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Nutritional, phytochemical and in vitro inhibitory activities of chow-chow (*Sechium edule)*: a common home garden crop of Northeast India.

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ABSTRACT

Sechium edule belonging to the *Cucurbitaceae* family is commonly consumed as vegetable and also used as feed for livestock, is one of the important home garden crops grown in North East (NE) Indian States. It is reported to have a wide range of therapeutics properties. We evaluated the nutritional content, phytochemicals and antiproliferative effects of two common genotypes of *Sechium edule* from Meghalaya. The proximate composition (CP, CF, EE, NFE and TA) was in range as reported by other workers. The phenol content of DG genotype (6.76 mg) was higher ($p<0.05$) as compared to the LG genotype (4.62 mg) in terms of gallic acid equivalent (GAE/100g). But the flavonoids content variation of 21.27 - 22.62 mg between the two genotypes was not significant in terms of catechin equivalents (CE)/100g. The total antioxidant capacity as acid equivalent antioxidant capacity (AEAC) was significantly higher ($p < 0.05$) for DG (76.67 mg) as compared to LG (41.67 mg) genotypes. Aqueous extract of both varieties showed in vitro growth inhibition of cancerous cells in a concentration dependant manner but the LG genotype has greater growth inhibition compared to the DG genotype in both the MCF-7 and SAS cells which was correlated with the findings of the apoptotic activity determined in terms of percentage by flow cytometry.

1. Introduction

Many medicinal plants have been used for natural treatment to recover health problems by indigenous people around the world for centuries. About 80% of the world's population depends on plants and their active compounds, which are applied directly or after synthetic modification to use the optimal pharmacological activity (Cadena-Iniguez et al., 2013). *Sechium edule* commonly called as chayote or chow-chow belongs to the *Cucurbitaceae* family along with melons, cucumbers, pumpkin and squash. It is commonly consumed as fruits, young leaves, shoots, stems and tuberous roots around the world and also used as pig feed or cattle fodder. Both fruit and seed are rich in amino acids and vitamin C (Singh et al., 2002; Mishra & Das, 2015) and the tuber starch is comparable with traditional potato starch (Hernandez-Uribe et al., 2010). The plant is one of the important home garden crops grown extensively and regarded as an important vegetable in the daily diet of people of North East (NE) Indian States (Mishra & Das, 2015). Though it is a native of Mexico, considerable diversity is found in north eastern hill region particularly Meghalaya, Nagaland, Mizoram and Sikkim. Based on fruit colour, there are four major genotypes i.e. pale yellow, light green, green and dark green grown in the NE region. The chow-chow accessions of Sikkim varied in their morphological characters and also differ in their biochemical composition (Kapoor et al., 2014). Phytochemical analysis revealed that *S. edule* contains peroxidases, sterols, alkaloids, saponins, phenols, polyphenols, flavonoids, and cucurbitacins (Cadena-Iniguez et al., 2007; Aguiniga-Sanchez et al., 2017). The leaves and fruit are reported to have diuretic, cardiovascular, anti-inflammatory and hypotensive properties. Decoctions of the

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leaves or fruits is been used to relieve urine retention and burning urination or to dissolve kidney stones and as a complementary treatment for arteriosclerosis, hypertension and kidney disorders (Flores, 1989). It also helped to relieve intestinal and cutaneous inflammation (Dire et al., 2003; Cadena-Iniguez et al., 2007) and promote ulcer cauterization (Andres-Del et al., 2017). The plant is grown in a wide range of climate conditions from regions at sea level to altitudes of 1300-2000 m MSL (Aung & Flick, 1976). Various workers documented the use of *S. edule* to have anti-allergenic, anti-viral, anti-inflammatory, anti-bacterial, anti-oxidant, anti-neoplastic and anti-mutagenic (Cadena-Iniguez et al., 2013; Jayaprakasam et al., 2003; Ordonez et al., 2003, 2006; Sibi et al., 2013; Setzer & Setzer 2003; Yen et al., 2001). Pharmacological studies have confirmed the diuretic properties of the leaves and seeds as well as the cardiovascular and anti-inflammatory properties of the leaves and fruits (Lozoya, 1980). The methanolic fruit extract of *S. edule* var. *nigrum spinosum* contains flavonoids, phenolic acids and cucurbitacins which can eliminate tumour cells while protecting normal bone marrow cells. Thus, it has been considered an emerging natural agent for the treatment of various diseases without any harmful side effects (Aguiniga-Sanchez et al., 2017). Therefore, the objective of the present study is to evaluate the nutritional qualities and phytochemical contents of aqueous extracts of two commonly prevalent genotypes/variety (light green and dark green colour fruits) of *S. edule* found in Meghalaya and also its anti-proliferative effects in cancerous cell lines - squamous carcinoma of tongue (SAS) and breast cancer cell (MCF-7) of human origin.

