also produced significant cytotoxic activity against HT-29 cells with the computed half maximal Inhibitory Concentration (IC_{50}) significantly lower than 5-fluorouracil. Effect on HDFn showed that three yacon extracts produced significantly lower cytotoxicity compared to drug controls with the DCM extract showing the least toxicity. The extracts of leaves showed significant cytotoxic effect against breast (hexane, methanol and DCM extracts) and colon cancer cells (DCM extract) while exhibiting non-cytotoxic activities on the normal human cells compared to existing cytotoxic drugs.

Key words: Yacon, Alamar, breast, colon, cancer, MCF-7, HT-29

INTRODUCTION

Cancer is one of the leading causes of mortality not only in the Philippines but worldwide with an incidence rate that is observed to be linearly increasing through time (CRUK., 2016). Numerous studies have investigated on every aspect of malignancy including types, causes, clinical presentation, pathologic basis, genetics, prognosis, diagnosis and treatment. These scientific inquiries have led to significant improvements on cancer management. However, the modern era is still facing the constant dilemma of treatment toxicity.

Yacon (*Smallanthus sonchifolius*) is a perennial plant that forms underground tuberous roots. This member of the sunflower family is a native herb found in the Andean regions and is currently being cultivated in the Mountain Province. Fresh yacon tubers are edible, yellowish white, crisp and juicy similar to apple or sinkamas with sweetness that increases with storage. The root crops are usually eaten raw but can also be prepared into syrups, jams and other foodstuff (Graefe *et al*., 2004). Aside from household consumption as food, there are a number of ethnomedical uses for yacon. The tubers were eaten raw in South America as diuretic for urinary ailments. Similarly in Bolivia, decoctions of the leaves were used as home remedy for cystitis, kidney and even liver problems. Peruvians alternatively prepare leaves into a warm poultice for treatment of muscle and joint pains (Graefe *et al*., 2004). In Brazil, leaves of yacon were taken in the form of tea for control of diabetes (Genta *et al*., 2009).

Yacon tubers consists mainly of fructans with a structure that is of the inulin type, i.e., β(2→1) fructofuranosyl saccharose (Ojansivu *et al*., 2011). This

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content makes yacon tubers marketable as sucrose substitutes and are considered dietetic. Additionally, fructans have favourable influence on the human intestinal flora and can modify certain types of lipid disorders. Since, humans have no enzyme capable of hydrolysing the β(2→1) bond, these fructans also serve as dietary fiber (Ojansivu *et al*., 2011). Recently, oligofructans have been classified as prebiotics (Pedreschi *et al*., 2003). These compounds are transported to the colon and fermented by selected species of gut micro-flora, especially, Bifidobacterium and Lactobacillus, both indicators of a balanced gut flora. The prebiotic effect of yacon tuber extracts has been demonstrated by their fermentation by these gut bacteria, *Lactobacillus plantarum*, *L. acidophilus* and *Bifidobacterium bifidum* (Valentova *et al*., 2003). Studies have shown that prebiotic consumption favorably modifies gut flora composition and its metabolic activities. Perhaps in a similar manner, yacon tuber consumption also modulate lipid metabolism, calcium absorption and immune response. β(2→1) fructans are related to β-glucans, native polysaccharides found in yeast and fungi, serving as non-specific immunostimulators (Valentova *et al*., 2003). They bind to macrophages, activate them and initiate the immunity cascade. β-glucans are recommended for the treatment of immunity defects, infections, allergies, chronic fatigue syndrome, high cholesterol levels, stomach problems and as an adjuvant in carcinoma therapy. Yacon tubers are also rich in free fructose, glucose and sucrose (Valentova *et al*., 2006).

The Fructooligosaccharides (FOS) extracted from yacon roots were also found to have hypolipidemic effects on diabetic rats. A significant decrease in fasting plasma triacylglycerol and very low-density lipoprotein levels were observed, along with increased insulin-positive pancreatic cell mass distributed in small cell clusters within the exocrine parenchyma (Habib *et al*., 2011). The positive metabolic effects of yacon root extracts were further tested in diabetes. Aqueous extracts were effective in controlling water and food consumption, hyperglycemia and dyslipidemia and promote the reduction of liver enzymes, suggesting a hepatoprotective effect in rats with drug-induced diabetes mellitus Type 1 (Oliveira *et al*., 2013). FOS extracted from yacon roots was also found to have preventive effect against *Salmonella typhimurium* enteric infection. When given orally up to 30 days, FOS from yacon enhanced non-specific immunity such as increasing the total IgA which improves the immunological intestinal barrier, thereby preventing pathologic colonization by *S. typhimurium* (Velez *et al*., 2013). The high concentration of fructans in yacon roots was also discovered to have potential for colon cancer prevention. A significant reduction in number and multiplicity of aberrant crypt foci and in number of invasive adenocarcinomas was observed in the groups orally treated with 1% yacon and its symbiotic formulation (yacon plus *L. casei*) (De Moura *et al*., 2012). Extracts of yacon were also shown to inhibit progression of acute pancreatitis. The inhibitory effect of 1% of yacon extract on dibutyltin dichloride-induced pancreatitis in rats was interpreted based on decreased levels of inflammatory mediators such as tumor growth factor and cyclooxygenase-2 in yacon-treated subjects (Choi *et al*., 2012).

