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Anti-inflammatory and Anticancer Activity of Tamarillo (*Cyphomandra betacea*) an Underexploited Fruit of Nilgiris District, Tamil Nadu, India

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ABSTRACT

Background: Cervical cancer of the uterus is a vital global health concern affecting women over the age of 25 years. The focus of the research was to assess the anticancer and anti-inflammatory properties of Tamarillo (*Cyphomandra betacea*).

Methods: Tamarillo fruit was ultrasonicated and the biologically active synthesis comprises of whole phenolic content, complete total flavonoids, total glycosides and combined carotenoids were quantified by UV-Spectrophotometer. Protease inhibition and protein denaturation inhibition were used to test the anti-inflammatory efficacy. MTT assay was employed to compare the anticancer effects of tamarillo, additionally flow cytometer was performed to find out the percentage of cell population, cell death and ROS.

Result: Tamarillo exhibited the highest inhibition at a concentration of 40-50 µl. The whole phenolic content was 95 mg/100 g, total flavonoids was 21 mg/g DW, glycosides were 10.6 mg Q/g DW and carotenoids were 96 mg βCE/g. The percent inhibition of protease and protein denaturation assay of tamarillo extracts of 10-50 µg/ml ranged from (15-64.7%) and (18.5-77%) respectively. The anticancer activity of tamarillo fruit was evaluated for human cervical cancer cell line (HeLa) which revealed IC50 of 39.04, 31.52 and 29.29, with corresponding cytotoxicity values of 56.11, 70.23 and 70.71 at 24, 48 and 72 hours. Flow cytometry revealed about 90% of cells in the G1-Mitotic phase, which indicate cell cycle inhibition and apoptosis effects. Elevated ROS levels in treated cells highlighted increasing oxidative stress, inhibiting cell proliferation. In conclusion, tamarillo fruit extracts show varying anti-inflammatory effects and anticancer activity due to differences in bioactive compounds.

Key words: Anticancer, Anti-inflammatory, Cytotoxicity, Flow cytometer, ROS, Tamarillo.

INTRODUCTION

According to WHO cancer is the second largest cause of death worldwide (Maodaa *et al.,* 2024). Uterine cervical cancer is the most prevalent malignancy affecting women globally, with significant mortality rates observed in developing countries (Bray *et al.,* 2018). It arises from the growth of tumour cells in the cervix and place as the 4th familiar and majority of cancer among women in the universe. In 2020, projected the incidence of uterine cervical cancer were 604,127 while deaths numbered 341,831 worldwide. There is no medicine for treating cancers. Hence there is a powerful support for the usage of natural remedies which contain no toxic impact for the environment. Novel biological compounds contribute for developing an innovative drug with the plants are mostly used as a anticancer drug at present (Maodaa *et al.,* 2024).

Inflammation is a natural physiological process essential for the defense and tissue repair mechanisms of the body. It is usually activated by factors such as infections, injuries, diseases or allergic reactions. Although, whenever inflammation persists and becomes persistent, it can play a crucial role in the growth of illnesses like cardiovascular diseases, autoimmune conditions, neurological disorders and cancer. This process may occur within the connective tissues of blood vessels or be directly initiated by the immune system (Stromsnes *et al.,* 2021).

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Cyphomandra betacea Cav., known as tamarillo or tree tomato is a tropical indigenous fruiting plant from South America characterized by the rich level of bioactive compounds like phenolics, flavonoids, carotenoids and glycosides showing diverse therapeutical properties (Yang *et al.,* 2021). This red fruit is very much in demand around the world, mainly because of its unique flavour and nutritional importance. Apart from its use as a culinary component, tamarillo has also received attention for its potential health benefits by exhibiting anti-inflammatory

and anticancer properties (Espin *et al.,* 2020). Phenolic compounds are group of secondary metabolites present in plant-based foods which helps to protect the body from oxidative cell damage (Bhattacherjee *et al.,* 2023). Recent studies also tested its anticancer effects, focusing especially on oxidative-stress-induced cell injuries, which are one of the important mechanisms of cancer development (Salim *et al.,* 2019). This study attempts to probe into the anti-inflammatory and anticancer properties of tamarillo focusing on its bioactive compounds and their effects on HeLa cervical cancer cells.

