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Full Length Research Paper

Antioxidant and anti-proliferative activities of Roselle juice on Caov-3, MCF-7, MDA-MB-231 and HeLa cancer cell lines

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Roselle (*Hibiscus sabdariffa* Linn) extract has been scientifically proven to possess high antioxidant activity, anti-proliferation and anti-carcinogenic properties. This study was conducted to evaluate the antioxidative capacity of commercialized Roselle juice (RJ) at three storage periods and its anti-proliferative effect on breast (MCF-7 and MDA-MB-231), ovarian (Caov-3) and cervical (HeLa) cancer cell lines. The antioxidant activity of 1 week (WRJ), 1 month (MRJ) and 1 year (YRJ) juice samples each at 0.001 to 10% concentration range were determined through 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay with L-ascorbic acid as positive control. EC_{50} values of WRJ, MRJ, and YRJ were found to be 3.733±0.247, 3.717±0.637 and 3.383±0.711%, respectively. These values were compared to 0.217±0.616% for positive control. The difference in antioxidant activity between different storage periods of RJ was not significant ($p>0.05$) but all samples exhibited increasing activity with increasing concentrations. RJ at the same concentrations were tested using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay on the four cell lines to obtain the percentage viability of the cells. The cells were incubated for 72 h after inoculation with RJ and the control group was without treatment. The IC_{50} was found to be highest for Caov-3 cells (2.267±1.193%) whereas MCF-7 cells exhibited the lowest (0.432±0.278%) IC_{50} value after treatment with MRJ. All determinations were analyzed using ANOVA through SPSS with $p<0.05$ considered as significant. Increasing concentrations of sample corresponded to lower percentage viability of cells for all samples, however the interaction within and between cell type and storage period was not significant ($p>0.05$). The study showed that commercialized Roselle juice has strong antioxidant capacity and anti-proliferative activity on the four cancer cell lines despite different storage periods. However, further study should be conducted to establish its anti-cancer mechanisms.

Key words: *Hibiscus sabdariffa*, antioxidant activity, anti-proliferation properties, anti-carcinogenic properties, breast cancer cell line, cervical cancer cell line, ovarian cancer cell line.

INTRODUCTION

Since ancient times, plant-based traditional medicine has played a major role in the therapy of a spectrum of diseases. The use of herbal extracts and nutritional supplements either as alternative or complementary medicine for treatment of cancer is well documented in various cultures such as Ayurveda in India (Dahanukar et al., 2000), and in Chinese Traditional Medicine (TCM) system (Chiu et al., 2003). According to the World Health Organization, approximately 80% of the world's

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inhabitants rely mainly on traditional medicines for their primary health care (Farnsworth et al., 1985).

Cancer is a much-feared disease in the modern society, and is one of the leading causes of mortality worldwide. It is a disease of genes; a disorder which occurs in the normal processes of cell division. Various carcinogens such as viruses, chemical carcinogens, and radiation cause aberration of the genetic material (DNA) of cells and ultimately the uncontrolled autonomous cell proliferation that defines cancer. The National Cancer Registry of Malaysia (NCR, 2004) reports a total of 21,464 cancer cases diagnosed in Peninsular Malaysia alone.

Many human cancers are caused by exposure to food mutagens and carcinogens. Although complete elimination of daily exposure to dietary carcinogens may not always be possible, chemoprevention is an alternative approach to decreasing the carcinogenic effect. Natural chemopreventive agents have exhibited inhibitory effects on the initiation, promotion, and progression stages in carcinogenesis (Wattenberg, 1985) and these agents are present in our diet (Wattenberg, 1992). With up to 60% of all cancers being related to dietary factors, diet and nutrition are perhaps the most important aspects of any cancer prevention program.

Strong epidemiological evidence suggests that a diet rich in vegetables and fruits can notably reduce the risk of diverse human cancers (Block et al., 1992). The presence of vitamins and provitamins, such as ascorbic acid, tocopherols and carotenoids, and phenolic substances in fruits and vegetables is well-established (Loliger, 1991). The principle role of antioxidants is in delaying the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions by free radicals, and thus reduces oxidative damage to the human body (Namiki, 1990). Antioxidants therefore inhibit the process of carcinogenesis to a certain level (Kim et al., 1994).