2. Materials and Method

Sample collection and preparation

Two varieties - dark green (DG) and light green (LG) colour fruits of *Sechium edule* (Figure 1) were collected from the Horticulture Farm in the Division of System Research and Engineering, ICAR-RC for NEH Region, Umiam, Meghalaya. The fruits were dried and powdered for proximate analysis of nutrient contents and others. The extracts @100 mg/ml (w/v) concentration with water was prepared, homogenized and clarified by centrifugation at 10,000 rpm for 30 min at 4°C. The aqueous extract was collected and filtered with a 0.45 µm syringe filter for further in vitro studies. One gram of the powder was extracted overnight in 80% methanol and filtered. The filtered extract was used for the estimation of total antioxidant capacity (TAC) and total phenols, respectively.

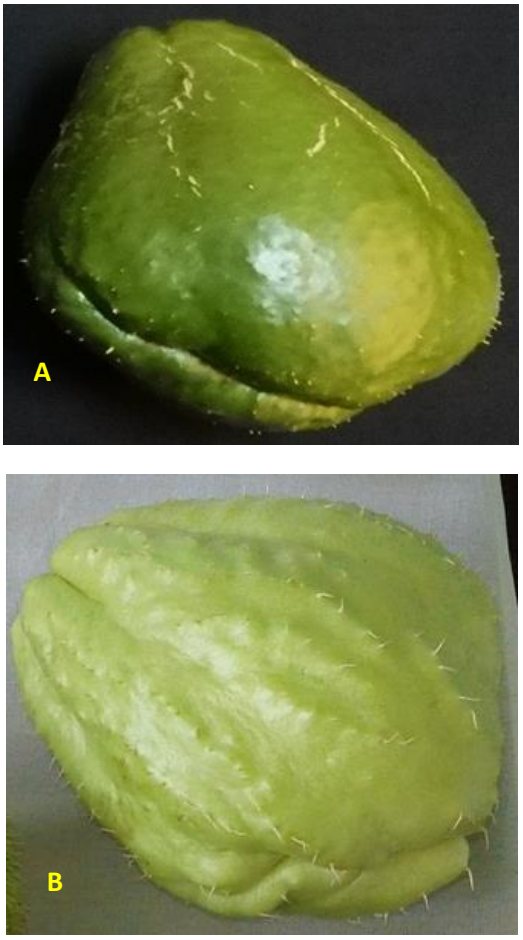

Figure 1. Representative photograph of *Sechium edule* fruit varieties used in the study. A. Dark green (DG**)** B. Light green (LG)

Proximate analysis of nutritional content of *Sechium* fruits

The proximate analysis of *Sechium* fruits was done as per the method prescribed by (AOAC,1980) for estimating the nutritional content: dry matter (DM), total ash (TA), crude protein (CP), ether extract (EE) and crude fibre (CF). The nitrogenous free extract (NFE) was calculated from 100 - (% moisture + % CF + % CP + % EE + % TA).