This work concentrates on the multipotential functionality of tamarillo in utilizing natural remedies for the treatment of cancer especially cervical cancer and its general anticancer and anti-inflammatory properties. Drug development that considers the effort of enhancing the efficacy and decreasing the cytotoxicity of anti-cancer drugs is crucial in enhancing the disease progress and lowering the load of this diseases, particularly in low-resource settings where access to preventive measures and quality treatment is restricted. The previous studies reported the biological potential of tamarillo including antioxidant and antiproliferative activity on breast cancer cells. Hence the present study has been carried out to find the effect of tamarillo on cervical cancer and it may act as a novel biomolecule to provide a better remedy for other recent lifestyle diseases with its anti-inflammatory and antioxidant properties (Gobikanila and Jeyaramraja, 2024).

MATERIALS AND METHODS

Sample collection and preparation of extract

The study obtained Institutional Human Ethical Clearance and the study conducted at Advance Research Laboratory, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamil Nadu, India during January to July month on 2023. Tamarillo fruits were sourced in Gudalur, The Nilgiris District, Tamil Nadu, India. The entire tamarillo plant was authenticated by Botanical Survey of India, Tamil Nadu Agricultural University. Ripe fruits were made cleaned, cut into small pieces, weighed and mixed with 100 ml of methanol and 10 g of tamarillo fruit. The mixture was soaked for 48 hours and the extract was obtained using ultrasonication, centrifuged and filtered. The extracts were then stored at below 5°C for future usage (Goufo *et al.,* 2020).

Quantification of biologically active compounds

Evaluation of phenolic content

The Tamarillo fruit's phenolic content was estimated by the gallic acid equivalence method. 5 g of sample was homogenized and incubated for 60 minutes with 50 mL of 50% methanol in a centrifuge container. The precipitate was assembled after centrifugal separation at 1500 RCF for 15 minutes was transferred to a volumetric flask. The residue was again extracted with 50 mL of 70% acetone and the same process was repeated. Combine extract was diluted to 10 mL with Milli-Q water. 1 mL of the solution was used to dilute to 10 mL to prepare the final extracted sample solution. 500 µL of Folin-Ciocalteu reagent was added to the solution and let it stand for a period of 5 minutes. A volume of 1.5 mL of 20% (Na_2CO_3) mix was added and the suspension was incubated in the darkness for 120 minutes. The immersion was then weighed at 765 nm with a UV-spectrophotometer. The outcome of the reaction were quantified derived from a gallic acid standard grade curve and expelled as milligrams of gallic acid equivalents (GAE) per 100 g of the sample (Diep *et al.,* 2020 and Wafa, 2024).

Total flavonoid content

The amount of flavonoids in the sample was analysed by applying aluminum trichloride method. The suspension was developed by combining 0.5 mL of each sample to test tubes, followed by 1.5 mL of 95% ethanol, 0.1 mL of 10% of $AlCl_3$, 0.01 mL of 1.0 M CH_3CO_2K (potassium acetate) and 2.8 mL of deionized H_2O. The sample was vortexed to ensure thorough mixing as well as left the sample at moderate temperature (30°C) in the darkness for 40 minutes. The immersion was weighed at 415 nm with a UV-Vis spectrophotometer, with deionized H_2O serving as the control. The content of flavonoid was measured as mg of quercetin equivalent (QE)/g of tamarillo (Rohilla, 2021).

Total carotenoids content

As described by Giuffrida *et al.* (2018), 5 g of sample underwent sequential extraction using 3 × 10 mL of hexane (holding 0.1% butylated hydroxytoluene, BHT) and 3 × 10 mL of ethyl acetate (also with 0.1% BHT). After each purification movement, the suspension was ultrasonicated for fifteen minutes at moderate temperature in a darker environment and then centrifugal separation at 3000 rpm for fifteen minutes. The outcome of the suspension was percolated through a 0.45 mm syringe filter. The collected suspension was subsequently evaporated to dryness under vacuum and re-dissolved in a specified volume of methanol.

Total glycoside content

The quantitative determination of glycosides was performed by combining a 10% of fruit extract, make ready to 10 mL of Baljet's reagent and add to it, which consisted of 95 mL of 1% picric acid and 5 mL of 10% sodium hydroxide. After allowing the suspension to react for 1 hour, it was watered down to 30 mL with purified H_2O and its immersion was measured at 495 nm with a Shimadzu UV-VIS spectro-photometer. A standard curve was created using 10 mL of securidaside prepared from varying concentrations, as described by Dibulo *et al.* (2017).