Yacon leaves were also extensively studied for physiologic effects on animals. A review on the characteristics of yacon as a functional food (Grethel *et al*., 2013) states that yacon leaves contain several phenolic compounds that enhance growth of intestinal bacteria with good metabolic properties, inhibiting the attack of pathogens. Hydro-ethanolic crude extracts (400 mg/kg) of yacon leaves given orally to diabetic Wistar rats for 3, 7, 10 and 14 days were shown to significantly decrease fasting and post-prandial serum glucose (Baroni *et al*., 2008). This finding was further confirmed by another study that utilized methanol, butanol and chloroform extracts, given to Wistar rats at 50, 10 and 20 mg/kg body weight for 8 weeks (Genta *et al*., 2010). This study measured for oral glucose tolerance test and serum insulin, aside from fasting and post-prandial blood glucose. Results showed effective hypoglycemic activity and increased insulin levels. Another study utilized normoglycemic mice and concluded that 100 mg/kg oral dose of yacon leaf tea extract and ent-kaurenoic fraction were both effective in lowering blood glucose levels (Raga *et al*., 2010). The methanolic extract of yacon leaves yielded ent-kaurenoic acid and related diterpenoid substances. Recently, ent-kaurenoic acid from yacon was found to possess significant antibacterial and antifungal activities (Padla *et al*., 2012). Extracts of leaves were also found to have *in vivo* radical scavenging activity. Peroxidation of lipids was significantly inhibited, protecting the liver of rats against oxidative injury (Valentova *et al*., 2003).

Sesquiterpene lactones, namely enhydrin, uvedalin and sonchifolin were also isolated from the leaves of yacon (Siriwan *et al*., 2011a, b). Sesquiterpene lactones are plant products extensively studied for their wide array of biological activities such as anti-inflammatory, neurocytotoxic and anticancer potentials (Cho *et al*., 2004). The ones isolated from yacon leaves, specifically enhydrin and uvedalin are demonstrated to have potent anticancer activity against cervical cancer cell line, specifically by inducing apoptosis-mediated proliferation inhibition via. caspase and deactivation of NF-κB (Siriwan *et al*., 2011b). Another study have also shown

chemopreventive properties of the sesquiterpene lactones isolated from yacon leaves with enhydrin, uvedalin and sonchifolin showing stronger chemopreventive activity than parthenolide (Siriwan *et al*., 2011a, b). The latter is a reference sesquiterpene lactone that has been proven to possess potent chemopreventive properties and is now included in cancer clinical trials (Ghantous *et al*., 2010). A study exploring on trypanocidal activity of sesquiterpene lactones isolated from yacon revealed that enhydrin, uvedalin and polymatin B efficiently inhibited both the epimastigote and the replicative intracellular amastigotes of *Trypanosoma cruzi* (Frank *et al*., 2013).

Yacon has also been investigated on its action against colon cancer and melanoma. Scientists used 1,2-dimethylhydrazine to induce colon carcinogenesis in male Wistar rats. Those administered with dried extract of yacon root and a mixture of yacon with a probiotic showed significant reduction in number and multiplicity of aberrant crypt foci and decreased number of invasive adenocarcinomas (De Moura *et al*., 2012). Another study investigated the anti-oxidant and anti-cancer activities of different organic solvent fractions of yacon root. Hexane fractions showed high growth inhibitory activities against cancer cells (Min *et al*., 2008). Another study explored the potential of yacon for melanin synthesis inhibition. Yacon leaf extracts exhibited significant anti-melanogenic activity to suppress melanin synthesis in mouse B16 melanoma cells (Ishikawa *et al*., 2010).

This study aims to establish reliable data on the anticancer activity of yacon extracts, specifically against breast and colon cancer cell lines. Future scientific ventures on acute toxicity, subacute toxicity and human clinical investigations on yacon will greatly benefit from the output of this study. The information generated from this research can also be used in further identification of active components which will eventually aid in the discovery and synthesis of a novel, plant-derived drug with superior cytotoxic activity and acceptable side effect profile.

MATERIALS AND METHODS

Collection of plant material: Yacon leaves were collected from a farm in Misamis Oriental under the management of Doalnara Multi-Purpose Cooperative. Samples of the leaves were sent to the Bureau of Plant Industry for taxonomic identification. The leaves were cleaned and shade dried for more than 4 weeks in average ambient temperature of 32°C and humidity of 64%. The dried materials were ground into powder using a blender and stored in airtight plastic containers and labeled accordingly.

