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Antiproliferative activity of Ontario grown onions against colorectal adenocarcinoma

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ABSTRACT This study evaluated the antiproliferative activity of five Ontario grown onion varieties, namely Lasalle, Ruby Ring, Fortress, Safrane, and Stanley. Cellular proliferation was evaluated by

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the MTS assay while cytotoxicity was evaluated by the lactate dehydrogenase activity (LDH) assay. Apoptosis was analyzed by measuring the fluorescent intensity of caspase 3/7 activation. All tested varieties showed similar antiproliferation, cytotoxic, and proapoptotic activity. Although similar, wells treated with the extract from Stanley variety had the highest concentration of LDH in the supernatant and greatest number of apoptotic cells. Of the three tested concentrations, only the extracts having a total flavonoid concentration of greater than 0.01 mg of quercetin equivalent/g of onion exerted a physiologically relevant biological response. Total flavonoid content alone did not explain the observed trends suggesting the presence of other bioactive compounds in the onion extracts.

Keywords: Onions; antiproliferative activity; lactate dehydrogenase; caspase-3/7; Caco-2; pressurized low polarity water extraction; flavonoids;

INTRODUCTION Phytonutrients are non-nutrient, bioactive plant based compounds. They are found in commonly consumed fruits and vegetables (Schreiner & Huyskens-Keil, 2006) and are grouped according to their chemical structure and functional properties. Some examples of phytonutrients include carotenoids, polyphenols such as flavonoids, saponins, phenolic acids, organosulfides, and polysterols, of which flavonoids are the most commonly found phytochemical (Caridi et al., 2007). The health promoting benefits of phytonutrients in diets have been extensively studied and continue to be an active area of research.

Clinical and epidemiological studies have shown a decreased risk of many chronic diseases such as diabetes, cardiovascular disease and cancers associated with a higher consumption of fruits and vegetables, specifically those rich in dietary flavonoids (Hui et al., 2013; He et al.,2014; Liu *et al.,* 2014; Guercio et al.,2016). Metabolically, flavonoids are known to have antioxidant activity, free radical scavenging capacity, inhibit membrane lipid peroxidation, inhibit enzyme activity, and are cation chelators (Peng & Kuo, *2003;* Leopoldini *et al.,* 2006).

Dietary flavonoids have also exhibited anti-carcinogenic properties against many cancer types including breast (Yamazaki et al., 2014), colorectal (Linsalta et al., 2010; He et al., 2014), liver (Yang et al., 2004), and upper digestive tract (Guercio et al., 2016). They have been reported to exert strong antiproliferative properties by inducing apoptosis and cell cycle arrest on different cancer cell lines (Wenzel *et al.,* 2000; Ren *et al.,* 2015).

Many fruits and vegetables are dietary sources of flavonoids. These include berries, leeks, onions, apples, citrus fruits, broccoli, and tea. Onions, however, are one of the richest sources of flavonoids and considerably contribute to the overall intake of flavonoids in the human diet (up to 1.2g/kg fresh wt; Manach *et al*., 2004; Suleria *et al.,* 2015). Three flavonoids found in onions are quercetin, myricetin, and kaempferol. Of the three, quercetin is the flavonoid with the highest concentration in onions (Sellappan & Akoh *et al.,* 2002). Globally, onions are also one of the most widely produced and consumed vegetables (Sellappan & Akoh*,* 2002; Slimestad *et al.,* 2007). However, due to the existence of varietal and environmental diversity, the composition and concentration of health promoting substances such as flavonoids may vary between cultivars. Consequently, there may be differences between the health promoting benefits of onion varieties grown outside of Canada and those cultivated in Ontario. Hence, an investigation of the antiproliferative capacity of Ontario grown onions is warranted.

The objectives of this study were to examine the functional properties of 5 Ontario grown varieties by:

1) Assessing the antineoplastic capacity of the onion extracts on adenocarcinoma growth,

2) Determining the cytotoxic activity of the extracts on cancer viability, and

3) Investigating their ability to induce apoptosis and ultimately cell death on cancer cells.

MATERIALS AND METHODS

Materials A human colorectal adenocarcinoma cell line (Caco-2) was obtained from the American Type Culture Collection (Rockville, MD). Minimum Essential Medium (MEM), fetal bovine serum (FBS), D-glucose, quercetin, myricetin, kaempferol, and L-glutamine were purchased from Sigma Aldrich (St Louis, MO). TrypLE Express, non-essential amino acid solution, and CellEvent™ Caspase-3/7 Green Detection Agent were bought from Invitrogen Corp. (Carlsbad, CA). 100X penicillin with streptomycin antibiotic solution and F12:RPMI 1640 media were obtained from GE Healthcare Life Sciences (Logan, UT). MTS cell proliferation and LDH-cytotoxicity assay kits were purchased from BioVision Inc. (Milipitas, CA). Onions were graciously donated by the Holland Marsh Growers' Association in Bradford, Ontario.

Pressurized low polarity water extraction of Ontario onions. Pressurized water extractions were implemented by colleagues at the Guelph Research and Development Centre. Briefly, freeze dried onion powder (5 gm) from five Ontario grown varieties, namely Lasalle, Ruby Ring, Fortress, Stanley, and Safrane were mixed in 80 mL of 0.1% formic acid in milliq water (v/v). Extractions of the mixtures were carried out using an automated Spe-ed SFE NP model 7100 instrument (Applied Separation Inc., Allentown, PA, USA), equipped with a pump (Module 7100) and 10 mL thick-walled stainless cylindrical extractor vessel. The extraction of onion phytochemicals was executed using the following parameters: temperature at 60°C, pressure at 150 bar, extraction time of 60 min.