Evaluation of anti-inflammatory activity

Protease inhibition assay

The proteinase inhibitory effects of tamarillo fruit were evaluated by preparing a mixture of 2 mL of 0.06 mg trypsin, 1 mL of 20% Tris-Hydrochloric acid buffer (pH 7.4) and 1 mL of the test sample (comprising 0.02 mL of extract and 0.98 mL of methanol). The solution was incubated at 38°C for five minutes, after which 1 mL of 0.8% (w/v) casein was mixed, along with an additional incubation period of twenty minutes. To terminate the reaction, 2 mL of 70% $HClO_4$ (perchloric acid) was added. The resulting suspension was separated centrifugally and the suspension of the extract was recorded at 210 nm, with the buffer serving as a blank. Phosphate buffer solution was taken as the control (Gunathilake *et al.,* 2018).

Protein denaturation inhibition assay

Denaturation of protein inhibition assay was assessed by the procedure reported by Sakat *et al.* (2010). A 1% bovine serum albumin (BSA) solution was prepared and 500 µL of this solution was mixed with varying volumes (10, 20, 30, 40 and 50 µL) of tamarillo fruit extract. The suspension was incubated at ambient temperature for ten minutes and subsequently heated at 51°C for 20 min. Followed to chill the sample to normal temperature, immersion was recorded at 660 nm with a UV-VIS spectrophotometer. Aspirin served as the standard and the analysis was carried out in triplicate performance to ensure reliability. Turbidness was weighed at 660 nm after chilling and the resulting formazan crystals were watered down in dimethyl sulfoxide (DMSO). Absorbance was then recorded at 570 nm using a microplate reader. Phosphate-buffered saline (PBS) was served as the control. Finally, the denaturation of protein inhibition percentage was recorded.

Anticancer effects of tamarillo

The MTT test was employed to assess the possibility of anticancer effects of tamarillo fruit extract. HeLa cervical cancer cells were plated at a density of 1×10^4 cells per well in 96-well and the analysis conducted with varying concentrations of tamarillo extract (10, 20, 40 and 50 µL) for 24-72 hours. Following the treatment period, 20 µl of [3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide] MTT reagent (5 mg/mL) was dropped to each well and the plates were incubated for four hours. Formazan crystals were developed and it was watered down in (DMSO- dimethyl sulfoxide) and the immersion was recorded at 570 nm with a microplate reader. The anticancer effects of tamarillo fruit extract was estimated to that of the standard chemotherapeutic drug etoposide (Akhtar *et al.,* 2022).

Statistical analysis

IBM SPSS statistical software version 25 was applied to analyze the data. Differences in cell viability among the five concentrations were reported as mean values obtained from triplicate experiments. A one-sample t-test was employed to assess anti-inflammatory properties, comparing percent inhibition between the standard and sample groups with notable levels of *p>0.014 and *p>0.010, respectively.

RESULTS AND DISCUSSION

Quantitative phytonutrients analysis

The levels of carotenoids, flavonoids, glycosides and total phenolics in tamarillo fruit extracts were examined in this study. The results showed a carotenoid concentration of 96 mg per 100 g and a significant quantity of phenolic content of 95 mg GAE per 100 g. Tamarillo is rich in carotenoids and β-carotene, compounds that are linked to lowering a thread of coronary artery disease and cancer. As a potent antioxidant, β-carotene has demonstrated the ability to alleviate oxidative stress, inflammation and apoptosis. (Giuffrida *et al.,* 2018). It also protects against chronic illnesses caused by free radicals. The carotenoids in tamarillo are responsible for the unique pigments present in the fruit.