Total phenols content

The total phenol content was determined with the Folin-Ciocalteau reagent by the method described by Singleton *et al.*, (1999). The volume of 0.5 ml of extract was initially made up to 3 ml with 80% methanol. 1 ml of DMSO and 1 ml of 10% Folin-Ciocalteu reagent were added and mixed. 3 ml of 1% Na_2CO_3 was finally added after 3 min, and the tubes were incubated for 2 hr at room temperature. A standard curve was plotted using different concentrations of gallic acid (50-300 µg/ml). Absorbance was measured at 760 nm. The phenolic contents were calculated based on the

calibration curve of gallic acid (50-300 ug/ml) and expressed as gallic acid equivalents (GAE), in milligrams per gram of dry weight.

Total flavonoids

Total flavonoid was estimated using the method previously described (Chun *et al*., 2003). Ethanolic extract (1 ml) of the sample was mixed with 0.3 ml of 5% $NaNO_2$, 0.3 ml of 10% $AlCl_3$ and 3.4 ml of 4N NaOH at 2 min intervals. The absorbance of the sample was read at 510 nm against reagent blank after 30 min and expressed as catechin equivalent (mg CE/100 g).

Total Antioxidant Capacity

Total antioxidant capacity was estimated with phosphomolybdenum reagent by the method described by Prieto et al*.,* (1999). To 0.3 ml of sample extract, 3 ml of phosphomolybdenum reagent solution (0.6 M sulphuric acid, 28 mM potassium phosphate and 4 mM ammonium molybdate) was added. The tubes were then incubated at 95^{o}C for 1 hr. After cooling at room temperature the absorbance was measured at 695 nm. A standard curve of Ascorbic acid was plotted (20-100 µg/ml) to calculate the values. Total antioxidant capacity was expressed as Ascorbic acid equivalents (AAE) in milligrams per gram of dry weight.

Anti-proliferative assay

Two-fold serial dilution of the aqueous extract was prepared and up to five dilutions (100, 50, 25, 12.6 and 6.25 mg/ml) were used for studying the anti-proliferative effects in MCF-7 and SAS cells by 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide (MTT) assay as per Mosmann, 1983 with slight modification. The cells @ $1x10^6$/ml in Earle's Minimum Essential Medium (EMEM), (Sigma-Aldrich, USA) with 10% FBS were suspended in a 96 well-plate @ 100 µl/well and 100 µl of each dilution were added in triplicates with mitogen-Concanavalin A (Con A) and Phytohaemagglutinin A (PHA) @ 10 mg/ml as a positive control. The cells were incubated at 37°C with 5% CO_2 for 72 h. 10 µg MTT dye was then added to each well and further incubated for 4 h. The supernatants were removed and dimethyl sulfoxide (DMSO) @150 µl was added to each well and incubated at room temperature by gentle shaking for 30 min. The supernatants were collected and the absorbance was measured at 570 nm with an ELISA reader (Lab systems Multiskan Plus, Thermo Fisher Scientific, USA).

Apoptosis assay

The apoptosis assay was done using Annexin V-FITC apoptosis detection kit (Calbiochem, Germany) in flow cytometer (BD – LSR Fortessa, USA). Two-fold five serial dilution (100, 50, 25, 12.6 and 6.25 mg/ml) of the aqueous extract was prepared for studying apoptosis in treated cells of MCF-7 and SAS along with non-treated control. The cells @$1x10^6$/ml in Earle's Minimum Essential Medium (EMEM), (Sigma-Aldrich, USA) with 10% FBS were suspended in a 96 well- plate @100 µl/well and 100 µl of each dilution were added in triplicates. The control wells were treated with 100 ml PBS (pH 7.2). The cells were incubated at 37°C with 5% CO_2 for 24 hr. The cells were then processed using apoptosis detection kit following the manufacturer's instructions. The percentage of apoptosis was measured in a flow cytometer with a setting of unstained cells and cells stained with isotype control. Samples were acquired by taking 10000 cell counts.

Statistical analysis

The experiment was done in triplicates and the results were expressed as mean ± SEM. Statistical difference compared between two varieties were analyzed by one way analysis of variance (ANOVA) followed by the Tukey's test for significance. Statistical analysis was considered significant if $p \leq 0.05$.