Hibiscus sabdariffa Linn (Roselle) is an annual shrub commonly used to make jellies, jams and beverages. The brilliant red colour of its calyx makes it a valuable food product, apart from its multitude of traditional medicinal uses. Infusions of the leaves or calyces are regarded as diuretic, cholerectic, febrifugal and hypotensive, decreasing the viscosity of the blood and stimulating intestinal peristalsis (Morton, 1987). Roselle calyx extract is a good source of antioxidants from its anthocyanins (Ajiboye et al., 2011) and is associated with antitumour and inhibitory effects on the growth of several cancer cells (Kamei et al., 1995; Meiers et al., 2001; Nagase et al., 1998; Tsai et al., 2002). Recent study has revealed the ability of *H. sabdariffa* tea to lower blood pressure in prehypertensive and mildly hypertensive adults (McKay et al., 2010) while its 80% methanol extract was effective in inhibiting *Escherichia coli* O157:H7 isolated from food, veterinary and clinical samples. Ajiboye et al. (2011) reported on the antioxidant activity of *H. sabdariffa* anthocyanin extract as a result of its ability to effectively scavenge α-diphenyl-β-picrylhydrazyl (DPPH) radical, superoxide ion, and hydrogen peroxide, and to completely attenuated the CCl_4-mediated decrease in antioxidant enzymes. The extract of *H. sabdariffa* have also been reported to exert hepatoprotective and antioxidant activities when assessed against the CCl_4^- induced hepatocyte damage in fish (*Cyprinus carpio*) (Yin et al., 2011). Ramirez-Rodrigues et al. (2011) have reported on the phytochemical content of *H. sabdariffa* cold (25°C) and hot (90°C) water extracts prepared in various time-temperature combinations. The authors reported that equivalent anthocyanins concentration was obtained at 25°C for 240 min and 90°C for 16 min with better total phenolics extracted using hot water. The hot water extract also exerted high antioxidant capacity compared to the cold water extract. However, similar polyphenolic profiles were observed between fresh and dried hibiscus extracts. According to them, cold and hot extractions yielded similar phytochemical properties and among the bioactive compounds identified include hibiscus acid, hydroxybenzoic acids, caffeoylquinic acids, flavonols, anthocyanins, delphynidin-3-sambubioside and cyanidin-3-sambubioside. Recent findings indicated that Hibiscus sabdariffa was effective at all levels in inhibiting E. coli O157:H7; thus it possesses antimicrobial activity (Fullerton et al, 2011). This study was undertaken to examine the antioxidant activity of a commercial Roselle juice stored for one week, one month and one year, and to determine its anti-proliferative activity on human breast cancer cell lines (MCF-7 and MDA-MB-231), cervical cancer cell line (HeLa) and ovarian cancer cell line (Caov-3).

MATERIALS AND METHODS

Roselle juice

Roselle juice samples of three different stored periods (one week, one month, and one year) were provided by Monrose Sdn. Bhd., Bangi, Selangor. The juice was stored in a refrigerator at 4°C. The juice was prepared by adding distilled water to the pressed Roselle flowers in ratio of 30:70 (v/v) with no preservative added to the juice.

Cell lines

The estrogen-receptor positive and estrogen-receptor negative human breast cancer (MCF-7 and MDA-MB-231, respectively), human cervical cancer (HeLa), and human ovarian cancer (Caov-3) cell lines were purchased from American Type Culture Collection (ATCC), Rockville, Maryland, USA.

Cell culture

Cell lines were maintained in suitable media (Caov-3, HeLa and

MCF-7 cell lines in RPMI 1640 culture medium; and MDA-MB-231 in DMEM and RPMI 1640 in a 1:1 ratio), supplemented with 10% heat-inactivated FBS, and 1% antibiotics (100 IU/ml penicillin and 100 µg/ml streptomycin). Cells were grown in 25 cm^3 tissue culture flasks in a humidified atmosphere containing 5% CO_2 and 95% air, at 37°C.

The cells in a flask were plated into a 96-well microculture plate once the cells had reached 70 to 80% confluence at a concentration of 1×10^5 cells/ml. 100 µl of cell suspension of the above final concentration was dispensed into each well of the 96-well plate except for wells that were designated for blank. The 'blank' wells were dispensed with 100 µl of culture medium alone. The plate was then incubated overnight to allow for cell attachment at 37°C and 5% CO_2.

Treatment with Roselle juice

Working solutions of Roselle juice were prepared using a ten-fold serial dilution with the appropriate cell culture medium. For treatment of MCF-7, HeLa and Caov-3 cells, RPMI 1640 supplemented with 10% FBS and 1% penicillin-streptomycin was used to dilute the juice, and for MDA-MB-231 cells, RPMI 1640 and DMEM in a 1:1 ratio was used.

A working concentration of Roselle juice ranging from 0.002, 0.02, 0.2, 2 to 20% was prepared. The solutions were filtered using a 0.2 nm filter and dispensed appropriately into the partitioned 96-well plates previously incubated for cell attachment. An aliquot of 100 µl of working concentration was added into each designated well in replicates of six. As the wells already contained 100 µl of cells in medium, the final concentration range was 0.001 to 10% (the prepared dilution concentrations were halved). The control wells received 100 µl of culture medium, while the blank wells were added with the working concentrations accordingly. The plate was then incubated at 37°C and 5% CO_2 in a humidified atmosphere for 72 h.