The findings, illustrated in (Fig 2), indicated that the phytochemical profile of tamarillo is likely responsible for its various health-promoting properties. Phenolic compounds and flavonoids are the important stuff present in the fruit which exhibit the antioxidant properties by inhibiting the conversion of hydroperoxides into free radicals or by neutralizing lipid-derived free radicals. This study specifically analyzed the total phenolic (95 mg Gallic

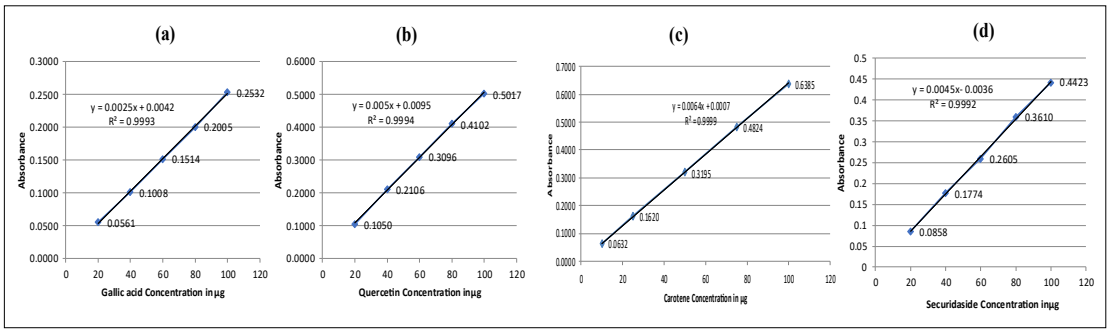


Fig 1: Standard graph of quantitative phytochemical analysis.

Acid Equivalence/100 g, compared on a gallic acid calibrate curve with (R^2 = 0.9993), total flavonoid, total glycoside and total carotenoid content in tamarillo fruits. Phenolic acids, carotenoids and flavonoids are vital secondary metabolites due to their antioxidant activities (standard graph given in Fig 1). Therefore, further studies should explore the correlation between phenolic and flavonoid content and antioxidant capacity to better understand their medicinal role in managing various disorders. The quantitative glycoside content, displayed in (Fig 2) and calculated with a securidaside calibration curve (y = 0.0045x - 0.0036, R^2 = 0.9992), showed tamarillo extract includes 10.6 mg of glycosides, which may help scavenge free radicals, modify inflammatory responses, not accelerate the spread of cancer cells and regulate apoptosis (Asih *et al.,* 2023).

Anti-inflammatory activity of tamarillo fruit

Protease inhibition assay

The study proposed that consuming tamarillo fruit may help alleviate inflammation. To explore this potential, a protein denaturation bioassay was conducted to assess the anti-inflammatory properties of tamarillo fruit extract. Methanolic extracts of tamarillo showed concentration-dependent inhibition of protein denaturation, with inhibition observed at concentrations ranging from 10 to 50 µg/mL. The proteinase inhibitory activity of the tamarillo fruit extract, detailed in (Table 1), showed inhibition rates between 15% and 64.7%. Notably, the tamarillo extracts exhibited significantly greater proteinase inhibition ($p<0.014$) compared to the standard aspirin (Stromsnes *et al.,* 2021).

Denaturation of protein inhibition

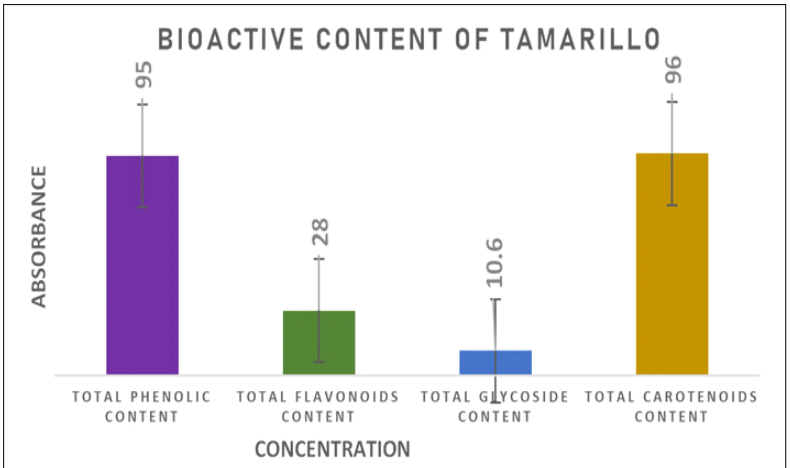
Methanolic extracts of tamarillo fruit effectively inhibited denaturation of protein in a concentration-dependent manner. The hindrance effects of tamarillo at concentrations ranging from 10 to 50 µg/mL are presented in (Table 2). The denaturation of protein inhibition per cent of tamarillo fruit ranged from 18.5% to 77% within this concentration range. Notably, tamarillo fruit demonstrated a significantly higher inhibition level ($p<0.010$) compared to the standard protease inhibitor, aspirin.