3. Result

Nutritional content

The proximate composition of *Sechium* fruits (DG and LG) is presented in table 1. There is no significant variation in the composition of crude protein, crude fibre, ether extract, nitrogen free extract and ash content between two varieties.

Table 1. Determination of total protein, fiber, lipid and ash content of *S. edule* fruit extracts

Fruit Varieties	Crude protein (CP)	Crude fiber (CF)	Ether extract (EE)	Nitrogen free Extract (NFE)	Ash content
Dark green (DG)	9.69± 0.0190	10.08± 0.0309	0.77± 0.0918	74.85± 0.0007	4.61± 0.0031
Light green (LG)	10.45± 0.0081	12.03± 0.0118	0.97± 0.0583	70.71± 0.0006	5.84± 0.0206

Values are mean of triplicates ± SEM

Biochemical constituent

The content of phenols and flavonoids was estimated as presented in table 2. The phenol content of the DG variety (6.76 mg) was higher ($p < 0.05$) as compare to the LG variety (4.62 mg) in terms of gallic acid equivalent (GAE/100g). But the flavonoids content variation of 21.27 - 22.62 mg between the two varieties was not significant in terms of catechin equivalents (CE)/100 g.

Table 2. Phytochemical composition of *S. edule* fruit extract

	Phenols (mg GAE/100g)	Flavonoids (mg CE/100g)	DPPH (mg AEAC/100g)
Dark green (DG)	6.76 ± 0.0042	22.62 ± 0.0009	76.67± 0.0011
Light green (LG)	4.64 ± 0.0183	21.27 ± 0.0060	41.67 ± 0.0039

Values are mean of triplicates ± SEM

Antioxidant capacity

The total antioxidant capacity measured and expressed as acid equivalent antioxidant capacity (AEAC) in the methanolic extract was 76.67 mg for DG and 41.67 mg for LG varieties (Table 2). The antioxidant capacity in the DG variety was significantly higher ($p < 0.05$) as compare to the LG variety.

Anti proliferative activities

The anti-proliferative effects by 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide (MTT) assay with two-fold serial dilution of the aqueous extract of DG and LG variety showed growth inhibition in concentration dependent manner. The LG variety has greater growth inhibition compared to the DG variety (Fig. 2 & 3) in both the MCF-7 and SAS cells

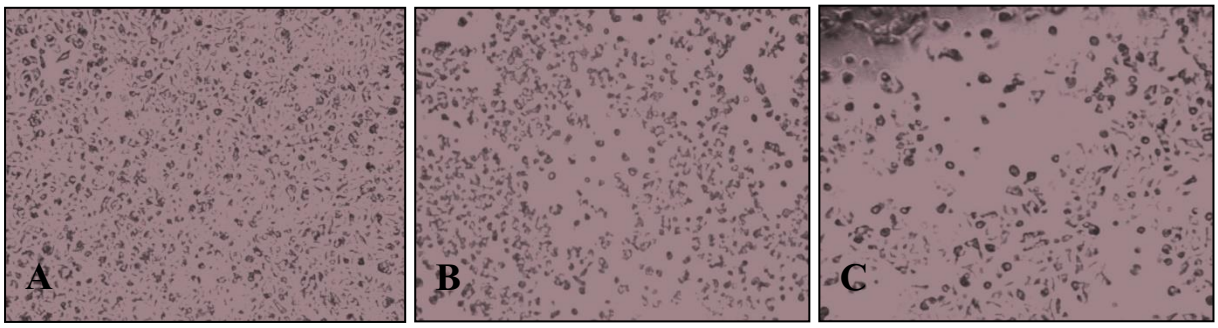

Figure 2. Microscopic photographs of growth inhibition with aqueous extract of *S.edule* in MCF-7 cells at 48 hrs (10x). A – Non-treated control. B - with DG extract @ 50 mg/ml. C - with LG extract @ 50 mg/ml.