Total flavonoid analysis. A colorimetric method using aluminum chloride was used to determine the total flavonoid content in the five varieties. Briefly 0.5 mL of the onion extracts were mixed with 0.1ml of 10% aluminum chloride and 0.1mL of 1M potassium acetate. This mixture was diluted with 2.8mL of distilled water. Samples were incubated at room temperature in the dark for 30 minutes. Absorbance was measured at 415 nm using a spectrophotometer (xMark Microplate Absorbance Spectrophotometer, Bio-Rad Laboratories Inc., Hercules, CA). The total flavonoid content was determined as quercetin equivalent per gram of onion. Experiments were done in triplicates.

Culture of Caco-2 Cells. Caco-2 cells were grown in MEM media supplemented with 10% heat inactivated FBS, 4 mM L-glutamine, 1% non-essential amino acids, 100 IU/mL penicillin and 100 µg/mL streptomycin. Glucose supplementation was increased to 4 g/L. Caco-2 cells were maintained in 75-cm^2 culture flasks in a humidified atmosphere at 37°C and 5% CO_2. Media was changed 3 times per week. Cells were passaged at approximately 70-80% confluency. Cells were harvested by trypsinization using TrypLE Express (12 min) and then centrifuged at 200 g for 5 minutes. Caco-2 cells at passage 28 – 35 were used for experiments.

Measurement of Inhibition of Cellular Proliferation of Caco-2 Cells. The antiproliferative efficacy of five onion extracts was assessed by measuring the inhibition of Caco-2 proliferation. Antiproliferative activity was determined by the MTS colorimetric assay. Caco-2 cells were seeded in a 96-well flat-bottom plate at a density of 1.5×10^4 cells per well. After a 24-hour incubation period to allow the cells to attach to the plate, the growth media was removed. Media containing various concentrations of onion extracts were added to the cells. Wells containing 100 mM of quercetin, myricetin, and kaempferol dissolved in DMSO and diluted in growth media were used as a baseline to assess the efficacy of the onion extracts. The final concentration of DMSO in the positive control wells was 0.5%. Cell proliferation was determined by the ability of viable cells to reduce 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfenyl)-2H-tetrazolium (MTS) to formazan at 72 hours. The MTS absorbance was read at 490 nm for each treatment and compared to the untreated control wells using three replicates for each sample after a 3-hour incubation period.

Lactate Dehydrogenase (LDH) Cytotoxicity Assay. Changes in the integrity of Caco-2 cell membranes were assayed after exposure to the onion extracts and positive control flavonoids. Leakage of LDH in the supernatant due to loss of cell membrane integrity was measured to assess cell death. Caco-2 cells were seeded in a 96-well flat-bottom plate at a density of 1.5×10^4 cells per well and left for 24 hours to allow for cell attachment. Old growth media was changed with growth media containing the different onion extracts as described before. LDH activity was assessed 72 hours post treatment. Briefly, cells were centrifuged at 600 g for 10 minutes to precipitate the cells. 10 µL of the supernatant was transferred to a clear 96 well microplate. 100 µL of the LDH reaction mix containing a water soluble tetrazolium (WST) salt dye was added to the supernatant samples. The absorbance was read at 450 nm after 30 minutes of incubation.

Effects of Onion Extract Dosing on Caspase-3/7 Activity. The activation of executioner caspases is considered as one of the many essential steps in a series of highly organized, complex, and orchestrated cascade of events leading to the degradation of cellular components and eventually cell death. Fluorescent intensity of Caspase-3/7 activity in Caco-2 cells exposed to different concentrations of onion extracts was measured using CellEvent™ Caspase-3/7 Green Detection Agent. Cells were seeded at a concentration of 1.5×10^4 cells per well and left for 48 hours. Growth media was removed and replaced with growth media containing onion extracts as previously described. After a 24-hour incubation period, the media was replaced with 100 µL of phenol free RPMI:F12 (1:1 ratio) media containing 5% FBS, 2 mM l-glutamine, and the caspase detection reagent at a final concentration of 7.5 µM. Cells were incubated for 30 minutes at normal cell culture conditions. Fluorescent readings and imaging was carried out using a Cytation 5 multimode plate reader (Biotek, Winooski, VT).

RESULTS

Total flavonoid content of extracted onion solutions. Analysis of the five onion varieties showed varietal differences in total flavonoid content. Of the five varieties, Stanley a yellow onion, and Ruby Ring a red onion, contained the highest total flavonoid content at 0.34 ± .04 and 0.3 ± 0.03 mg of quercetin equivalent/g of onion, respectively. This translated to approximately a 2-3-fold higher concentration than Safrane, Fortress, and Lasalle all of which are yellow onions. This highlights the diversity in phytonutrient content among onions even for those of the same color. Figure 1 shows the total flavonoid content of the pressurized water extractions obtained from the five onion varieties. The results from the analysis of flavonoid content of extract onion solutions are also being used in another study (Manohar *et al.,* 2016) for antioxidant properties assessment.

