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CYTOTOXIC POTENTIAL OF CASHEW APPLE (*ANACARDIUM OCCIDENTALES* L.) JUICE ON ROOT MERISTEMATIC CELLS OF *ALLIUM SATIVUM*

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ABSTRACT
The cytotoxic effect of cashew apple juice (red and yellow varieties) on Allium sativum root tip cells was evaluated with a view to studying their mutagenic and anti-mitotic potentials. The extracted raw juice (100%) was diluted to concentrations of 25%, 50% and 75% with distilled water, while distilled water without the juice served as the control for the study. The root tips of Allium sativum grown in the treatments and control for 24 hours were harvested between 8:30am and 9:30am CAT for cytological studies. Qualitative phytochemical analysis of the juice was also carried out. Phytochemical analysis revealed the presence of flavonoids, phenols, tannins, saponins, alkaloids and glycosides in juice from both varieties. The results showed chromosomal aberrations with different concentrations of the cashew apple juice. The six chromosomal aberrations associated with different concentrations of juice from the red apple variety were faulty polarity, C-mitosis, sticky chromosomes, spindle disturbance, star metaphase and binucleate cells while three aberrant conditions observed in juice obtained from yellow apple variety were binucleate cells, C-mitosis cells and sticky chromosomes. The results obtained in this study revealed that the yellow cashew juice showed lesser chromosomal abnormalities. Also the cashew apple juice has anti-mitotic properties despite the reported mutagenic potentials. Yellow cashew apple with lesser chromosomal aberrations would be better for consumption than the red cashew apple.

Key words: Cytotoxic, Phytochemical, Aberration, Anti-mitotic, Mutagenic

1.0 INTRODUCTION
The cashew (*Anacardium occidentale* L.) is native to the coast of Brazil. Cashew is a hardy tree that can grow on poor soils and under various climatic conditions (Hammed *et al*., 2008).The large, fleshy and juicy fruits according to Michodjehoun *et al*. (2009) are commonly called cashew apple. Cont and Porto (2014) reported that the major cashew producing countries are Tanzania, India, Mozambique, Srilanka, Kenya, Madagascar, Thailand, Malaysia, Indonesia, Nigeria, Senegal, Malawi and Angola.

Ripe cashew fruit has a light yellow or red spongy flesh, which is very juicy, slightly acidic and slightly astringent, when eaten raw but highly astringent when green or unripe (Zhang *et al*., 2010; Awe *et al*., 2013). According to Alves *et al.* (2010) and Pellegrini *et al*. (2007), cashew fruits contain carotenoids, chlorophyll and a wide variety of secondary metabolites such as simple phenolics compounds, flavonoids, glycosides and complex polymeric tannins (eprocyanidins and gallotannins). In line with this, there exists a plethora of literature suggesting positive association between consumption of fruits rich in diet and reduction in the incidence of diseases such as cancer, cardio-vascular hypertension (Agte *et al.,* 2005). In the opinion of Sivagurunathan *et al.* (2010) the high tannin content gives an astringent taste and a dry-mouth feeling, making it less appealing for consumption

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in comparison to other fruit juices. Ross (2000) attributed the suspected risk of developing acute myeloid leukemia after high consumption of cashew juice to the presence of high flavonoids in the juice. Lowor and Agyente-Badu (2009) opined that fruits rich in tannins like the cashew are considered to be of low nutritional value. However the study of tannins strongly indicated that the major effect of tannins was not due to their inhibition on food consumption or digestion rather a decrease in the efficiency to convert the absorbed nutrients to ones needed by the body (King-thom *et al.,* 1998). Of all the tropical fruits trees in Western Nigeria, cashew is the most widely domesticated, ahead of mango and pawpaw trees (Daramola, 2013). Cashew tree (*Anacardium occidentale* L.) belonging to the family Anacardiaceae is an extremely hardy tree that grows on poor soil under various climatic conditions. The tree (*Anacardium occidentale* L.) is native to the coast of Brazil and Adou *et al.* (2012*b*) reported that cashew produced at season as the popular fruits and nuts. Cashew according to Emelike *et al.* (2015) consists mainly of false fruit commonly called cashew nut and cashew apple with the nuts containing an embryo. According to Akinwale (2000) the juicy content of the cashew apple is approximately 85%.