MTT cell proliferation assay

The MTT cell viability assay was performed, with slight modifications, according to instructions in the Cell Proliferation Kit 1 (MTT) manual from Boehringer Mannheim (Cat. No. 1465 007). After incubation for 72 h, 10 µl of the MTT labeling reagent (final concentration 0.5 mg/ml) was added into each well except the empty wells. The microtiter plate was then incubated for four hours in a humidified atmosphere, at 37°C and 5% CO_2.

Following that, 100 µl of the solubilization solution was pipetted into each well. The plate was then allowed to stand overnight in the incubator in the same conditions. The plate was checked for complete solubilization of the purple formazan crystals under the inverted phase microscope. If no crystals were detected in the wells, the spectrophotometrical absorbance of the samples was measured using the microtiter plate (ELISA) reader at the wavelength of 550 nm. The reference wavelength was set at 690 nm. The number of viable cells was proportional to the extent of formazan production.

The cell viability was expressed as the optical density ratio of the treatment to control. The percentage of cell viability was calculated with the following formula:

$$\text{Cell viability (\%)} = \left(\frac{OD_{sample} - OD_{blank}}{OD_{control}} \right) \times 100\%$$

In which OD_{sample} represents the optical density (absorbance) of the samples, OD_{blank} as the optical density of the blank (of the respective concentration solutions), and $OD_{control}$ as the optical density of the control wells. A dose-response curve (percentage of cell viability versus sample concentration) was plotted and the sample concentration that inhibits 50% of the cell viability (IC_{50}) was determined from the graph. The procedure was repeated for each sample and cell line.

DPPH· free radical scavenging activity assay

The effect of the three samples on DPPH· radicals was estimated according to the procedure described by Yen and Hsieh (1998). Roselle juice sample solutions were prepared using serial dilution into concentrations of 0.001, 0.01, 0.1, 1 and 10% with absolute ethanol. L-Ascorbic acid (vitamin C) was used as positive control and prepared with ethanol in the same concentration range. The reagent, 0.45 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH·), was prepared in absolute ethanol and 1 ml of the reagent was added into aliquots of 0.5 ml of RJ samples and L-ascorbic acid in 4 ml glass reaction tubes. The mixture in the tubes, with a final volume of 1.5 ml and concentration of 0.3 mM, was shaken vigorously. Following that, the tubes were left to stand in the dark at room temperature for 30 min. A negative control was prepared by adding 0.5 ml absolute ethanol in place of the samples with DPPH·. The optical density of the remaining DPPH· was measured spectrophotometrically at 517 nm. For each sample concentration tested, the percentage of DPPH· remaining at the steady state was calculated as follows:

$$\text{\% Antioxidant activity} = \left(\frac{OD_{negative\ control} - OD_{sample}}{OD_{negative\ control}} \right) \times 100\%$$

where OD_{sample} is the optical density of samples or positive control, and $OD_{control}$ as optical density of negative control.

These values were plotted versus concentration of samples to obtain the amount of antioxidant necessary to decrease the initial DPPH· concentration by 50% (EC_{50}). The kinetics of the antioxidant reaction in the presence of Roselle juice was compared with L-ascorbic acid as an antioxidant reference. The assay was performed in triplicate.

Statistical analysis

All determinations were expressed in mean ± SEM. The free radical scavenging activity values of the samples were analyzed through one-way analysis of variance (ANOVA) with SPSS version 12.0 to determine the difference between samples and control. The cell treatment with RJ was carried out three times at three different days. The MTT assays for each sample were carried out three times. The cell viability and IC_{50} values for different samples and cell lines were analyzed via two-way ANOVA. Significant differences between means were determined by Duncan's multiple-range tests. In all statistical analyses, a probability of $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

DPPH· radical scavenging assay

The scavenging effects of Roselle juice samples on the DPPH· radical were evaluated and found to be strongly

Table 1. EC_{50} values of Roselle juice according to storage time*.

Sample	EC_{50} (%)
1 week	3.733 ± 0.247[a]
1 month	3.717 ± 0.637[a]
1 year	3.383 ± 0.711[a]
Positive control	0.217 ± 0.161[b]

*Values are means of three replicate samples (n = 3). Data is presented as mean ± SEM. Means followed by different letters within a column are significantly different according to DMRT ($p<0.05$).

concentration-dependent, thus the scavenging activity of the juice on inhibition of the DPPH· radical, was proportional to the amount of juice added into the working concentrations. The positive control (L-ascorbic acid) showed a similar trend, but with a significantly higher scavenging activity ($p<0.05$) as compared to the samples. All three samples were not significantly different from each other in terms of antioxidant activity ($p>0.05$). The three samples, nevertheless, exhibited a potent scavenging effect on free radicals.