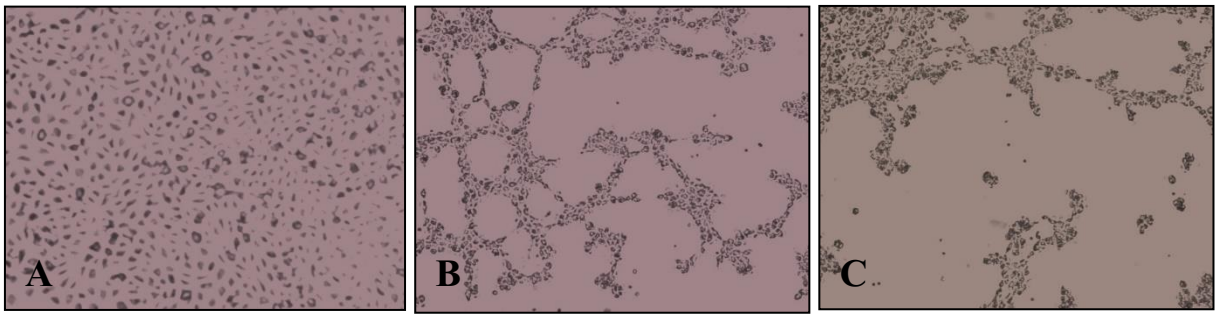

Figure 3: Microscopic photographs of growth inhibition with aqueous extract of *S.edule* in SAS cells at 48 hrs (10x). A – Non-treated control. B –with DG extract @ 50 mg/ml. C - with LG extract @ 50 mg/ml

Apoptosis activity

The apoptosis activity was determined using Annexin V- FITC with two-fold five serial dilution @100, 50, 25, 12.6 and 6.25 mg/ml of the aqueous extract in MCF-7 and SAS cell lines along with non-treated control showed the concentration dependant apoptotic activity in both the cell lines(table 3). Representative apoptotic activity determined as shown in Fig. 4.

Aqueous concentration of *S. edule* (mg/ml)	**Apoptosis (%) Light green var**	**Apoptosis (%) Dark green var**
100	51.8	20.3
50	25.8	12.0
25	20.5	8.7
12.5	10.6	6.3
6.25	9.4	6.4
non-treated control	7.0	6.3

Table 3. Flow cytometry analysis of apoptosis in SAS cells treated with aqueous extract of the LG and DG varieties of *S. edule* in serial two-fold five dilution along with non-treated control showed the apoptotic activity decreases with decrease concentration of *S. edule* extract.

It was found the apoptotic activity was shown to be higher with the LG variety as compared to the DG variety. The percentage apoptotic activity was also shown to be more in SAS cells compared to MCF cells.