The anti-inflammatory effects of tamarillo fruit were likely attributed to its rich content of flavonoids and phenolic compounds. The primary constituents of tamarillo include phenolic compounds, flavonoids and carotenoids, which are well-known for their significant biological properties (Hossain *et al.,* 2021). In this study, the observed anti-inflammatory effects of tamarillo can be linked to its polyphenol content, potentially resulting from the synergistic interactions of its bioactive components. By preventing protein denaturation, tamarillo demonstrates promise as a candidate for anti-inflammatory drug development. Tamarillo, a widely consumed fruit, is abundant in phenolic, flavonoid and carotenoid compounds, which are key contributors to its anti-inflammatory properties (Rahman *et al.,* 2020). Phenolic and polyphenolic compounds exhibit antioxidant properties that enhance their anti-inflammatory

Table 1: Protease inhibition assay of tamarillo fruit.

Concentration	% of Inhibition protease standard (Aspirin)	% Inhibition of tamarillo
10 µg	17.43	15.00
20 µg	47.86	23.30
30 µg	58.80	35.10
40 µg	77.43	47.40
50 µg	87.69	64.70
't' value	4.716	4.213
p value	0.009	0.014

Protease inhibition assay of tamarillo fruit extract. The inhibitory activity was represented in percentage, the experimental sample was differentiated with the standard aspirin *$p<0.014$ considered highly significant.



(a) total phenolic, (b) total flavonoids, (c) total carotenoids and (d) total glycosides.

Fig 2: Quantitative phytonutrients content of tamarillo.

effects. Flavonoids, in particular, neutralize reactive oxygen species produced by neutrophils and macrophages while inhibiting the enzymes responsible for ROS production, thereby exerting potent antioxidant and anti-inflammatory activity (Sharma, 2021).

Anticancer activity of tamarillo fruit

The data revealed the cytotoxic effects of different concentrations of tamarillo fruit compared to a control-untreated cell and a standard drug (etoposide). The concentrations of the substances were (25-125 µg), respectively. The optical density (OD) of triplicate values were measured after 24 hours, 48 hours and 72 hours. The OD values are indicative of cell viability and cytotoxic effects of tamarillo fruit.

Table 2: Denaturation of protein inhibition on tamarillo.

Concentration	% Inhibition of standard (Aspirin)	% Inhibition of tamarillo
10 µg/ml	24.91	18.50
20 µg/ml	35.61	33.00
30 µg/ml	48.82	45.00
40 µg/ml	54.34	60.10
50 µg/ml	69.23	77.00
't' value	6.095	4.577
p value	0.004	0.010

Protein denaturation assay of tamarillo fruit extract. The inhibitory activity was represented in percentage, the experimental sample was differentiated with the standard aspirin *p<0.010 considered highly significant.

The population of cell viability and cytotoxicity was determined by comparing the OD values of treated cells to those of the control cells (untreated). As shown in (Table 3), cells treated with tamarillo fruit exhibited a significant decrease in cell viability, underscoring its notable antiproliferative activity. Furthermore, the percentage of cell viability decreased even further following 24-hour and 48-hour treatments with tamarillo fruit (Prasada *et al.,* 2024).

Cancer initiation arises from the activation of multiple signalling pathways, research has been conducted on the temporal regulation of events that influence cell proliferation and division. The impact of tamarillo fruit on cell cycle distribution was investigated to elucidate the underlying mechanism of its anti-proliferative properties against cancerous cells and the microscopic structure shown in Fig 3. Cell cycle analyses were conducted to assess potential of hinderance of cell cycle induced by tamarillo fruit in a cervical cancer cells. The study pointed to explored the effectiveness of tamarillo fruit on various stages of the cell cycle through flow cytometry analysis.