The EC_{50} value of the samples, defined as the mean concentration of 50% free radical scavenging activity against the 250 µM DPPH· radical, or the amount of juice necessary to decrease the initial DPPH· radical concentration by 50%; were presented in Table 1 to compare the antioxidant activities between samples and control. The EC_{50} of L-ascorbic acid was shown to be the lowest (0.217 ± 0.161%), indicating that the scavenging activity of the control was significantly the strongest in comparison to the samples ($p<0.05$). Although the one-year Roselle juice exhibited the strongest activity among samples, the difference was not significant ($p>0.05$) in terms of radical scavenging activity. The EC_{50} values of all three samples, however, were significantly higher than that of the positive control ($p<0.05$). The antioxidant level of Roselle juice was significant considering that the crude extract in the juice was compared to ascorbic acid, a pure antioxidant compound. Tanaka et al. (1988) noted that the antioxidant properties are concomitant with the development of the reducing power. Thus, the marked antioxidant activities of the Roselle juice may be concerned with their reducing power.

The work conducted by previous investigators supports the findings in this experiment. Roselle calyx extract was found to be approximately 16 to 25% as active in antioxidant capacity as green tea, concluding that Roselle petals are a good source of antioxidants (Tsai et al., 2002). The antioxidant activity of Roselle juice also does not seem to reduce over time, as the radical-scavenging activity for the juice from different storage periods is similar. This finding agrees with Tsai et al. (2002) in that the antioxidant activity of Roselle extract is relatively stable over a storage period, probably due to the contribution from the formation of polymeric phenols throughout the storage duration (Tsai et al., 2002). The antioxidant activity of the juice is therefore not lost in processing; instead, the activity of the extract is retained. The high levels of phenolic compounds in Roselle calyx may also contribute to the antioxidant activity of the extract and juice (Duh and Yen, 1997).

According to Duh and Yen (1997), the Roselle extract is an electron donor and can react with free radicals to convert them into more stable products and terminate radical chain reactions. Besides demonstrating the potent antioxidant activity of the extract, Duh and Yen (1997) revealed that the extract does not only induce mutagenicity, but possibly play an important role as dietary antioxidants after ingestion, in the chemical protection against oxidative damage of cell membranes. Thus, the extract may be a strong chemopreventive agent in carcinogenesis.

Antioxidant activity of the Roselle extract correlated strongly to its anthocyanin content (Tsai et al., 2002; Mizukami, 1993). Anthocyanins (extracted from the dried calyx of *H. sabdariffa*) are natural polyphenolic compounds in the red pigments of Roselle and several other intensely-coloured plants. The *Hibiscus* anthocyanins were found to possess antioxidant bioactivity both *in vivo* and *in vitro* (Tseng et al., 1997; Wang et al., 2000). Du and Francis (1973) identified delphinidin-3-sambubioside (Dp3-Sam) and cyanidin-3-sambubioside as major anthocyanins, and clarified their chemical structure. Delphinidine is found to be the major component in the *H. sabdariffa* L. anthocyanin (Chang et al., 2005). Extraction of the Roselle anthocyanins has been investigated for the application of the pigments as food colouring (Mizukami, 1993). The nutritional benefit of Roselle as an antioxidant will ultimately depend on the bioavailability of the anthocyanins (Tsai et al., 2002).

Apart from the antioxidant activity contributed by the anthocyanin content in Roselle calyx extracts, the extract was also found to be very high in ascorbic acid content (Falade et al., 2005). Ascorbic acid, or ascorbate, is a well-known natural antioxidant and excellent reducing agent (Buettner and Jurkiewicz, 1996). Thus, the high level of ascorbic acid in Roselle juice may also serve to maintain its substantial antioxidant activity. However, whether this nutrient level is maintained in the juice at increased duration of storage period is a subject for further investigation.

MTT cell viability assay

After 72 h of incubation, the survival fraction of cancer cells was investigated by MTT assay. MTT assay is a simple and reliable technique which measures cell

