Mediterranean Journal of
Pharmacy & Pharmaceutical Sciences
www.medjpps.com

ISSN: 2789-1895 online
ISSN: 2958-3101 print

SHORT COMMUNICATION article

Cytotoxicity study of aqueous extract of Asam Gelugur (*Garcinia cambogia)* against *Vero* cell line: Implications for nutraceutical safety

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Article number: 199, **Received:** 12-02-2025, **Accepted:** 04-04-2025, **Published online:** 07-04-2025

HOW TO CITE THIS
Sulong et al. (2025) Cytotoxicity study of aqueous extract of Asam Gelugur (*Garcinia cambogia)* against *Vero* cell line: Implications for nutraceutical safety. Mediterr J Pharm Pharm Sci. 5 (2): 36-42. [Article number: 199].
https://doi.org/10.5281/zenodo.15164035

Keywords*: Garcinia cambogia*, herbal supplements, nutraceuticals, Taxol, Vero cell line

Abstract: *Garcinia cambogia*, commonly known as Asam Gelugur, has entrenched itself as a traditional herbal medicine, renowned for its applications in treating obesity and its integration into global nutraceutical formulations. The bioactive compounds within, particularly hydroxy citric acid, mediate various effects. This study aims to assess the *in vitro* cytotoxicity of Taxol, a cytotoxic drug used as a control, and the aqueous extract from *Garcinia cambogia* against the Vero cell line - a kidney-like cell. Cytotoxicity was evaluated using the 3-(4,5-dimetyl-2-2thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) assay. The assay relies on mitochondrial succinate dehydrogenase's potential to reduce MTT, providing a colorimetric indication of cell viability. The results revealed an IC_{50} value exceeding 500 µg/mL for the aqueous extract from *Garcinia cambogia* significantly higher than Taxol's 0.0581 µg/mL. This higher IC_{50} value implies lower toxicity, positioning the *Garcinia cambogia* extract as a safe component in nutraceuticals and herbal supplements. The aqueous extract of *Garcinia cambogia* demonstrates low cytotoxicity, reinforcing its safety profile for use in nutraceuticals, which contributes valuable insights into the safety considerations of incorporating *Garcinia cambogia* into herbal supplements.

Introduction

Medicinal plants are an important natural resource and are considered potentially safe medicines. They have made significant contributions to reducing human misery throughout time [1]. Herbal medications are used in rural primary health care systems, and in distant highland places, more than 70% of the population rely on folklore over the conventional medical system [2]. Medicinal plants always provide new substances, e.g. antibiotics, alkaloids, cardiac glycosides, quinines, phenols, flavonoids, and saponins, which have many biological active functions [3, 4]. *Garcinia cambogia*, a tropical fruit native to Southeast Asia, India, and Africa, has garnered significant attention for its potential health benefits, particularly in the realm of weight management. The fruit's active component, hydroxy citric acid (HCA), has extensively been studied for its biochemical effects, including the inhibition of citrate lyase, an enzyme critical for fatty acid synthesis, and the modulation of serotonin levels, which may influence appetite suppression. These mechanisms have