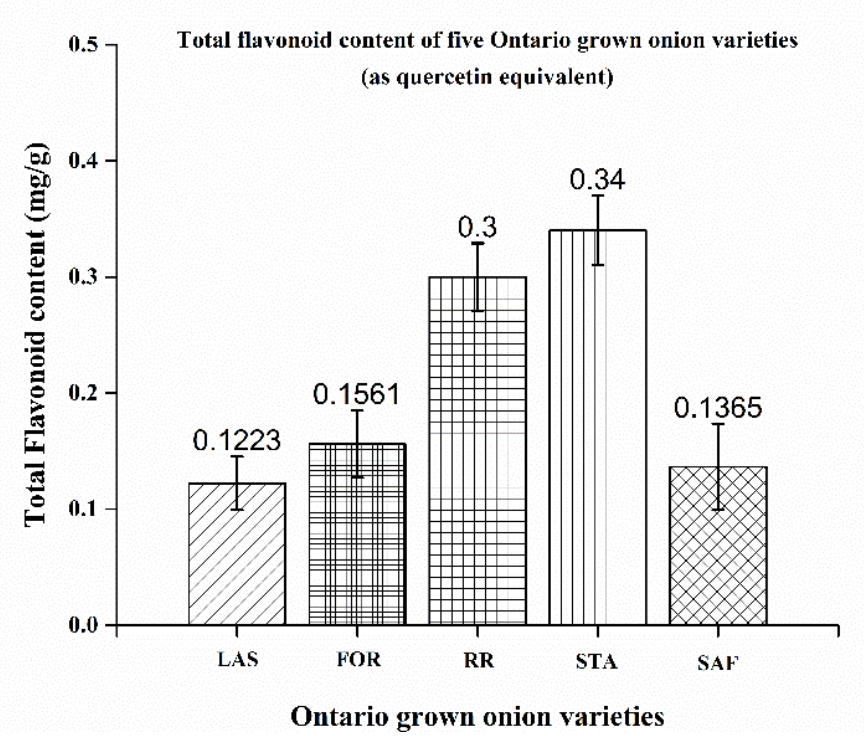

Figure 1: Total flavonoid content of Ontario grown onion varieties. Total flavonoid content is reported as miligram equivalents of quercetin per gram of onion. **LAS**: Lasalle; **FOR**: Fortress; **RR**: Ruby Ring; **STA**: Stanley; **SAF**: Safrane. RR and STA approximately have a 2 to 3 fold higher concentration of flavonoid content compared to LAS, FOR, and SAF.

Inhibition of Human cancer proliferation. The antiproliferative capacity of the onion extracts was evaluated by measuring the inhibition of Caco-2 proliferation. The concentrated phytochemical extracts were supplemented to Caco-2 cells at three final dilution factors: 10, 50, and 100. Table 1 summarizes the final flavonoid concentrations for each variety used in each of the dilution factor treatments. Figure 2 demonstrates the antiproliferative activity of the phytochemical extracts at an extract dilution factor of 10. No appreciable differences in Caco-2 proliferation were observed at higher dilution factors, 50 and 100. Phytochemical treatments were not physiologically relevant at these concentrations as cellular growth was similar to the untreated control wells. Jeong *et al.* (2009) reported that most antitumor studies using quercetin, the major flavonoid found in onions, used high concentrations (25-200 µM) of the pure flavonoid. However, pharmacokinetic studies of quercetin bioavailability in humans revealed a peak concentration of only 10 µM after supplementation with onion extracts containing 68±13 mg quercetin equivalents (Holland *et al., 1997)*. Furthermore, onion extracts are a heterogeneous mixture of various flavonoid and non-flavonoid phytochemicals; they are not a pure mixture of any one component. Consequently, the extracts contain an array of other flavonoid compounds that may or may not exhibit any antiproliferative activity. Table 1 reports total flavonoid content as quercetin equivalents and not the concentration of quercetin alone. Hence, treatments at dilution factors of 50 and 100 are at too low of a concentration to exert any effective antitumor activity. DMSO supplemented at 0.5% (v/v) did not negatively impact proliferation of Caco-2 cells. However, treatment of Caco-2 cells at total flavonoid concentrations ranging from 0.012 and 0.034 mg/g (dependent on onion variety) yielded the most promising results. The antiproliferative efficacies of all varieties at these treatment concentrations (dilution factor of 10) were comparable to the pure flavonoid extracts (quercetin, myricetin, and kaempferol) supplemented at 100 µM. Furthermore, a five-fold decrease in the absorbance of the treated onion extract samples was observed compared to the untreated control wells indicating strong antiproliferation activity of Caco-2 cells.

Table 1: The final flavonoid concentrations used against Caco-2 for each onion variety at 10, 50, 100 times dilution. Total flavonoid units are reported as mg of quercetin equivalent per gram of onion.