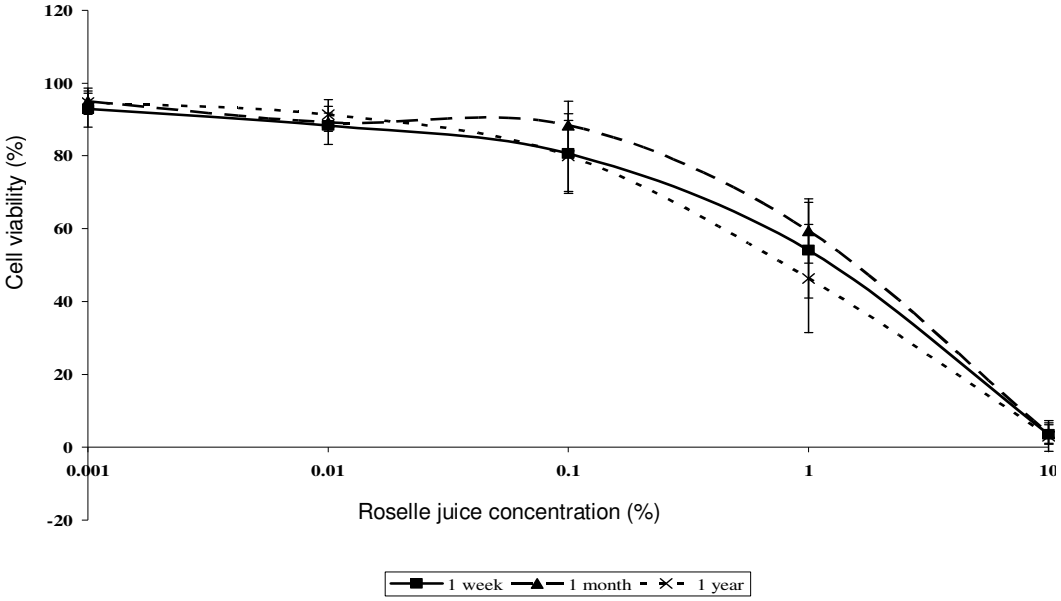

Figure 1. The effect of Roselle juice on the viability of Caov-3 cells after 72 h incubation. Data represent means ± SEM of three replicates.

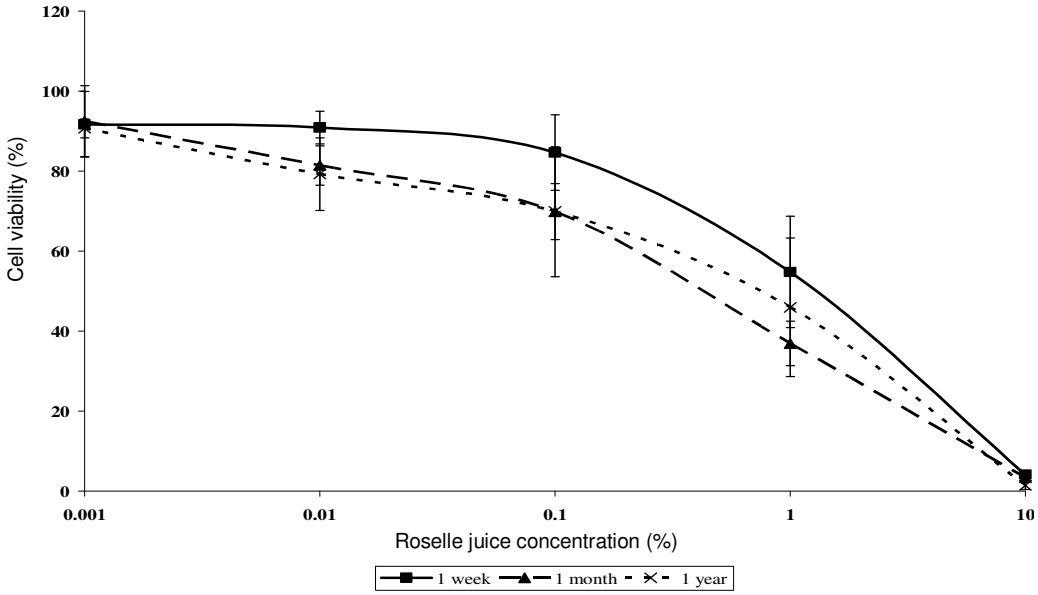

Figure 2. The effect of Roselle juice on the viability of MCF-7 cells after 72 h incubation. Data represent means ± SEM of three replicates.

viability, and can be used for screening of anti-proliferative agents (Manosroi et al., 2005). As inferred from Figures 1 to 4, Roselle juice at different storage periods inhibited cell proliferation in a dose-dependent manner for all cell lines.

Figure 1 shows the cell viability reduction in Caov-3 ovarian cancer cells after treatment with Roselle juice. At increasingly higher concentrations, the one-year Roselle juice indicated a potency superior to that of the other two samples, although the inhibitory activity at more diluted concentrations (0.001, 0.01 and 0.1%) was almost similar for each sample. The proliferation of MCF-7 breast cancer cells, on the other hand, was most strongly inhibited by the one-month juice at higher concentrations in comparison to other samples (Figure 2).