Preparation of plant extract: The finely ground leaves of *Smallanthus sonchifolius* (278.62 g) was exhaustively extracted for 6 consecutive days (2 days for each type of solvent) with solvents in increasing polarity starting with hexane, followed by dichloromethane and lastly with methanol. For every extraction, the collected crude extracts were concentrated in vacuo using a Buchi rotavapor at a maintained temperature of 45°C. Each extraction afforded three crude extracts labeled as SsH for the hexane extract, SsD for the dichloromethane extract and SsM for the methanol extract. Small amounts of each crude extract (0.2029 g for SsM, 0.6832 g for SsD and 0.3888 for SsH) were prepared into 100 μg/mL using 0.2% Dimethyl Sulfoxide (DMSO) in complete Dulbecco's Modified Eagle Medium (DMEM) as solvent. This working concentration was then serially diluted (two-fold) to 50, 25, 12.5, 6.25, 3.125, 1.563 and 0.781 μg/mL during treatment on the different cell lines.

Cell culture: Three cell lines were used for this study namely, breast cancer (MCF-7), colon cancer (HT-29) and normal Human Neonatal Dermal Fibroblast (HDFn) cells. The cells were maintained in Dulbecco's Modified Eagle Medium (DMEM, Invitrogen, USA) supplemented with 10% fetal bovine serum (FBS, Invitrogen, USA) and 1% antibiotic antimycotic (Invitrogen, USA) in tissue culture flasks (Falcon, USA) and incubated at 37°C, 5% CO_2 and 95% relative humidity. Cell counts were obtained by the trypan blue exclusion method to calculate cell densities to a final of 1×10^4 viable cells per mL. Experiments were performed in flat bottom 96-well microplates (Falcon, USA) seeded with cell densities of 1×10^3 cells per well. The cells were incubated for 24 h before the drug or plant extracts were added. Untreated cells served as negative controls while 0.2% DMSO in complete DMEM as negative vehicle control. After treatment, the cells were incubated for 48 h prior to analysis with the AlamarBlue® assay.

AlamarBlue® assay: Ten microliters of AlamarBlue® wasere aseptically added to each well. The plates were shaken carefully to thoroughly mix the contents. These were then further incubated at 37°C, 5% CO_2 and 95% relative humidity for 4 h. Viable cells in culture reduce blue resazurin in AlamarBlue® into red resorufin which has maximum absorbance measured at 570 nm using a microplate reader (EL×800, Biotek, USA).

Methotrexate, colchicine, tamoxifen and 5-fluorouracil were used as standard drug controls. Concentrations of these drugs were prepared similarly as that of the extracts using complete DMEM as solvent. The assay was done in triplicates. The percentage of

inhibited growth was computed as: 100-[(absorbance of treated cells/absorbance of untreated cells)×100].

Statistical analysis: The data were expressed as mean optical density ±SD. ANOVA was used to assess significant differences between controls and plant extracts. IC_{50} for extracts and controls were computed from the generated dose-response curves.

RESULTS AND DISCUSSION

MCF-7 cell line: The next set of tables and figures shows the cytotoxicity effect of increasing concentrations of yacon leaves extracts and controls on breast cancer cells. The measured optical densities are tabulated in Table 1 and plotted against concentration in Fig. 1. The computed percentages of cell viability inhibition are shown in Table 2 and Fig. 2. There is an observed linear decrease in optical density and increase in cellular growth inhibition with increasing concentration of the three extracts. ANOVA analysis revealed significant difference ($p<0.001$) from negative control for the three extracts at concentrations 12.5, 25 µg/mL (except for hexane extract), 50 and 100 µg/mL and no significant difference from the positive controls at all concentration levels, except for tamoxifen.

The IC_{50} were computed using log-linear regression dose-response curve and are shown in Table 3, along with measure of linearity (R^2), slope and their respective confidence intervals. The three extracts significantly reduced viability of cells in dose-dependent manner,with the DCM extract being the most potent. The IC_{50} for the hexane and methanol extracts were 32.08 and 37.44 µg/mL, respectively. These values are higher than the computed IC_{50} for colchicine and 5-fluorouracil but are significantly lower than the IC_{50} of tamoxifen and methotrexate. The DCM extract has the lowest IC50 at 23.77 µg/mL which is significantly lower than the positive controls, except for colchicine.