Fiskesjö (1985) and Rank *et al.* (2002) reported that *Allium* test is the most commonly used to determine the level of toxicity in the laboratories because of the easy storage and growing peculiarities of *Allium*. Abu and Mba (2011) opined that *Allium* test is one of the few direct methods of measuring damage in system that are exposed to mutagens or potential carcinogens, and enables the evaluations of these damages through the observation of chromosomal aberrations. According to Gadano *et al.* (2002) Mitotic Index (MI) and Replication Index (RI) which indicate adequate cell proliferation can be measured by plant test system especially *Allium* test. Toxicity test using this plant test system *in vivo*, such as *Allium* were validated by several researchers like Vicentini *et al*. (2001) and Teixeira *et al*. (2003) who performed animal testing *in vivo* and the results obtained were similar, providing valuable information for human health assessment. Fiskesjö, (1988) reported that *Allium* test also correlated well with data obtained from prokaryotic systems. It is an established bio-assay, validated by the International Programme on Chemical Safety (PCS) for efficient and standard test of chemical and *in situ* monitoring of the environment (Kumari and Mukherjee, 2009). Therefore *Allium* testing systems can be used to assess the possible fundamental risks of substances on living organisms including humans.

Winterhalter *et al.* (1991) reported that cashew apple juice is converted into alcoholic and non-alcoholic beverages, candied fruit, fresh juice, jam, jelly, syrup and pectin. Many people suck the juice in the fruits/apples directly. Despite this aforementioned uses and worldwide acceptance of cashew fruit and its juice, reports on the possible toxicity of the juice on the genetic system has been limited. The main aim of this study therefore was to examine the effects of cashew apple juice (red and yellow varieties) on the mitotic cell division of the root tips of *Allium sativum* with a view to reporting the mutagenic and anti-mitotic potentials.

2.0 MATERIALS AND METHODS

2.1 Collection of Materials

The two cashew varieties (red and yellow apple) considered in this study were gotten from Faculty of Agricultural Science, Kogi State University, Anyigba between the months of December to February, 2015 while *Allium sativum* (garlic) used were purchased from Anyigba Market, Kogi State.

2.2 Preparation of Test Materials

The juices from each of the two cashew varieties considered were pressed out from the apples and the stock juice extracts at 100% was serially diluted to 25% 50% and 75% with distilled water. Distilled water served as the control for the study. The treatments at each concentration were

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replicated five times and arranged in Complete Randomized Design (CRD).

Allium sativum bulbs were rinsed with tap water and the outer scales were carefully removed in order not to destroy the root primordial. These set of garlic bulbs were allowed to grow roots by placing the reduced stems in contact with water in beakers. The bulbs were separated into cloves and only cloves with properly root growth were selected. The garlic roots were allowed to grow in each solution for 24 hours while root tips measuring about 2cm in length were harvested into vials between the hours of 8 to 9am CAT and the study proceeded in series of stages according to the method outlined by Akinleye (2007).

2.3 Fixation

The fixative comprised of glacial acetic acid and absolute ethanol in a ratio of 1:3. The fixative helps to keep the cells in their natural condition. The specimens were appropriately labeled and all the vials were kept in the refrigerator.

2.4 Hydrolysis

After 24 hours, the root tips were taken out of fixatives and washed thoroughly in distilled water. Hydrolysis of root tips was carried out using 10% HCl solution. The hydrolysis was done at 60^{o}C in water bath to soften the root tips.

2.5 Squashing and Staining

The hydrolyzed root tips were washed with distilled water and each tip was placed on a clean glass slide. The meristematic tips were cut with blade and the remaining part discarded. Two drops of aceto-carmine stain were put on the roots tips and covered with cover slips. Squashing was done using the broader flat end of a cylindrical search needle until a turbid suspension was seen. Excess stain was drained off using a filter paper and gently applying pressure. Transparent finger nail polish was applied to the edges of the cover slip to prevent air from entering. Five slides were prepared in this manner for each treatment.

2.6 Chromosome Observation

The slides were mounted and observed under the Celestron Digital model of the light microscope. The X4, X10, X40 objectives were used for viewing the slides. Photomicrographs of the normal mitotic stages and aberrant cells were taken at X40 objectives.