The reduction in viability of MDA-MB-231 breast cancer

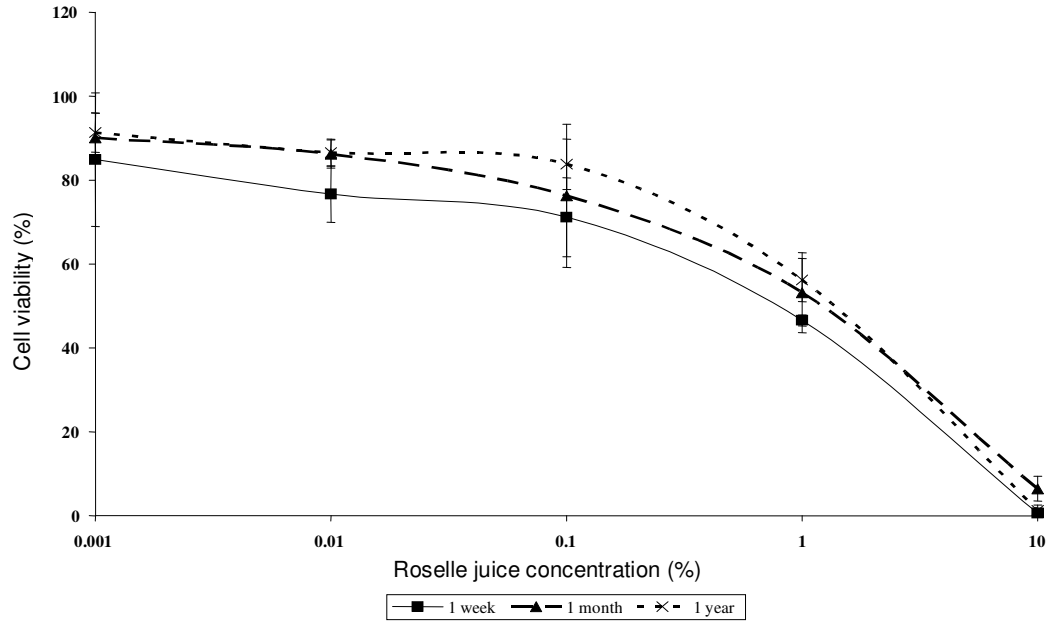

Figure 3. The effect of Roselle juice on the viability of MDA-MB-231 cells after 72 h incubation. Data represent means ± SEM of three replicates.

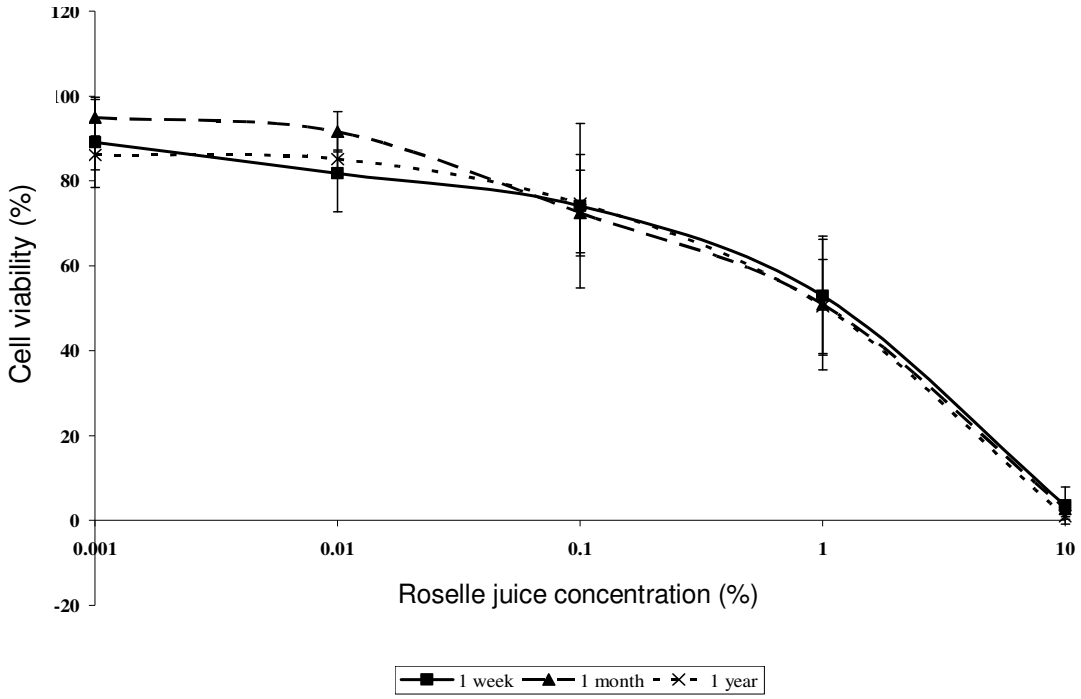

Figure 4. The effect of Roselle juice on the viability of HeLa cells after 72 h incubation. Data represent means ± SEM of three replicates.

cells shown in Figure 3 showed a consistent stronger inhibition by the one-week Roselle juice as compared to the two other samples; however the proliferation of HeLa cervical cancer cells was inhibited in a similar manner by all three juice samples (Figure 4). Table 2 presents the IC_{50} values of Roselle juice samples in the four cancer

Table 2. IC_{50} values (%) of Roselle juice samples on four cancer cell lines[a].