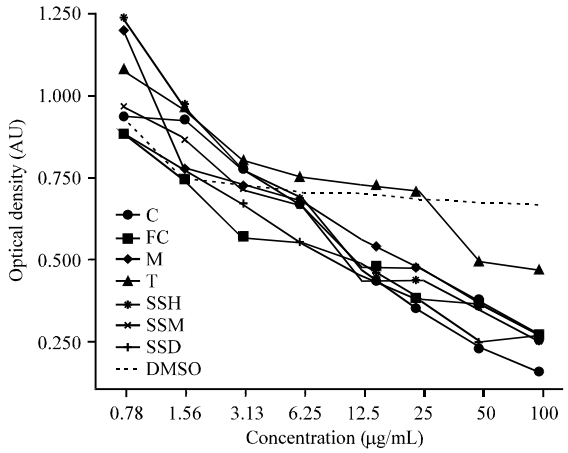

Fig. 1: Mean optical density (AU) vs. concentration (µg/mL)-MCF-7

Table 1: Mean Optical Density (AU) from MCF-7 at different concentrations (µg/mL) of smallanthus sonchifolius leaves extracts and controls

Controls/	Optical density (AU)* at different concentration (µg/mL)							
Plant extracts	0.780	1.560	3.130	6.250	12.500	25.000	50.000	100.000
C	0.93+0.013	0.92+0.021	0.77+0.034	0.67+0.071	0.45+0.021	0.33+0.018	0.23+0.020	0.15+0.003
FC	0.87+0.023	0.73+0.023	0.55+0.031	0.55+0.043	0.48+0.016	0.37+0.015	0.36+0.020	0.26+0.005
M	1.18+0.482	0.76+0.050	0.72+0.020	0.66+0.036	0.55+0.032	0.47+0.003	0.37+0.001	0.27+0.001
T	1.07+0.385	0.95+0.257	0.80+0.067	0.74+0.012	0.72+0.016	0.70+0.027	0.48+0.014	0.46+0.017
SSH	1.22+0.215	0.96+0.029	0.77+0.019	0.66+0.001	0.44+0.019	0.44+0.043	0.35+0.047	0.24+0.042
SSM	0.96+0.026	0.86+0.008	0.70+0.051	0.66+0.034	0.47+0.016	0.47+0.007	0.36+0.002	0.26+0.006
SSD	0.88+0.009	0.76+0.033	0.66+0.031	0.55+0.023	0.44+0.034	0.37+0.005	0.24+0.036	0.27+0.004
DMSO	0.92+0.234	0.74+0.023	0.72+0.030	0.70+0.021	0.70+0.019	0.68+0.032	0.67+0.032	0.66+0.038

Table 2: Percentage of inhibited MCF-7 at different concentrations (µg/mL) of smallanthus sonchifolius leaves extracts and controls

Controls/	Percentage cell inhibition at different concentration (µg/mL)							
Plant extracts	0.780	1.560	3.130	6.250	12.500	25.000	50.000	100.000
C	-22.09+1.64	-17.74+2.63	3.19+4.28	15.71+8.84	40.99+2.74	56.57+2.39	69.85+2.55	80.46+0.33
FC	-13.68+3.01	6.07+2.88	30.48+3.88	31.58+5.43	37.64+2.08	50.98+1.98	52.46+2.57	65.75+0.59
M	-54.51+63.06	2.09+6.37	9.70+2.46	16.79+4.56	28.07+4.18	38.88+0.38	51.98+0.15	65.05+0.13
T	-39.87+50.30	-22.05+32.89	-.13+8.40	6.85+1.52	6.14+2.14	7.99+3.47	37.21+1.77	39.55+2.28
SSH	-59.91+28.12	-23.03+3.74	3.27+2.36	17.67+0.13	43.08+2.42	42.77+5.60	54.64+6.10	68.29+5.53
SSM	-25.05+3.36	-10.60+1.04	11.29+6.41	17.46+4.27	38.34+2.11	38.93+0.91	52.90+0.20	66.32+0.83
SSD	-15.12+1.17	1.97+4.17	16.75+3.84	31.62+2.82	42.34+4.39	51.90+0.60	68.28+4.67	65.09+0.55
DMSO	-20.74+30.61	4.91+2.88	9.15+3.72	12.16+2.68	8.83+2.54	11.10+4.17	12.33+4.14	12.75+4.95

*Mean±SD, n = 3; C = Colchicine, FC = 5-Fluorouracil, M = Methotrexate, T = Tamoxifen, SSH = Hexane extract, SSM = Methanol extract, SSD = Dichloromethane extract, DMSO = Solvent

Table 3: IC_{50} values and other dose-response curve parameters against MCF-7 for Smallanthus sonchifolius leaves extracts and controls

Controls/ Plant extracts	R^2	Intercept	95% CI Lower	95% CI Upper	Slope (coefficient for dose)	95% CI Lower	95% CI Upper	IC_{50}(µg/mL)
C	0.979	-21.813	-25.850	-17.775	23.030	21.533	24.527	22.61
FC	0.916	0.646	-4.670	5.962	14.692	12.721	16.664	28.77
M	0.687	-24.588	-40.962	-8.215	20.351	14.279	26.424	39.06
T	0.610	-28.313	-42.651	-13.975	15.042	9.724	20.359	182.42
SSH	0.889	-35.148	-45.512	-24.783	24.551	20.707	28.394	32.08
SSM	0.969	-15.996	-19.870	-12.122	18.217	16.780	19.653	37.44
SSD	0.960	-4.906	-9.151	-0.662	17.329	15.755	18.903	23.77
DMSO	0.285	-3.943	-12.838	4.951	4.706	1.408	8.005	95092.08