Cell cycle

Fig 4 depicts that untreated cells were uniformly distributed across all stages of the cell cycle suggesting the absence of any cell cycle. Cells exposed to tamarillo fruit were arrested in the G0-G1 phase, demonstrating the inhibitory effect of tamarillo fruit on mitosis progression. Conversely, cells treated with tamarillo exhibited arrest at the G0-G1-S phase, pointed, the tamarillo can arrest the cells during mitosis. Analysis of cell death distribution after exposure of tamarillo fruit was conducted to further elucidate these findings (Suganya *et al.,* 2022). Hence, tamarillo fruit

Table 3: Cell viability and cytotoxicity of tamarillo fruit.

Samples (Concentration)	24 hours (125 µg)	48 hours (125 µg)	72 hours (125 µg)
	OD values (triplicate)- % of viability		
Control cells (without treatment)	100%	100%	100%
Etoposide (standard drug)	35.59	19.07	15.28
(Fresh fruit + Methanol)	39.04	31.52	29.29
	OD values (triplicate)- % of cytotoxicity		
Control cells (without treatment)	0%	0%	0%
Etoposide (standard drug)	64.41	80.93	84.72
(Fresh fruit + Methanol)	56.11	70.23	70.71

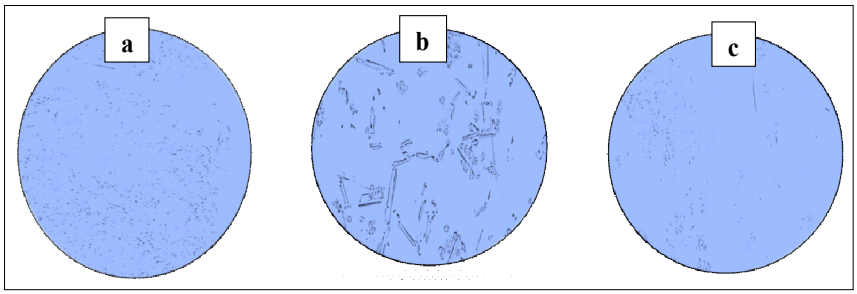


Fig 3: Microscopic view of treated cancer cells of (a) control, (b) etoposide and (c) fresh tamarillo fruit.

demonstrated the potential to hinder the cell cycle of cervical cancer cells indicated a potential promising approach for the progression of innovative curative agents for tumors (Chang *et al.,* 2015).

Cell death

Examine the process of cell apoptosis achieved by tamarillo fruit, FITC Annexin V/PI staining was performed and evaluated using flow cytometry. The outcome of the analysis demonstrated that tamarillo fruit treatment notably raise the count of cells in the early apoptotic phase, as shown in Fig 5. In the group explored with tamarillo, the percentage of early apoptotic cells was more than that of late apoptotic cells. Furthermore, the proportion of cells undergoing apoptosis in the tamarillo-treated grouping revealed a marked rise in early apoptotic cells differentiated from both the control and the standard drug, as illustrated in Fig 5.

Reactive oxygen species

It is well established that mitochondrial dysfunction is closely linked to the raising of intracellular production of reactive oxygen species (ROS). Tamarillo fruit treatment resulted in a decline in mitochondrial membrane potential, further investigation was conducted to determine if ROS generation could be the underlying cause. To explore this, cells were treated with tamarillo fruit and stained with

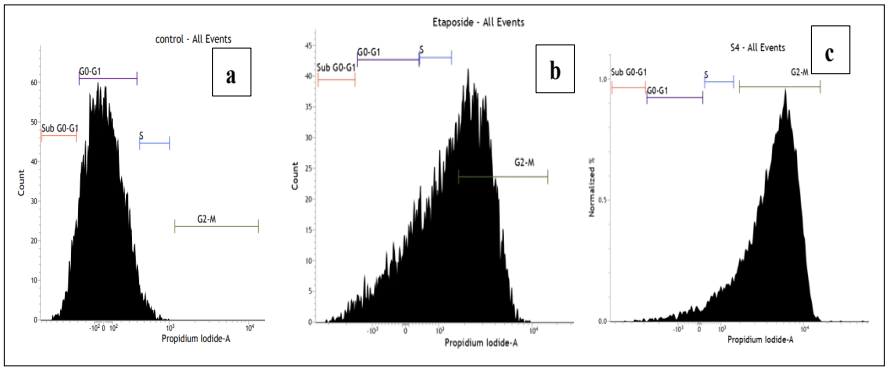


Fig 4: Cell cycle analysis for (a) control, (b) standard etoposide and (c) tamarillo fruit by flow cytometer.