Final Dilution Factor	***Total Flavonoid Content (mg/g)***				
	LAS (Lasalle)	FOR (Fortress)	RR (Ruby Ring)	STA (Stanley)	SAF (Safrane)
10	0.01223	0.0156	0.03	0.034	.01365
50	0.002446	0.00312	0.006	0.0068	.002730
100	0.001223	0.00156	0.003	0.0034	0.001365

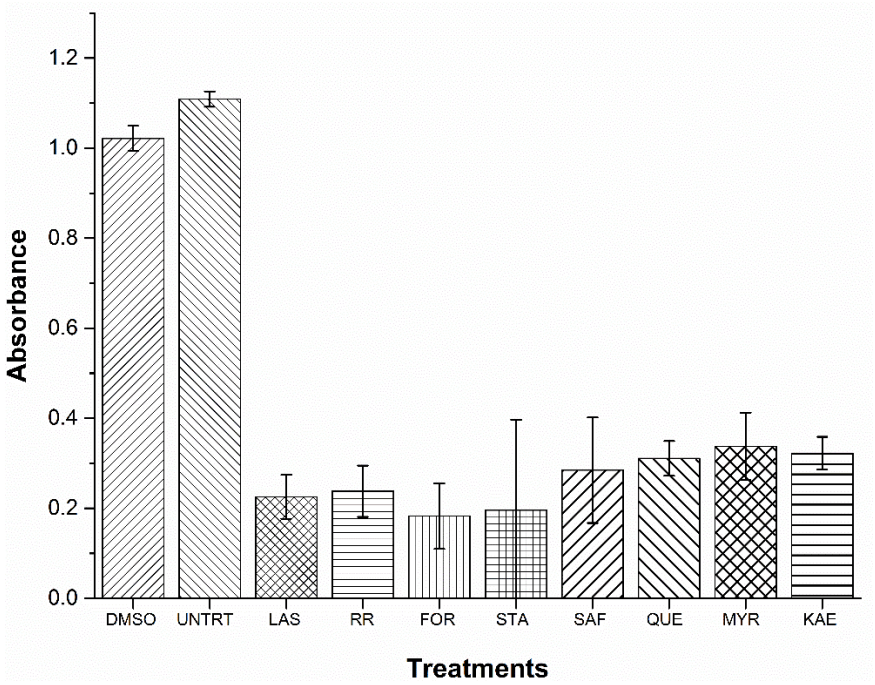

Figure 2: Inhibition of Caco-2 cells treated with onion extracts at a final dilution factor of 10 (DMSO:0.5% v/v; UNTRT: Untreated; LAS: Lasalle, 0.01223 mg/g; RR: Ruby Ring, 0.03 mg/g; FOR: Fortress, 0.0156 mg/g; STA: Stanley, .034 mg/g; SAF: Safrane 0.01356 mg/g; QUE: Quercetin, 100 µM; MYR: Myricetin, 100 µM; KAE: Kaempferol, 100 µM). Caco-2 growth was dramatically inhibited when compared to the untreated wells. A five-fold decrease in absorbance was observed compared the untreated control wells indicating a strong antiproliferation activity of the onion extracts on Caco-2 growth.

Cytotoxic evaluation of onion extract dosing on Caco-2 cancer cells. Lactate dehydrogenase (LDH) is an enzyme usually present in the cytosol of the cell. Detection of LDH in the supernatant is an indication of loss of membrane integrity and cellular death. The efficacy of onion extracts in killing Caco-2 cancer cells was assessed. This was done to determine whether or not the phytochemicals extracted have the capacity for inducing Caco-2 death and not just slowing down their growth. Similar to the cellular proliferation measurements, LDH indications of cytotoxicity were not affected at dilution factors 50 and 100. Treatment at the highest tested dose, showed a four-fold increase in LDH measurements for both Stanley and Fortress when compared to the

untreated control wells. Meanwhile, a three-fold increase in LDH measurements was observed for the remaining varieties. The observed results indicated the capacity of the onion extracts to induce cytotoxicity and are shown in figure 3.

Activation assessment of executioner caspases in live cells. Apoptosis is an organized series of biochemical events by which cells undergo cell death. The process is characterized by several morphological changes in the dying cell. One of the key events that occur during apoptosis is the activation of executioner caspase such as caspase 3 and 7. These are a family of proteases that play an essential role in the apoptosis cascade. Assessment of caspases 3/7 allow insight into the intracellular changes that occur during treatment with compounds of interest. This assessment is important because it allows an estimation of the cells that are alive but are undergoing the process of cell death. A significant increase in the number of apoptotic cells compared to the untreated control wells was observed. There was approximately a 3.5 – 4-fold increase in the number of cells undergoing apoptosis in the wells treated with the onion extracts. Wells treated with the phytochemical extracts from the Stanley variety showed a slightly higher number of apoptotic cells compared to the other extracts. It is interesting to note that kaempferol was the most potent of pure extract treatments. Treatment with kaempferol appears to induce apoptosis more readily compared to the other positive control treatments as the number of apoptotic cells is many folds higher. The results are clearly shown in figure 4.