C = Colchicine; FC = 5-Fluorouracil; M = Methotrexate; T = Tamoxifen; SSH = Hexane extract; SSM = Methanol extract; SSD = Dichloromethane extract; DMSO = Solvent

Table 4: Mean optical density (AU) from HT-29 at different concentrations (µg/mL) of Smallanthus sonchifolius leaves extracts and controls

Controls/ Plant extracts	Optical density (AU)* at different concentration (µg/mL) 0.780	1.560	3.130	6.250	12.500	25.000	50.000	100.000
C	0.875+0.017	0.758+0.003	0.652+0.005	0.550+0.004	0.445+0.023	0.327+0.015	0.269+0.010	0.172+0.014
FC	0.862+0.008	0.763+0.001	0.655+0.006	0.552+0.011	0.448+0.016	0.338+0.018	0.245+0.008	0.132+0.008
M	0.866+0.010	0.781+0.022	0.738+0.020	0.651+0.026	0.630+0.020	0.467+0.006	0.431+0.020	0.241+0.013
T	0.837+0.025	0.749+0.016	0.725+0.029	0.648+0.002	0.558+0.012	0.550+0.024	0.545+0.022	0.523+0.021
SSH	0.843+0.033	0.751+0.007	0.746+0.011	0.642+0.010	0.533+0.011	0.521+0.014	0.514+0.015	0.495+0.011
SSM	0.854+0.004	0.741+0.008	0.734+0.005	0.630+0.007	0.526+0.010	0.523+0.009	0.516+0.009	0.509+0.010
SSD	0.861+0.007	0.651+0.030	0.537+0.023	0.549+0.028	0.435+0.018	0.327+0.020	0.254+0.014	0.131+0.018
DMSO	0.845+0.033	0.746+0.025	0.732+0.017	0.683+0.011	0.654+0.004	0.648+0.004	0.638+0.019	0.635+0.019

*Mean±SD, n = 3; C = Colchicine, FC = 5-Fluorouracil, M = Methotrexate, T = Tamoxifen, SSH = Hexane extract, SSM = Methanol extract, SSD = Dichloromethane extract, DMSO = Solvent

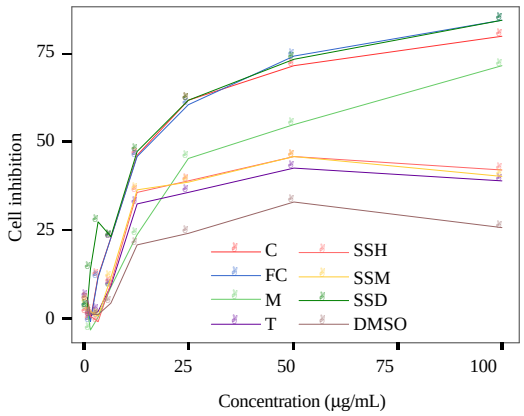

Fig. 2: Percentage cell inhibition vs. concentration (µg/mL)-MCF-7

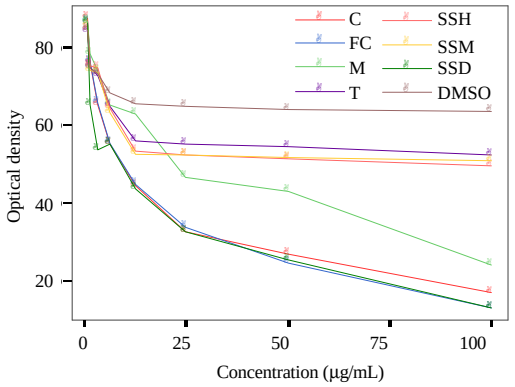

Fig. 3: Mean optical density (AU) vs. concentration (µg/mL)-HT-29

HT-29 cell line: Table 4 and Fig. 3 show cytotoxic effect of the Yacon leaves extracts and drug controls on colon cancer cells. Decreasing optical densities were observed in a linear fashion after treatment with increasing concentrations of the three Yacon extracts. Percentage cell growth inhibition was computed based on these values and are shown in Table 5 and plotted against increasing concentrations in Fig. 4. The DCM extract significantly reduced cell viability in a dose-dependent manner. ANOVA analysis showed significant difference ($p<0.001$) of optical density and cell viability from the negative control and no significant difference from positive controls (5-flurouracil and colchicine) starting from 3.125 µg/mL concentration of DCM extract.

Dose-response curve parameters were generated using log-linear regression to compute for the IC_{50} (Table 6). Hexane and methanol extracts did not exhibit significant cytotoxicity (IC_{50}>100 µg/mL). On the other hand, the IC_{50} for the DCM extract is 14.32 µg/mL which is lower than all the positive drug controls including 5-fluorouracil. This indicates potent cytotoxicity effect produced by the DCM extract.