2.7 Data Analysis

Ten counts each were taken from five different slides from each concentration for normal mitotic stages and aberrant cells. The percentage of Relative Division Rate (RDR), Active Mitotic Index (AMI) and Mitotic Index (MI) for the cells treated with different concentrations of cashew apple juice and control were calculated using the formula proposed by Malode *et al.* (2012) as given below:

$$Mitotic\ Index = \frac{Total\ number\ of\ dividing\ cell}{Total\ numberof\ cells\ examined}\ X\ 100$$

The number of dividing cells was taken as the number of prophase, metaphase, anaphase and telophase while the Relative Division Rate (RDR) was calculated using the formula below:

$$(RDR) = \frac{\%\ of\ dividing\ cells\ in\ treated\ root\ tips - \%\ of\ dividing\ cells\ in\ control\ root\ tips}{100 - \%\ of\ dividing\ cells\ in\ control\ root\ tips}\ X\ 100$$

Active Mitotic Index (AMI) was calculated using the formula below:

$$(AMI) = \frac{Number\ of\ Metaphase + Anaphase\ cells}{Total\ number\ of\ cells\ examined}\ X\ 100$$

Data pooled in this study were subjected to Analysis of Variance (ANOVA) and means with significant differences were separated using Duncan Multiple Range Test (DMRT) using SPSS Version 21software package.

2.8 Phytochemical screening

Qualitative secondary metabolite assessments of the juices from the two cashew apple samples were carried out according to the methods outlined by Evans (1996) modified by Evans (2002).

3.0 RESULT AND DISCUSSION

Table I shows the qualitative phytochemical

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results for juices from the red and yellow cashew apple varieties. It was observed that both cashew apple types produced juice containing flavonoid, phenols, tannins, saponins, alkaloids and glycosides. The red apple variety contained more phenols, alkaloid and glycosides while yellow apple variety contained more saponins.

Table I: Secondary Metabolites Constituents of Juices from the Red and Yellow Varieties of Cashew Apple

Phytochemicals	Red Variety	Yellow Variety
Flavonoid	+ +	+ +
Phenols	+ + +	+ +
Tannins	+ +	+ +
Saponins	+ +	+ + +
Alkaloid	+ + +	+ +
Glycosides	+ +	+

Keys:
+ + + Highly present
+ + Moderately present
+ Present in trace concentration

Ten out of thirteen cytological parameters studied showed statistical significant differences with respect to the different concentrations of red cashew apple juice considered (Table II). The attributes are number of prophase cells, metaphase cells, anaphase cells, telophase cells, C-mitotic cells, sticky chromosomes, spindle disturbance, star metaphase cells, binucleate cells and total number of aberrations. Total number of cells, number of interphase cells and number of faulty polarized cells did not show significant difference. The highest number of aberrant cells was recorded for 50 % concentration (14.80) while the least was recorded for 75 % concentration (3.50) as presented in Table II. No chromosomal aberration was recorded in the untreated root tips (control). No precise pattern of increase was observed in relation to variations in concentration of red cashew apple juice for all the cytological parameters studied (Table II).

Table II: Effect of Different Concentrations of Cashew Apple Juice (Red Variety) on some Cytological Attributes

Conc. (%)	TNC	NOI	NOP	NOM	NOA	NOT	NOB	NOC	NSC	NFP	NSD	NSM	TNA
Control	44.20	21.30	7.60^{a}	5.70^{b}	3.80^{c}	4.80^{b}	0.00^{a}	0.00^{a}	0.00^{a}	0.00	0.00^{a}	0.00^{a}	0.00^{a}
25	48.30	17.80	16.70^{b}	1.90^{a}	2.10^{a}	1.60^{b}	2.40^{a}	0.60^{ab}	5.10^{b}	0.00	0.00^{a}	0.00^{a}	7.10^{b}
50	59.40	20.10	18.10^{b}	2.00^{a}	0.50^{a}	4.90^{b}	5.00^{b}	0.80^{b}	9.00^{c}	0.50	0.00^{a}	0.00^{a}	14.80^{c}
75	37.10	14.60	14.90^{ab}	1.00^{a}	2.50^{b}	1.10^{a}	0.40^{a}	0.30^{ab}	1.20^{a}	0.90	0.00^{a}	0.20^{b}	3.50^{ab}
100	45.20	16.30	19.80^{b}	4.80^{a}	1.70^{ab}	1.00^{a}	1.20^{a}	0.60^{ab}	2.30^{a}	0.20	0.20^{b}	0.00^{a}	4.50^{b}
LSD Value	NS	NS	2.16	1.10	0.70	0.89	0.89	0.14	1.60	NS	0.04	0.04	2.48