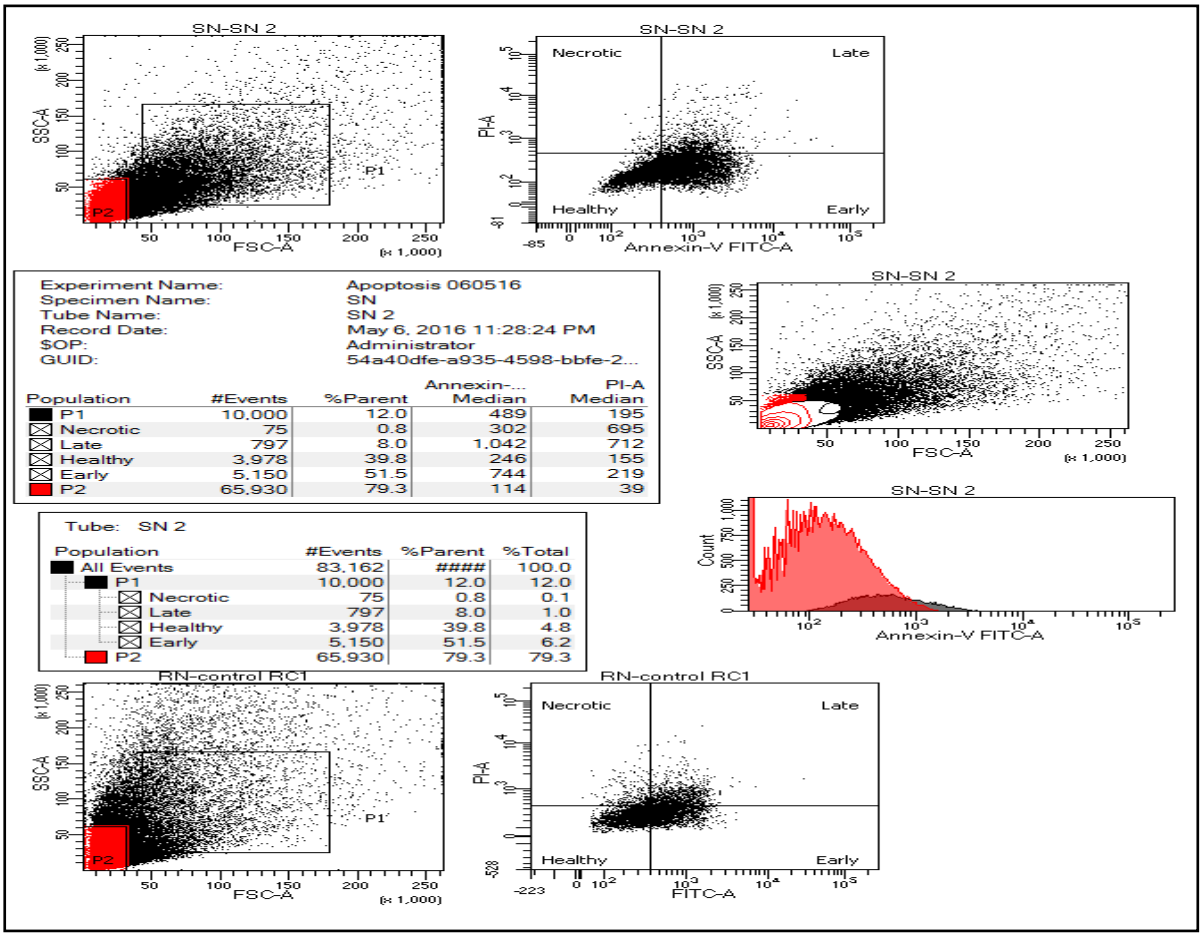

Figure 4. Representative flow cytometry analysis output of apoptosis in SAS cells treated with aqueous extract of *S. edule* showing fractions of cells undergoing apoptosis and unaffected cells.