Cell lines	WRJ IC_{50} (%)	MRJ IC_{50} (%)	YRJ IC_{50} (%)
Caov-3	1.800 ± 1.375	2.267 ± 1.193	1.117 ± 0.898
MCF-7	1.803 ± 0.357	0.432 ± 0.278	0.978 ± 0.760
MDA-MB-231	0.675 ± 0.106	1.475 ± 1.237	1.825 ± 0.672
HeLa	1.300 ± 1.212	1.783 ± 1.205	1.283 ± 1.247

[a]Data is presented as mean ± SEM (n = 3). WRJ, 1 week Roselle juice; MRJ, 1 month Roselle juice; YRJ, 1 year Roselle juice. Means are not significantly different (p>0.05).

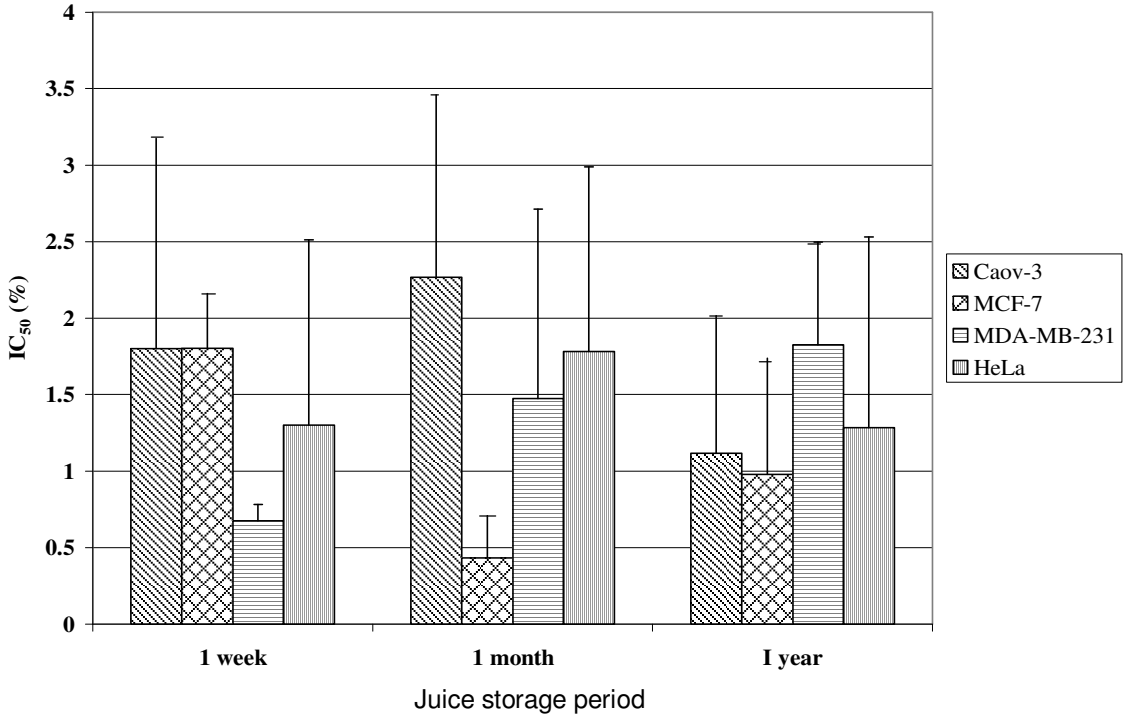

Figure 5. IC_{50} values of various cancer cell lines after 72-hour treatment with Roselle juice. Data is presented as mean ± SEM of three replicate analyses. The values are not statistically significant from each other according to ANOVA and DMRT (p>0.05).

cell lines. IC_{50} is the concentration of sample (juice) that inhibits the viability of cells by 50%, and the values were determined from the cell viability versus concentration graphs. As similarly shown in the previous dose-response curves, the Caov-3 cells were most sensitive to, or highly inhibited by, one-year Roselle juice (IC_{50} 1.117 ± 0.898%), MCF-7 cells by one-month Roselle juice (IC_{50} 0.432 ± 0.278%), MDA-MB-231 cells by the one-week Roselle juice (IC_{50} 0.675 ± 0.106%), and HeLa cells, although not significantly, by the one-year Roselle juice (IC_{50} 1.283 ± 1.247%). However, these differences were not found to be statistically significant (by ANOVA and Duncan Multiple Range Test, p>0.05). Figure 5 illustrates the IC_{50} of the juice on the various cell lines in a graphic presentation. All the IC_{50} values were in the range of 0.432 to 2.267%, indicating a strong anti-proliferation effect of the juice, irrespective of storage periods, on the four cancer cell lines.