Means with the same alphabets in the same column are not significantly different at P<0.05

KEY
CONC- Different concentrations
TNC- Total number of cells
NOI- Number of cells at interphase stage
NOP- Number of cells at prophase stage
NOM- Number of cells at metaphase stage
NOA- Number of cells at anaphase stage
NOT- Number of cells at telophase stage
NOB- Number of binucleate cells
NOC- Number of cells with C-mitosis
NSC- Number of cells with Sticky chromosomes
NFP- Number of faulty polarized cells
NSD- Number of spindle disturbance cells
NSM- Number of star metaphase cells
TNA- Total number of aberrant cells
NS- Not Significant

All the cytotoxic attributes in Table III showed significant differences in relation to different concentration of yellow cashew apple juice. No precise trend in relation to increasing concentration of yellow cashew apple juice was observed for all the cytological parameters (Table III). However, the highest total number of aberrations (8.70) was recorded for 50 % concentration and the least (4.60) was recorded for 25 %. This 4.60 recorded for 25 %

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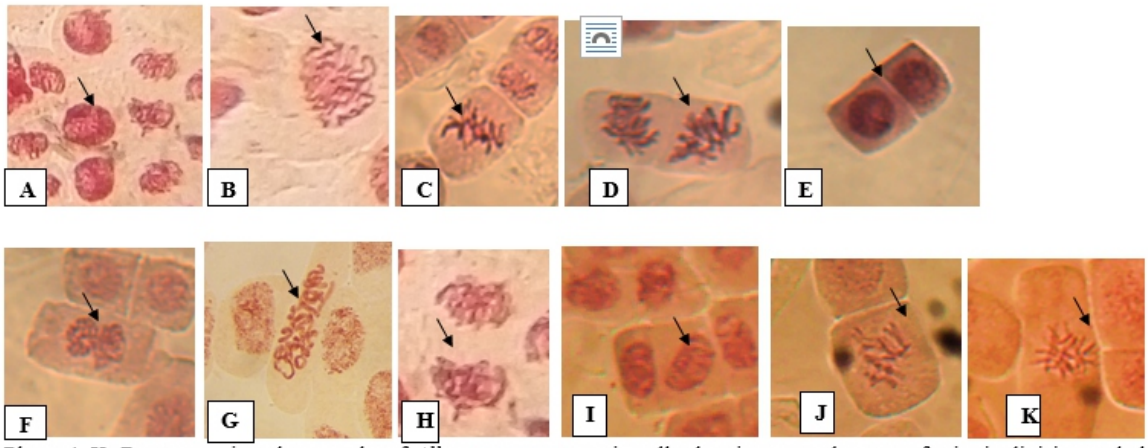
concentration is not significantly different for the 4.80 and 5.90 total aberrant cells recorded for 75 % and 100 % concentrations respectively. No chromosomal aberration was observed in the control.

Table III: Effects of Different Concentrations of Cashew Apple Juice (Yellow Variety) on Some Cytological Attributes

Conc. (%)	TNC	NOI	NOP	NOM	NOA	NOT	NOB	NOC	NSC	TNA
Control	44.20^{b}	21.30^{b}	7.60^{a}	5.70^{c}	3.80^{c}	4.80^{c}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
25	49.10^{b}	21.40^{b}	17.40^{b}	0.10^{a}	4.00^{a}	1.10^{ab}	2.60^{b}	0.70^{ab}	1.20^{ab}	4.60^{b}
50	50.50^{b}	24.20^{b}	14.60^{b}	2.4^{b}	1.10^{a}	0.60^{ab}	2.40^{b}	2.20^{c}	3.90^{d}	8.70^{c}
75	22.40^{a}	11.00^{a}	3.60^{a}	0.10^{a}	2.00^{a}	1.80^{b}	1.80^{b}	1.30^{bc}	1.70^{bc}	4.80^{b}
100	15.10^{a}	4.40^{a}	3.21^{a}	0.10^{a}	1.29^{a}	0.20^{a}	2.50^{b}	0.60^{ab}	2.80^{cd}	5.90^{b}
LSD Value	7.00	3.70	2.56	1.09	0.73	0.81	0.48	0.37	0.67	1.40