HDFn cell line: Effect on normal human cell line was assessed by treating HDFn with the same concentrations

Table 5: Percentage of inhibited HT-29 at different concentrations (µg/mL) of smallanthus sonchifolius leaves extracts and controls

Controls/ Plant extracts	Percentage cell inhibition at different concentration (µg/mL)							
	0.780	1.560	3.130	6.250	12.500	25.000	50.000	100.000
C	1.65+1.92	-0.01	11.77+0.68	22.81+0.53	46.09+2.75	61.62+1.73	71.68+1.10	79.86+1.61
FC	3.18+0.85	-0.93	11.37+0.82	22.58+1.54	45.80+1.90	60.33+2.14	74.25+0.88	84.58+0.95
M	2.70+1.17	-0.55	0.18+2.73	8.74+3.66	23.77+2.46	45.23+0.68	54.67+2.11	71.78+1.52
T	5.92+2.86	0.75+2.06	1.85+3.94	9.16+0.32	32.45+1.47	35.45+2.79	42.60+2.31	38.76+2.42
SSH	5.28+3.70	0.57+0.88	0.62	9.91+1.46	35.47+1.38	38.85+1.66	45.89+1.58	42.00+1.29
SSM	4.08+0.47	1.85+1.05	0.72+0.67	11.59+0.99	36.32+1.15	38.62+1.04	45.72+0.90	40.40+1.17
SSD	3.30+0.81	13.77+3.91	27.29+3.17	22.95+3.93	47.34+2.20	61.62+2.36	73.23+1.47	84.66+2.13
DMSO	5.09+3.72	1.15+3.36	0.99+2.33	4.25+1.54	20.82+0.44	23.98+0.49	32.81+1.96	25.64+2.26

*Mean±SD, n = 3, C = Colchicine; FC = 5-Fluorouracil; M = Methotrexate; T = Tamoxifen; SSH = Hexane extract; SSM = Methanol extract; SSD = Dichloromethane extract; DMSO = Solvent

Table 6: IC_{50} values and Other dose-response curve parameters against HT-29 for Smallanthus sonchifolius leaves extracts and controls

Controls/ Plant extracts	R^2	Intercept	95%CI		Slope (coefficient for dose)	95%CI		IC_{50}(µg/ml)
			Lower	Upper		Lower	Upper	
C	0.9618	-3.57	-7.98	0.834	18.56	16.93	20.20	17.93
FC	0.9590	-4.15	-8.88	0.570	19.17	17.42	20.93	16.86
M	0.8931	-9.14	-15.69	-2.590	15.87	13.45	18.30	41.53
T	0.8214	-0.21	-5.59	5.160	9.67	7.68	11.67	179.89
SSH	0.8208	-1.38	-7.40	4.620	10.79	8.56	13.02	116.96
SSM	0.8337	-0.49	-6.09	5.110	10.51	8.43	12.59	122.00
SSD	0.9612	4.56	0.47	8.650	17.07	15.56	18.59	14.32
DMSO	0.7637	-0.17	-4.58	4.250	6.66	5.02	8.30	1868.76

C = Colchicine; FC = 5-Fluorouracil; M = Methotrexate; T = Tamoxifen; SSH = Hexane extract; SSM = Methanol extract; SSD = Dichloromethane extract; DMSO = Solvent