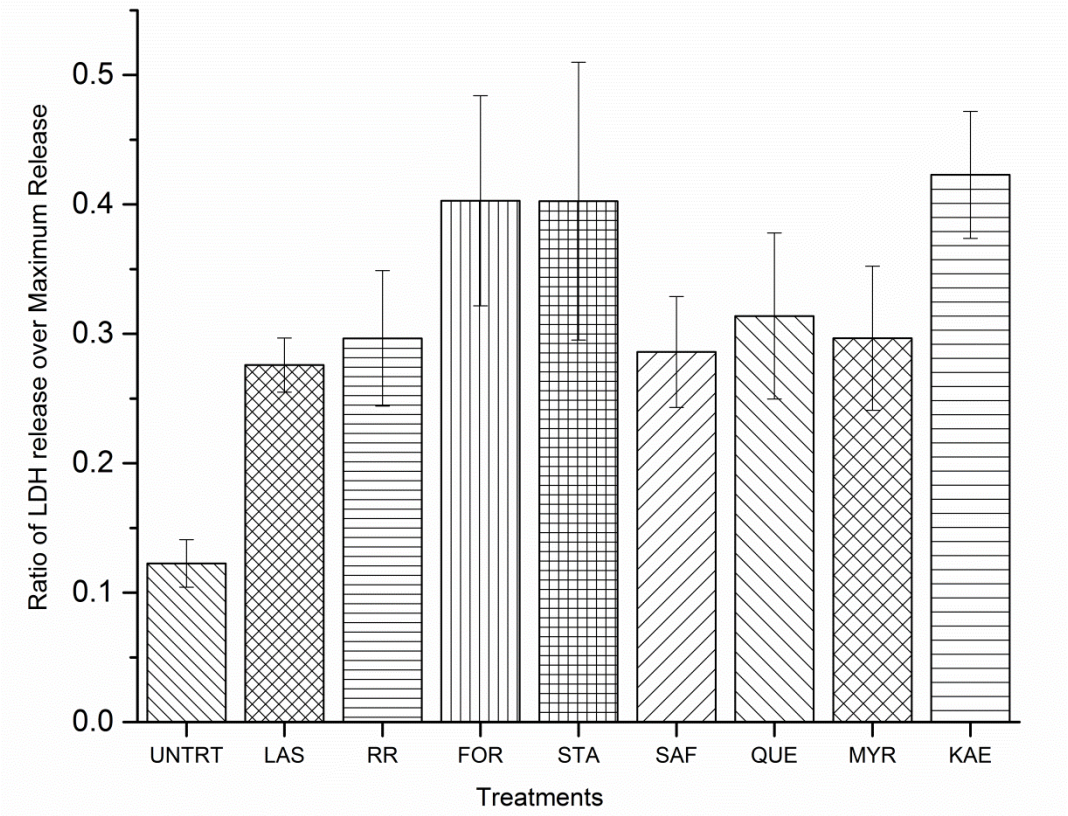

Figure 3: A 2.5 – 4-fold increase in LDH release was observed for the 10 times dilution factor treatment. Wells treated with extracts of Stanley and Fortress exhibited the highest levels of LDH release and are comparable to levels detected in quercetin and kaempferol treated wells. DMSO:0.5% v/v; UNTRT: Untreated; LAS: Lasalle; RR: Ruby Ring; FOR: Fortress; STA: Stanley; SAF: Safrane; QUE: Quercetin; MYR: Myricetin; KAE: Kaempferol

DISCUSSION

Our work has clearly demonstrated that phytochemicals present in Ontario grown onions have antiproliferative activities against Caco-2 cells. In assessing the different varieties, there were no appreciable differences in antigrowth activities found. All five tested varieties showed a five-fold decrease in cellular proliferation when compared to the untreated control wells. However, only one of the tested concentrations was physiologically relevant in inhibiting Caco-2 growth. In our study, a minimum treatment concentration of total flavonoid content at 0.01 mg/g of onion was needed to arrest cell growth. Inhibition of cellular proliferation was similar for all onion extract treatments even though total flavonoid content for the extracts differed. Other phytochemicals have yet to be characterized and may explain this observed discrepancy. These phytochemicals may also exert antitumor activity and the observed inhibition of Caco-2 growth is due to the collective action of both flavonoids and the other phytochemicals present in the onion extracts. It may also be that the differences in flavonoid concentrations among the varieties are not large enough to exhibit an observable increase in antiproliferation activity.