Means with the same alphabets in the same column are not significantly different at P<0.05

KEY
CONC- Different concentrations
TNC- Total number of cells
NOI- Number of cells at interphase stage
NOP- Number of cells at prophase stage
NOM- Number of cells at metaphase stage
NOA- Number of cells at anaphase stage
NOT- Number of cells at telophase stage
NOB- Number of binucleate cells
NOC- Number of cells with C-mitosis
NSC- Number of cells with Sticky chromosomes
TNA- Total number of aberrant cells



Plates A-K: Representative photographs of *Allium sativum* root tip cells showing normal stages of mitotic division and aberrant cells from root tips treated with both cashew apple juices considered in this study (A) Normal interphase (B) Normal prophase (C) Normal metaphase (D) Normal anaphase (E) Normal telophase. (F) Binucleate cells (G) C-mitosis (H) Sticky chromosome (I) Faulty polarity (J) Spindle disturbance (H) Star metaphase (Magnification ×400).

From Table IV, the highest Relative Division Rate value of +21.51% was observed in 100% concentration of juice from red cashew apple variety, the least value was recorded for 25% concentrations (-6.70%) while 50% and 75% had -13.12% and +5.92% Relative Division Rate (RDR) respectively. The highest percentage of Active Mitotic Index (AMI) was observed in control with 21.49% while the least number was observed in 50% concentration with 4.21%. Others include: 8.28%, 9.44% and 14.38% recorded for 25%, 75% and 100% respectively. The highest Mitotic Index (MI)

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observed from juice from red apple cashew was recorded for 100% concentration with 60.40% while the least value of 42.93% was recorded for 50% concentration. Mitotic Index values of 49.55%, 46.17% and 52.56% were observed for control (0 %), 25%, 50% and 75% concentrations respectively (Table IV).

Table IV: Effects of Differe nt Concentrations of Cashew Apple Juice (Red Variety) on Cell Division Indices of *Allium sativum*

Concentration (%)	Relative Division Rate (RDR)%	Percentage of Active Mitotic Index (AMI)%	Percentage of Mitotic Index (MI)%
Control	-	21.49	49.55
25	-6.70	8.28	46.17
50	-13.12	4.21	42.93
75	+5.92	9.44	52.56
100	+21.51	14.38	60.40

Table V shows that there was a progressive decrease in Relative Division Rate (RDR) with increasing concentrations of cashew apple juice (yellow apple variety). The highest value was recorded for 100% concentration (-35.21%) and the least value of -6.98% was recorded for 25% concentration. 50% and 75% concentrations had -24.82% and -31.83% respectively. The highest value of Active Mitotic Index was recorded in control (21.49%) while the least was recorded for 50% concentration (6.93%) of cashew apple juice (yellow apple variety). 8.35%, 9.38% and 9.21% values of Active Mitotic Index were recorded for 25%, 75% and 100% concentrations respectively. There was progressive decrease in Mitotic Index from control (49.55%) to 100% juice concentration with the least Mitotic Index (31.79%). The Mitotic Indices of 25, 50 and 70% concentrations of yellow apple juice were 46.03%, 37.03% and 31.79% respectively.

Table V: Effects of Different Conc entrations of Cashew Apple Juice (Yellow Variety) on Cell Division of *Allium sativum*

Conc. (%)	Relative Division Rate (RDR)%	Percentage of Active Mitotic Index (AMI)%	Percentage of Mitotic Index (MI)%
Control	-	21.49	49.55
25	-6.98	8.35	46.03
50	-24.82	6.93	37.03
75	-31.83	9.38	33.49
100	-35.21	9.21	31.79