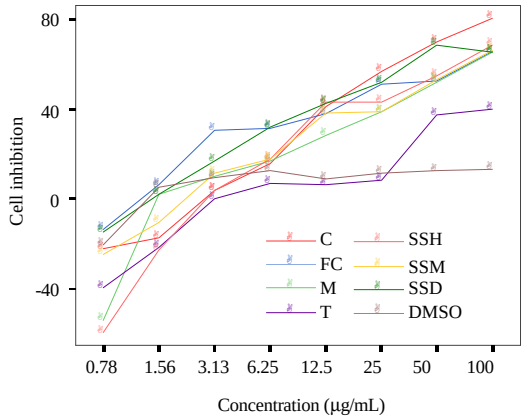

Fig. 4: Percentage of cell inhibition vs. concentration (µg/mL)HT-29

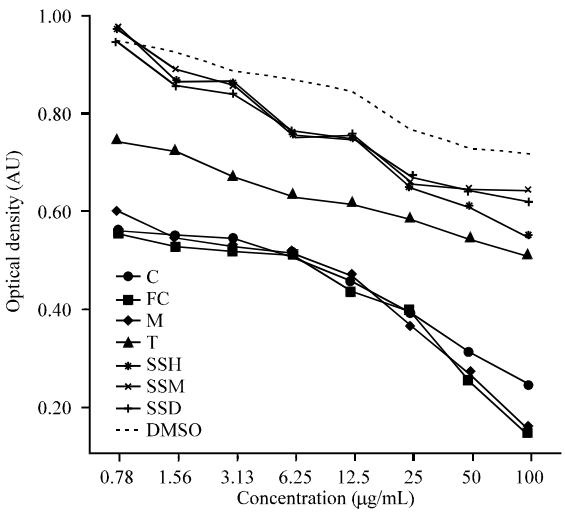

Fig. 5: Mean optical density (AU) vs. concentration (µg/mL)-HDFn

of Yacon extracts and controls used for MCF-7 and HT-29 cell lines. Table 7 and 8 (plotted as Fig. 5 and 6, respectively) show the cytotoxic effects of the extracts and controls on the normal cells. Results showed significant higher cytotoxicity effect of the drug controls on normal cells compared to the plant extracts. Data suggest that the Yacon extracts are non-cytotoxic to HDFn normal cells (IC_{50} >100 µg/mL).

Cancer treatment almost always includes chemotherapy and/or radiation and these cytotoxic processes can lead to life threatening conditions such as severe immune deficiency, cardiomyopathy and development of treatment-related malignancy. The search for safer treatment options continues to be an unrelenting challenge for the scientific community. Recent researches have ventured on plant products, uncovering several anti-cancer potentials from different extracts. Yacon has been found to have a great potential in prevention and cure of colon and skin cancer (De Moura *et al*., 2012; Ishikawa *et al*., 2010). However, there is scarcity of further studies to establishyacon's anti-cancer activity and safety parameters (Table 9).

Results of this study showed strong potential of the three yacon extracts to be further investigated as cytotoxic agents against breast cancer. The strong

Table 7: Mean optical density (AU) from HDFn at different concentrations (µg/mL) of Smallanthus sonchifolius leaves extracts and controls

Controls/ Plant extracts	Optical density (AU)* at different concentration (µg/mL)							
	0.780	1.560	3.130	6.250	12.500	25.000	50.000	100.000
C	0.556+0.016	0.548+0.014	0.543+0.018	0.502+0.066	0.453+0.097	0.385+0.058	0.304+0.019	0.244+0.03
FC	0.551+0.006	0.524+0.006	0.516+0.008	0.510+0.006	0.434+0.052	0.390+0.005	0.246+0.010	0.140+0.006
M	0.596+0.046	0.545+0.013	0.527+0.006	0.513+0.017	0.462+0.044	0.358+0.018	0.263+0.013	0.148+0.022
T	0.740+0.009	0.716+0.018	0.667+0.030	0.628+0.018	0.613+0.005	0.582+0.010	0.541+0.035	0.503+0.002
SSH	0.967+0.020	0.864+0.005	0.859+0.004	0.755+0.006	0.744+0.012	0.643+0.039	0.606+0.054	0.541+0.008
SSM	0.967+0.012	0.886+0.018	0.851+0.036	0.747+0.015	0.754+0.001	0.652+0.002	0.640+0.015	0.638+0.016
SSD	0.942+0.028	0.851+0.036	0.839+0.034	0.751+0.013	0.745+0.009	0.665+0.020	0.636+0.002	0.619+0.010
DMSO	0.944+0.027	0.924+0.051	0.886+0.021	0.866+0.017	0.840+0.015	0.763+0.003	0.728+0.011	0.715+0.004

Table 8: Percentage of inhibited HDFn at different concentrations (µg/mL) of smallanthus sonchifolius leaves extracts and controls

Controls/ Plant extracts	Percentage cell inhibition at different concentration (µg/mL)							
	0.780	1.560	3.130	6.250	12.500	25.000	50.000	100.000
C	42.229+1.613	36.574+1.633	38.889+1.990	44.371+7.262	47.991+11.080	60.110+5.977	66.557+2.085	74.145+3.387
FC	42.783+0.648	39.313+0.658	41.929+0.910	43.485+0.668	50.210+5.999	59.662+0.511	72.871+1.047	85.185+0.635
M	38.145+4.809	36.883+1.528	40.616+0.640	43.152+1.906	46.919+5.031	62.905+1.856	70.999+1.395	84.339+2.277
T	23.157+0.923	17.091+2.100	24.850+3.405	30.491+2.043	29.659+0.578	39.717+0.995	40.382+3.872	46.737+0.220
SSH	1.644	0.544	3.266+0.491	16.353+0.610	14.619+1.379	3.471+4.002	3.260+5.927	42.787+0.855
SSM	0.78	-0.546	4.129+4.087	17.239+1.677	13.471+0.133	32.540+0.158	29.479+1.668	32.451+1.681
SSD	2.181+2.878	1.505+4.130	5.556+3.773	16.870+1.445	14.428+1.035	31.194+2.094	29.919+0.229	34.462+1.017
DMSO	1.973+2.823	-1.089	0.225+2.343	4.134+1.887	3.559+1.767	21.014+0.359	19.860+1.157	24.339+0.382