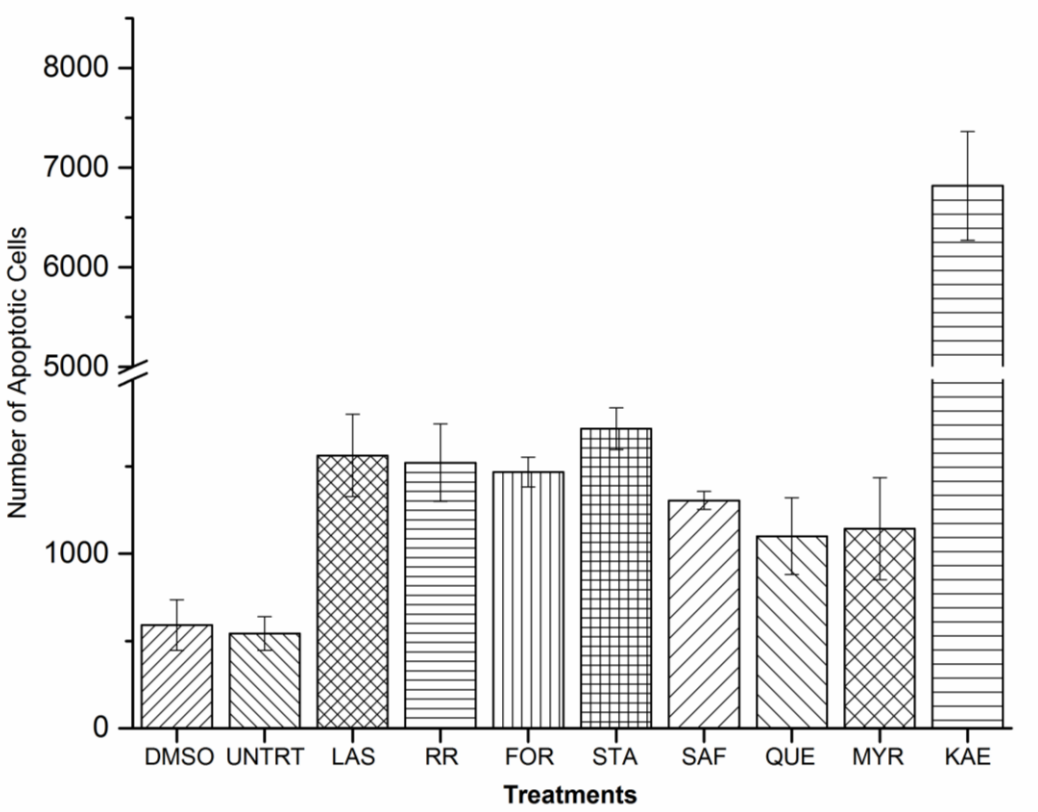

Figure 4: The number of cells undergoing programmed cell death in the 10 times dilution factor treatment showed a three-fold increase in the number of cells undergoing apoptosis compared to the untreated control wells. DMSO: 0.5% v/v; UNTRT: Untreated; LAS: Lasalle; RR: Ruby Ring; FOR: Fortress; STA: Stanley; SAF: Safrane; QUE: Quercetin; MYR: Myricetin; KAE: Kaempferol

Assessment of LDH release into the supernatant is in agreement with the proliferation assay. Only the 10-fold dilution treatment was physiologically relevant to induce cell death. As previously mentioned, detection of LDH in the supernatant is an indicator of cell death and a measure of the extracts' efficacy in inducing cell death. While no differences were observed among the extracts for inhibition of cellular proliferation, two varieties Fortress and Stanley, on average showed a higher presence of LDH in the supernatant of their respective wells. Lasalle, Ruby Ring, and Safrane on

average had lower but similar LDH levels detected in their supernatant samples. Total flavonoid content alone cannot explain why the Fortress variety had a comparable LDH concentration to the Stanley variety. Additionally, it cannot explain why Ruby Ring had a similar LDH concentration to the two varieties with the lowest total flavonoid concentrations. Nonetheless, all five varieties demonstrated their ability to induce cellular death on a level that is comparable to the pure flavonoid extracts. The ability to induce to cell death is important as this demonstrates the extracts' ability to not only slow down the growth of cancerous cells but also kill them. The results from both the proliferation and cytotoxicity assays show the extracts' potential in preventing further proliferation of cancer cells as well as inducing their death.

Measurements of cells undergoing caspase 3/7 activation are an important indicator of the extracts' ability to induce apoptosis in cancer cells. A hallmark of cancer cells is their ability to evade apoptosis. By triggering apoptosis in abnormally dividing cells, cancer growth and metastasis can be put in check. The induction of apoptosis in Caco-2 cells was studied for the various onion extracts at different supplementation concentrations. The fluorescence of cells undergoing programmed cell death was analyzed and the observed trends are in agreement with the previous assays. Supplementation at the highest tested concentration showed approximately a three-fold increase in the number of apoptotic cells compared to the untreated control. Wells treated with the Stanley variety had a slightly higher number of apoptotic cells than wells treated with the other extracts which is consistent with LDH levels observed in the cytotoxicity assay. Lasalle, Ruby Ring, and Fortress had a similar number of apoptotic cells even though wells treated with Fortress had a higher LDH concentration present in their supernatant than the other two varieties. This may indicate that the kinetics of cell death in Fortress treated wells are faster than the other two varieties or that the mechanism for inducing apoptosis is different for the varieties. The differences in total flavonoid content do not correlate well with the differences observed in the induction of apoptosis, LDH release, nor cellular proliferation. As previously mentioned, the presence and bioactivity of other phytochemicals present in the onion extracts can also exert antiproliferative and cytotoxic activities in Caco-2 cells. Xiao *et al.* (2005) demonstrated both antiproliferative and proapoptotic activity from organosulfur compounds. The authors found that mitotic arrest of the cancer cells was due to the disruption of microtubule assembly, followed by loss of mitochondrial membrane potential, and the induction of apoptosis. Additionally, review of anticancer studies using organosulfur compounds by Herman-antosiewicz *et al.* (2007) found that cycle arrest mainly occurred in the G2/M phase of cell cycle and that induction of apoptosis was mediated by the intrinsic pathway (mitochondrial-mediated pathway) of apoptosis. The anticancer activity of phytosterols was also reported. Similar to organosulfur compounds, phytosterols have been reported to induce cell cycle arrest at the G2/M transition point of the cell cycle and have demonstrated potent apoptotic activity in different human cancer cell lines (Bradford & Awad, 2007).

Possible mechanisms on the mode of action of flavonoids responsible for cell growth arrest and apoptosis have also been reported. Ren *et al.* (2015) have demonstrated cell cycle arrest in ovarian cancer cells following flavonoid treatment. The authors observed a shift in the distribution of cells in the cell cycle with a marked increase in the number of cells in the G_0/G1 phase of the cell cycle. Linsalta *et al.* (2010) reported that treatment of a colon cancer cell line with flavonoids affected polyamine biosynthesis, consequently slowing down polyamine metabolism, and the rate of cell proliferation. However, Wenzel *et al.* (2000) have identified changes in mRNA levels of cell cycle and apoptosis related genes in a different human colon cancer cell line. The precise mechanism of different phytochemicals has yet to be elucidated as their mode of action is likely dependent on their chemical structure, bioavailability in the body, and cancer types they come into contact with.