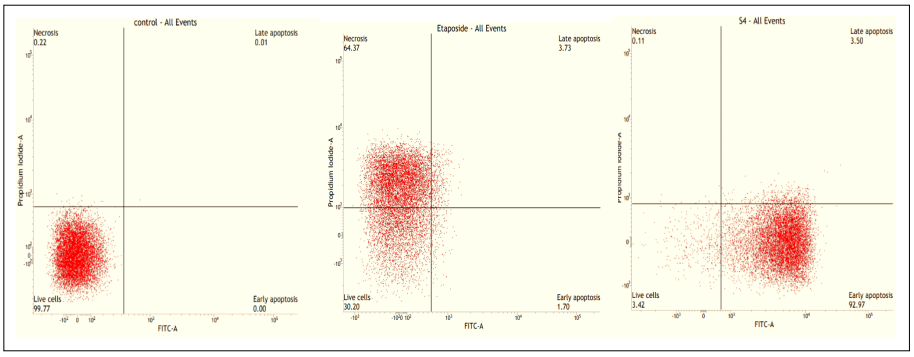


Fig 5: Cell death or cell apoptosis analysis for control, standard etoposide and tamarillo fruit by flow cytometer.

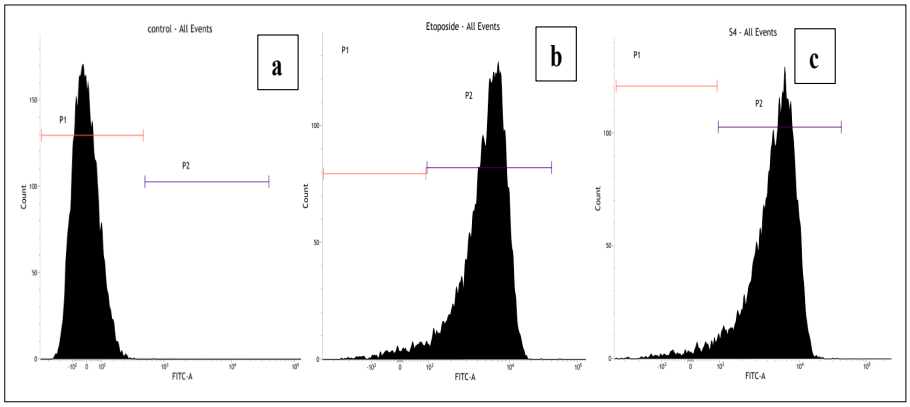


Fig 6: Reactive oxygen species analysis for control, standard etoposide and tamarillo fruit by flow cytometer.

2,7-dichlorodihydrofluorescein diacetate. A significant increase in ROS production was observed in the tamarillo-treated group, which is likely to contribute to cell death, as shown in Fig 6. Elevated ROS levels can cause oxidative stress, harm cellular structures and stimulate apoptotic processes (Prasada *et al.,* 2024).

CONCLUSION

The study concluded the possibility of tamarillo as a reasonable food source of wholesome antioxidative, specifically phenolic, flavonoid and carotenoid composites, which exhibited potent anti-inflammatory properties, primarily by their ability to neutralize harmful free radicals and inhibit inflammatory enzymes. While significant advancements have been made in cervical cancer research, there remains a persistent need to bridge awareness gaps and prioritize prevention, treatment and medication strategies. The discovery of this research features the significance of incorporating tamarillo into a balanced diet to promote overall health and well-being. Further research work are warranted to explore the specific process of mechanism of these nutraceuticals and to assess their possible therapeutic applications in various inflammatory conditions and anticancer effects. This knowledge can be applied to further pharmacological applications of the fruit. Additionally, concerted efforts are necessary to raise awareness about cervical cancer risk factors, early detection methods and the importance of regular screening.

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Author contributions

Arivazhagan Suganya: Framing of methodology, analysing and interpretation of data, preparing the draft manuscript, statistical analysis.

Chinnappan A. Kalpana: Conduct supervision and guidance, provides opinion and reviewing, input and suggestions for writing manuscripts, editing and validating.

Conflict of interest

The authors do not have any conflict of interest for this study.

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