4. Discussion

The proximate composition of *Sechium* fruits (dark green and light green) showed no significant variation in the composition of CP, CF, EE, NFE and ash content. Similar studies were carried out by Mishra and Das (2015) for *S. edule* fruits of Garo Hills of Meghalaya. The CP, CF and ash content of the fruits was 0.873, 5.308 and 0.287 respectively, which is lower than the values found in this study. The CP, CF, EE, NFE and ash content detected by Lalthangsa and Samanta (2015) in *S. edule* fruit samples of Mizoram were 14.88, 7.53, 0.83, 70.79 and 5.97, respectively. The CP value was higher in comparison to the values found in our study but other values were comparable to that found in this study. These differences might be due to different variety of the plant used and the growing conditions of different geographical climate. The phenol content of the dark green variety (6.76 mg) was higher as compared to the light green variety (4.62 mg) in terms of gallic acid equivalent (GAE/100g). But the flavonoids content variation of 21.27-22.62 mg between the two varieties was not significant in terms of catechin equivalents (CE)/100g. Phenolic acids, such as gallic, chlorogenic, vanillic, p-hydroxybenzoic, caffeic and p-coumaric acids (0.072, 0.823, 0.032, 0.020, 0.091 and 0.032 mg/g of extract, respectively) and flavonoids as phloridzin, naringenin, phloretin and apigenin (0.005, 1.556, 0.018 and 0.292 mg/g of extract, respectively) in *S. edule* var. *nigrum spinosumm* extract was differentiated by HPLC methods by Aguiniga-Sanchez et al., (2017). The combined phenolic and flavonoid values were lower than the values detected in our study which may be due to the method of estimation. Salazar-Aguilar et al., (2017) reported the presence of only terpenes and flavonoids in the *S. edule* fruit extracts with the flavonoid content of 1.5 g. 100 g^{-1} of fruit extract. In our present study, the flavonoid content was higher at 22.62 and 21.27 mg CE/100g for the dark green as well as light green varieties, respectively. The total antioxidant capacity as acid equivalent antioxidant capacity (AEAC) in the methanolic extract was 76.67 mg for dark green and 41.67 mg for light green varieties. The antioxidant capacity in dark green variety was significantly higher ($p \leq 0.05$) as compared to the light green variety. The presence of an anti-oxidative function is indicated by the presence of polyphenols in the plant. This is due to their inherent high redox potentials which make them efficient reducing agents, hydrogen donors and singlet oxygen quenchers (Zheng & Wang, 2001). Many researchers reported the correlation between the phenolic and flavonoid contents with their antioxidant activities. We have observed significant difference in total antioxidant capacity in the DG variety (76.67 mg AEAC/100g) as compared to LG (41.67mg AEAC/100g). The antiproliferative effects showed that both the DG and LG *S. edule* extracts inhibit the breast cancer cell (MCF-7) and carcinoma cell (SAS) in concentration dependant manner but the LG extracts showed greater inhibition. The apoptotic activity corroborates with the antiproliferative effects. It was observed that the aqueous extracts @100 mg/ml concentration of LG exhibited 51.8% apoptosis while DG showed 20.3% with decreasing percentage as concentration decreases in both varieties. The dose dependent growth inhibition was also reported by Aguiniga - Sanchez et al., (2015) in the crude extract of chayote hybrid inducing apoptosis in leukaemic cells- P388, J774 and WEHI-3 cells. It also reported the difference in growth inhibition with different cell lines (Cadena-Iniguez et al., 2013; Aguiniga-Sanchez et al., 2017) indicating the different varieties of species exhibit different activities across different tumor cell lines. We also observed that growth inhibition was more prominent in SAS cells as compared to MCF-7 cells. Aguiniga - Sanchez et al., (2015) found that *S. edule* var. *nigrum spinosum* extract inhibited the proliferation of both leukemic P388 cells but not normal cells. It also was shown to have antiproliferative activity in HeLa, L929 or P388 cell lines validating their previously established anti-tumor properties in alternative medicine and becoming a new therapeutic target in cancer studies (Cadena-Iniguez et al., 2013). The results indicates that *S. edule* could be a potential anti-cancer candidate as well as functional food for alleviating oxidative stress. Therefore, it could very well be integrated into crop production for management of traditional jhum (Khezhie and Ao., 2022) along with other crops. It can also augment the diversity of homestead garden (Das et al., 2023) in fulfilling the basic nutritional security since *S. edule* is having rich phytogenic components.

5. Conclusion

Sechium is a widely grown plant mainly for its fruits and shoots and has diverse cultivars which need to be explored and conserved. It is also relevant to bioprospecting genetic variants so that range of outstanding genotypes for the pharmacological application that is easy and safe to use could be broadened given the wide environmental adaptation of the species with rich phytochemicals and phytonutrients. The fruits can be considered nutraceutical agent or maybe the leaves and stem be exploited as biopreservatives in food applications, health supplements etc. It is also a potential candidate for therapeutic use in cancer treatment. Hence, it is desirable to have more impetus on research in *Sechium* exploring its beneficial properties.

6. Acknowledgement

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7. Conflict of Interest

The authors declare that there is no conflict of interest within themselves and others including the funding agency where the research was carried out.

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Cancer Statistics, 2017 Rebecca L Siegel[1], Kimberly D Miller[2], Ahmedin Jemal[3]