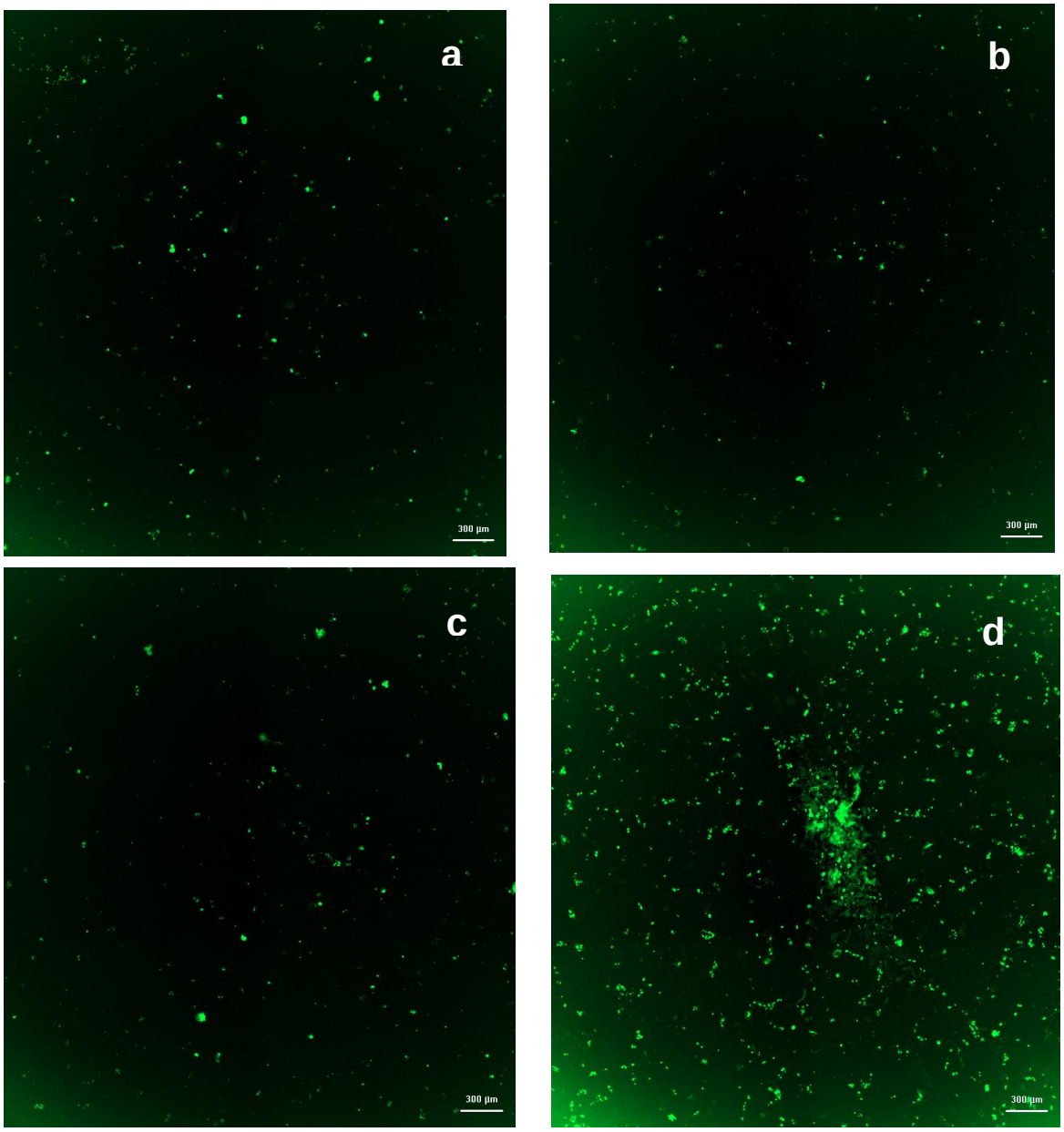

Figure 5: Fluorescent images of Caco-2 cells under different treatment conditions: a) control b) 100 dilution factor, c) 50 dilution factor, and d) 10 dilution factor. Fluorescence indicates cells undergoing apoptosis.

CONCLUSIONS

The present study has investigated the efficacy of five Ontario grown onions in suppressing colon cancer proliferation and apoptosis induction. Extracts supplemented at a total flavonoid concentration of 0.01 mg of quercetin equivalent/g of onion were successful in arresting cell growth and inducing apoptosis. Total flavonoid content did not correlate well with the observed trends suggesting the presence of other bioactive phytonutrients. All five tested varieties demonstrated similar antiproliferative and apoptotic activity.

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REFERENCES

Bradford, P. G., & Awad, A. B. (2007). Phytosterols as anticancer compounds. *Mol. Nutr. Food Res.,* 51, *1*61–170.

Caridi, D., Trenerry, V. C., Rochfort, S., Duong, S., Laugher, D., & Jones, R. (2007). Profiling and quantifying quercetin glucosides in onion (Allium cepa L.) varieties using capillary zone electrophoresis and high performance liquid chromatography. Food Chemistry, 105(2), 691–699.