*Mean±SD, n = 3, C = Colchicine; FC = 5-Fluorouracil; M = Methotrexate; T = Tamoxifen; SSH = Hexane extract; SSM = Methanol extract; SSD = Dichloromethane extract; DMSO = Solvent

Table 9: IC_{50} values and other dose-response curve parameters against HDFn Cells for Smallanthus sonchifolius leaves extracts and controls

Controls/ Plant extracts	R^2	Intercept	95% CI		Slope (coefficient for dose)	95% CI		IC_{50}(µg/ml)
			Lower	Upper		Lower	Upper	
C	0.769	34.875	29.935	39.816	7.565	5.732	9.397	7.38
FC	0.819	34.808	29.768	39.897	9.005	7.137	10.874	5.40
M	0.856	31.874	27.137	36.610	9.693	7.937	11.450	6.49
T	0.863	19.340	16.693	21.988	5.585	4.603	6.567	242.19
SSH	0.916	-2.942	-6.403	0.520	9.573	8.289	10.856	252.23
SSM	0.878	-1.878	-5.481	1.725	8.106	6.770	9.442	601.82
SSD	0.895	0.459	-2.650	3.568	7.598	6.445	8.751	678.76
DMSO	0.764	-4.663	-8.669	-0.656	6.050	4.564	7.536	8393.45

C = Colchicine; FC = 5-Fluorouracil; M = Methotrexate; T = Tamoxifen; SSH = Hexane extract; SSM = Methanol extract; SSD = Dichloromethane extract; DMSO = Solvent

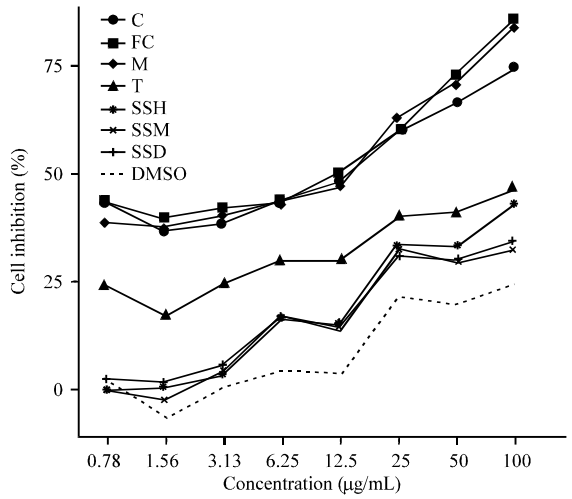

Fig. 6: Percentage of cell inhibition vs. concentration (µg/mL)-HDFn

cytotoxic activity of DCM extract against colon cancer also warrants further investigation. The IC_{50} for hexane, methanol and DCM extracts against MCF-7 were 32.08, 37.44 and 23.77 µg/mL, respectively. These are acceptable IC_{50} levels against MCF-7 compared to that observed from drug controls (colchicine with 22.61 µg/mL, 5-fluorouracil with 28.77 µg/mL, methotrexate with 39.06 µg/mL and tamoxifen with 182.42 µg/mL). The IC_{50} for hexane, methanol and DCM extracts against HT-29 were 116.96, 122.00 and 14.32 µg/mL, respectively. The IC_{50} observed from hexane and methanol extracts are significantly higher than the drug controls, indicating poor cytotoxic activity for this cancer cell line. However, the low IC_{50} of DCM (14.32 µg/mL) is noteworthy as it is significantly lower compared to the IC_{50} values from all the drug controls (colchicine with 17.93 µg/mL, 5-fluorouracil with 16.86 µg/mL, methotrexate with 41.53 µg/mL and tamoxifen with 179.89 µg/mL).

The three yacon extracts were also observed to be significantly non-cytotoxic to normal HDFn cells. The IC_{50} for colchicine, 5-fluorouracil and methotrexate were 7.38, 5.40 and 6.49 μg/mL, respectively. Tamoxifen produced the highest IC_{50} for the drug controls at 242.19 μg/mL but this value is still lower than those observed from the plant extracts. The IC_{50} for hexane, methanol and DCM extracts were 252.23, 601.82 and 678.76 μg/mL, respectively.

The DCM extract outstandingly produced lower IC_{50} levels compared to drug controls against MCF-7 (except to colchicine) and HT-29.. This extract showed the lowest IC_{50} against HT-29 (14.32 μg/mL), even lower than the IC_{50} observed from the current drug of choice against colon cancer, 5-fluorouracil (16.86 μg/mL).

CONCLUSION

Results of this study feature the potential anti-cancer activity of Yacon extracts, most exceptionally the DCM extract. These extracts showed significant cytotoxic effect against breast (hexane, methanol and DCM extracts) and colon cancer cells (DCM extract) while exhibiting non-cytotoxic activities on the normal human cells compared to existing cytotoxic drugs. Results of this study merit further investigation particularly on the cytotoxic mechanisms of the extracts which can also be utilized for development of new medicine against cancer.

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