It was found that the one-week Roselle juice showed a two- to three-fold selectivity towards MDA-MB-231 cells, whereas the one-month juice was most selective (three- to five-fold selectivity) towards MCF-7 cells. MCF-7 cells were also found to be very sensitive towards the one-year juice, with the sample exhibiting almost two-fold selectivity towards the estrogen-receptor positive cell line compared to the other cancer cells tested. Although the

difference in IC_{50} values between cell lines and samples were not significant ($p>0.05$), it may be deduced that Roselle juice generally exhibited the strongest anti-proliferative potency towards the MCF-7 human breast cancer cells in comparison to Caov-3, HeLa, and MDA-MB-231 cells. It was also found that the storage period of the juice did not decrease the anti-proliferative effect on cell lines.

The anthocyanins in Roselle calyx are found to not only possess antioxidant activity (Pool-Zobel et al., 1999; Tsuda et al., 2000), but also mediate other physiological functions related to cancer suppression (Kamei et al., 1995; Meiers et al., 2001; Nagase et al., 1998). *Hibiscus* anthocyanins (HAs) extracted from the Roselle calyx was found to have a concentration-dependent inhibitory effect on the growth of several cell lines, including MCF-7 cells (Chang et al., 2005). However, the strongest cytotoxicity of HAs was discovered in human leukemia HL-60 cells, and the least potency toward normal cells (NIH3T3 cells).

According to Hou et al. (2005), Dp3-Sam (a HA) inhibited cell proliferation in a dose-dependent manner. This finding concurred with the results in this study (Figures 1 to 4). Thus, it is suggested that HAs in the Roselle juice used in this study produced the pronounced anti-proliferative effect against the various cancer cells. The HAs extracted from Roselle calyces inhibits cell proliferation and possess cytotoxic activity possibly due to the anthocyanin properties of apoptosis-induction (Chang et al., 2005; Hou et al., 2005). The apoptotic mechanism was suggested to be through a ROS-mediated mitochondrial dysfunction pathway (Hou et al., 2005).

The sensitivity of the MCF-7 cell line to the anti-proliferative activity of Roselle juice may be due to several factors. The MCF-7 cell line retains several characteristics of differentiated mammary epithelium including ability to process estradiol through cytoplasmic estrogen receptors and the capability of forming domes. The cells express the WNT7B oncogene (Huguet, 1994) and also contain the Tx-4 oncogene. Growth of MCF-7 cells is inhibited by tumor necrosis factor alpha (TNF alpha). In the study by Chang et al. (2005), HL-60 cells were most sensitive to Roselle anthocyanins. It was suggested that the p53 null HL-60 cells exhibited this selectivity as the expression of p53 may be relevant to the cytotoxicity of Roselle anthocyanins. Therefore it may be deduced that MCF-7 cells may retain this characteristic as well.

Furthermore, Olaleye (2007) reported on the presence of several classes of phytochemical contituents, which are cardiac glycosides, flavonoids, saponins and alkaloids in *H. sabdariffa* aqueous-methanolic extract. In term of the antiproliferative mechanisms involved, several mechanisms could be suggested based on the bioactive compounds presence in each of the extracts. Flavonoids, particularly, have been shown to exert antioxidant activity (Robak and Gryglewski, 1988) and associated with possible role in the prevention of several chronic diseases involving oxidative stress (Lee et al., 2006) including cancer (Middleton et al., 2000). Flavonoids were capable of modulating the cell cycle arrest at the G1/S phase, inhibiting the cell-survival kinase and the inflammatory transcription factors, inducing the cyclin-dependent kinase inhibitors or the Ca^{2+}-dependent apoptotic mechanism, or down-regulating the anti-apoptotic gene products (Agarwal et al., 2006; Sergeev et al., 2006). On the other hand, saponins have been reported to induce apoptosis response on cancer cells by causing permeabilization of the mitochondrial membranes (Lemeshko et al., 2006), to cause necrotic cell death (Russo et al., 2005) or cell cycle disruption by decreasing the number of cells in G0/G1 phase, with initial increases in S and G2/M (Roy et al., 2007) or by inhibiting nuclear factor-kappa B (NF-κB) (Lee et al., 2006).

Overall, the Commercial Roselle juice at different storage periods: one year, one month, and one week, was found to exhibit fairly strong antioxidant activity, and this activity is not significantly different between the different samples ($p>0.05$). The juice also showed significant anti-proliferative activity on Caov-3, HeLa, MDA-MB-231, and MCF-7 cells, with the juice being most selective towards MCF-7 cells. However, the difference in IC_{50} values between samples and cell lines was not significant ($p>0.05$). The storage period does not affect the antioxidant activity or anti-proliferation effect of the juice on different cancer cell lines. In conclusion, the present study demonstrated that the Commercial Rosselle juice possesses antioxidant and antiproliferative activities that could be attributed to its flavonoids' and saponins' contents.

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