Guercio, V., Turati, F., La Vecchia, C., Galeone, C., & Tavani, A. (2016). Allium vegetables and upper aerodigestive tract cancers: a meta-analysis of observational studies. *Molecular Nutrition & Food Research,* 60(1), 212–222.

He, Y., Jin, H., Gong, W., Zhang, C., & Zhou, A. (2014). Effect of onion flavonoids on colorectal cancer with hyperlipidemia: An in vivo study. *OncoTargets and Therapy*, 7, 101–110.

Herman-antosiewicz, A., Powolny, A. A., & Singh, S.V. (2007). Molecular targets of cancer chemoprevention by garlic-derived organosulfides. *Acta Pharmaco Sin.,* 28(9), 1355–1364.

Hui, C., Qi, X., Qianyong, Z., Xiaoli, P., Jundong, Z., & Mantian, M. (2013). Flavonoids, Flavonoid Subclasses and Breast Cancer Risk: A Meta-Analysis of Epidemiologic Studies*. PLoS ONE*, 8(1).

Jeong JH, An LY, Kwon YT, Rhee JG, Lee YJ. (2009). Effects of low dose quercetin: cancer cell-specific inhibition of cell cycle progression. *J. Cell Biochem*., 106(1), 73–82.

Leopoldini, M., Russo, N., Chiodo, S., Toscano, M. (2006). Iron Chelation by the Powerful Antioxidant Flavonoid Quercetin. *J. Agric. Food Chem., 54, 6343-6351.*

Linsalata, M., Orlando, A., Messa, C., Refolo, M. G., & Russo, F. (2010). Quercetin Inhibits Human DLD-1 Colon Cancer Cell Growth and Polyamine Biosynthesis. *Anticancer Res*,30(9), 3508, 3501–3507.

Liu, Y., Zhan, J., Liu, X., Wang, Y., Ji, J., & He, Q. (2014). Dietary fl avonoids intake and risk of type 2 diabetes : A meta-analysis of prospective cohort studies. *Clinical Nutrition*, 33(1), 59–63.

Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jime, L. (2004). Polyphenols: food sources and bioavailability. *Am J Clin. Nutr.,* 79(5), 727-747.

Manohar, C., Xue, J., Murayyan, A., Neethirajan, S., Shi, J. (2016). Screening Ontario Grown Onion Varieties for Antioxidant Properties. *Proceedings of the 2016 CSBE Annual Meeting and Technical Conference*.

Peng, I.W. & Kuo, S.M. (2003). Flavonoid Structure Affects the Inhibition of Lipid Peroxidation in Caco-2 Intestinal Cells at Physiological Concentrations. *J Nutr.,*133(7), 2184–2187.

Reilly, J. D. O., Mallet, A. I., Mcanlis, G. T., Young, I. S., Halliwell, B., Sanders, T. A. B., & Wiseman, H. (2001). Consumption of flavonoids in onions and black tea : lack of effect on F 2 - isoprostanes and autoantibodies to oxidized LDL in healthy, *Am. J. Clin. Nutr*., 73(6), 1040–1044.

Ren, M. X., Deng, X., Ai, F., Yuan, G., & Song, H. (2015). Effect of quercetin on the proliferation of the human ovarian cancer cell line SKOV-3 in vitro, *Exp. Ther. Med*. 10(2), 579–583.

Schreiner, M. and Huyskens-Keil, S. (2006) Phytochemicals in fruit and vegetables: health promotion and postharvest elicitors. *Crit. Rev. Plant Sci*. 25, 267-278.

Sellappan, S., & Akoh, C. C. (2002). Flavonoids and antioxidant capacity of Georgia-grown Vidalia onions*. J of Agric and Food Chem*., 50(19), 5338–5342.

Slimestad, R., Fossen, T., Vågen, I.M. Onions: A source of unique dietary flavonoids. *J of Agric and Food Chem., 55(25),10067-10080.*

Suleria, H. A. R., Butt, M. S., Anjum, F. M., Saeed, F., & Khalid, N. (2013). Onion: Nature Protection Against Physiological Threats. *Critical Reviews in Food Science and Nutrition*, 55, 50-66.

Wenzel, U., Kuntz, S., Brendel, M. D., & Daniel, H. (2000). Dietary Flavone Is a Potent Apoptosis Inducer in Human Colon Carcinoma Cells. *Cancer Res.,* (60)14, 3823–3831.

Xiao, D., Pinto, J. T., Gundersen, G. G., & Weinstein, I. B. (2005). Effects of a series of organosulfur compounds on mitotic arrest and induction of apoptosis in colon cancer cells. *Mol Cancer Ther.,* 4(9) 1388–1398.

Yamazaki, S., Miyoshi, N., Kawabata, K., Yasuda, M., & Shimoi, K. (2014). Quercetin-3-O-glucuronide inhibits noradrenaline-promoted invasion of MDA-MB-231 human breast cancer cells by blocking β2-adrenergic signaling. *Archives of Biochemistry and Biophysics*, 557, 18–27.

Yang, J., Meyers, K., J., Heide, V., D., Liu, R.H. (2004). Varietal Differences in Phenolic Content and Antioxidant and Antiproliferative Activities of Onions. *J of Agric. and Food Chem*.,52, 6787-6793.