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DISCUSSION

Fruits and vegetables according to Daramola (2013) are important for good health because this class of food forms an important portion of healthy diet. Fruits contain hundreds of compounds with potential antioxidant activity, including the antioxidant vitamins C and E (Zhang *et al.*, 2010). Akinboro *et al.* (2013) opined that most plant extracts contained toxic phytochemicals that can interact with biomolecules in the cells to cause mutagenic, cytotoxic and genotoxic effects *in vitro* and *in vivo* plant and animal assays. No significant varietal difference was observed with respect to qualitative phytochemical composition of the two cashew apple juices varieties studied. Although the higher number of aberrations recorded in juice from the red variety compared with juice from the yellow variety may be attributed to the high alkaloid content. This finding agrees with the report of Abderrahman *et al.* (2016) that high concentration of alkaloid recorded from *Rubia cordifolia* roots induces significant number of chromosome aberrations in mice bone marrow cells. It is also very important that cashew apple should be allowed to ripen very well before consumption because Daramola (2015) reported presence of significantly higher level of phytochemical component for juice obtained from unripe fruits than juice obtained from ripped cashew apple.

This study revealed that ten out of the thirteen cytological parameters studied showed statistical significant differences to the different concentrations of cashew apple juice (red variety) while all the cytological attributes showed significant differences in relation to different concentration of cashew apple juice (yellow variety). This finding suggests that cashew apple juice irrespective of variety is cytotoxic. This finding on cashew apple juice agrees with the earlier reports of Akinboro *et al.* (2011) which states that most plant extracts contained toxic phytochemicals that can interact with biomolecules in the cells to cause mutagenic, cytotoxic and genotoxic effects *in vitro* and *in vivo* plant and animal assays.

The results obtained in this study indicated that cell division was normal in control while six aberrant conditions such as faulty polarity, C-mitosis, sticky chromosomes, spindle disturbance, star metaphase and binucleate cells were observed in cells treated with juice from the red cashew apple. Three aberrant conditions: binucleate cells, C-mitosis and sticky chromosomes were recorded in cashew apple juice (yellow variety) treated cells. The observed aberrant cells in this study are an indication that cashew apple juice is mutagenic to some extent. Although the red variety is more mutagenic since it produces more aberrations six (6) compared to the three (3) produced by the yellow variety. Apart from the varietal differences observed in relation to the number of aberrant cells in this study, maturity stage of the cashew apple before extracting the juice may be responsible for the mutagenic conditions of this juice. Adou *et al.* (2012*a*) reported that several factors such as variety, growing region, climate, cultural practices, maturity at harvest, the storage atmosphere, condition storage are known to affect the chemical composition of apple juice. Although the finding contradict the report of Lowor and Agyente-Badu (2009) that varietal and ecological differences do not affect most chemical compositions in cashew apple juice.

The relatively high Relative Division Rate (RDR), Active Mitotic Index (AMI) and Mitotic Index (MI) among the cells treated with apple juice from the red and yellow varieties observed in 100% concentration is an indication that this concentration is the most mutagenic without varietal difference.

The higher Relative Division Rate (RDR), Active Mitotic Index (AMI) and Mitotic Index values observed in the control cell compared to cells treated with 25%, 50% and 75% concentrations of apple juice from the red and yellow cashew varieties suggests that although cashew apple juice may be mutagenic but also exhibits the highest anti-mitotic potentials. Several

researchers have reported the anti-tumor and anti-microbial potentials (Cavalcante *et al*., 2005) and antioxidant activity (Alves *et al*., 2010) of cashew apple juice. These anti-mitotic properties according to Cavalcante *et al.* (2005) can be attributed to the presence of chemically active components in juices and may be considered a potential anti-cancer agent.

By comparison, little differences were observed between the results obtained on red and yellow cashew varieties for Relative Division Rate (RDR), Active Mitotic Index (AMI) and Mitotic Index (MI) except for the fact that more aberrant cells were recorded for the red cashew variety. This indicates that varietal difference in cashew does not have much effect on the level of cytotoxicity but the consumption of yellow cashew apple is safer.

4.0 CONCLUSION

The finding in this study indicates that although cashew apple juice showed some mutagenic potential, it also has anti-mitotic properties. This anti-mitotic property is therefore considered a promising anti-cancer potential if well harness. Also, varietal difference in cashew does not have much effect on the level of cytotoxicity but the consumption of yellow cashew apple is safer because of its lesser mutagenic ability. Further studies should therefore be directed towards considering the effects of juices from both apple varieties on animal cells (especially cancerous cells) (*in vivo* study) to complement the findings in this study.

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