ISSN: 2789-1895 online
ISSN: 2958-3101 print

positioned *Garcinia cambogia* as a key ingredient in numerous nutraceuticals and dietary supplements marketed for weight control and obesity management [5, 6]. However, despite its widespread use, questions about its efficacy and safety remain unresolved, with conflicting reports in the scientific literature. This underscores the necessity of further research into its biological effects, particularly its toxicological and cytotoxic properties. Cytotoxicity, the capacity of a compound to cause cellular damage or death, is a critical consideration in evaluating the safety of natural and synthetic substances. It plays a particularly important role in the development and assessment of pharmaceuticals, nutraceuticals, and other biologically active agents. In the context of chemotherapy, cytotoxicity is an intended property aimed at targeting rapidly dividing cancer cells. However, unintended cytotoxic effects on healthy cells can lead to significant adverse effects. Taxol (paclitaxel), a chemotherapy drug derived from the *Pacific yew* tree (*Taxus brevifolia*), illustrates this dual nature [7]. By stabilizing microtubules and disrupting cell division, Taxol effectively treats cancers such as ovarian, breast, and lung cancers [8, 9]. Nevertheless, its non-selective cytotoxicity often damages healthy tissues, causing side effects such as peripheral neuropathy, alopecia, and gastrointestinal disturbances [10]. Taxol's well-documented cytotoxic profile makes it a useful reference in toxicological studies. In contrast, the toxicological profile of *Garcinia cambogia*, a popular nutraceutical, remains poorly understood, particularly regarding its cytotoxic effects. Although HCA, the active compound in *Garcinia cambogia*, is recognized for its role in modulating fat synthesis and appetite, its impact on cellular health has not been extensively studied [11]. Reports of adverse effects, including gastrointestinal discomfort and potential hepatotoxicity, raise concerns about its safety, particularly when consumed in high doses or for prolonged periods. As *Garcinia cambogia* continues to gain popularity in consumer health products, understanding its cytotoxic effects is essential for ensuring safety and establishing appropriate regulatory standards. This study addresses this knowledge gap by comparing the cytotoxic properties of *Garcinia cambogia* and Taxol using the Vero cell line, a widely used model for evaluating toxicity in kidney-like cellular structures [12]. The research assesses cellular responses, including viability, proliferation, and morphological changes, to determine whether *Garcinia cambogia* exhibits significant cytotoxicity relative to Taxol, a compound with well-characterized cytotoxic effects. The study investigates also dose-dependent effects to identify toxicity thresholds for *Garcinia cambogia*. The findings aim to provide a detailed understanding of the cytotoxic properties of *Garcinia cambogia*, highlighting potential risks and benefits. This information is critical for promoting its responsible use in nutraceutical and pharmaceutical applications, ensuring that its health benefits are balanced against potential risks [13].

Materials and methods

Plant materials: The plant materials utilized in this study consisted of the fruit of *Garcinia cambogia*. The selection of this plant is based on its traditional uses and reputation in folk medicine, particularly in the treatment of obesity. The fruit's powder was procured from Sunpure Extracts Private Limited (99A, Sunpure, Dilshad Garden, Delhi, India) ensuring a reliable and standardized source. Taxol and Whatman No 4 filter paper (20-25 µm) were purchased from Merck KGaA (Frankfurter, Darmstadt, Germany). Vero cells were purchased from the American Type Culture Collection (ATCC) (ATCC, 1549, Manassas, USA).

Extraction procedure: The grounded fruit powder was mixed thoroughly with water with 1: 1 solid: solvent ratio, creating a homogenous mixture. This mixture underwent filtration 24 hrs intervals repeatedly through Whatman No 4 filter paper until to obtain a clear solution [14-16]. The extracted solution was condensed by a vacuum rotary evaporator. The filtrate, containing the water-soluble compounds from *Garcinia cambogia*, was then subjected to freeze-drying (-70ºC) [17]. This was further frozen at -70°C and shifted instantly to three weeks of successive freeze drying at -50°C using a bench top freeze dryer to give an ultimate yield [18]. This process ensured the removal of excess water, resulting in obtained water-free crude extracts for further analysis.

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ISSN: 2958-3101 print

Storage and handling: The obtained crude extracts were stored under controlled conditions to prevent degradation and maintain their stability. Proper labeling and documentation of the samples were implemented to facilitate traceability and ensure the reliability of subsequent analyses. This methodology outlines the systematic approach employed in obtaining and preparing plant materials from *Garcinia cambogia* for the subsequent cytotoxicity study. The emphasis on standardized extraction procedures and meticulous handling serves to maintain the integrity of the samples, providing a foundation for the forthcoming stages of the research.

Cell culture and MTT-assay assay for toxicity study: Vero cells were cultured in 25 t-flask and were maintained in Dulbecco‟s modified Eagle‟s medium supplemented with 100 IU/ mL penicillin, 100 µg/mL streptomycin, 10.0% fetal bovine serum at 37°C with 5% CO_2, 95% air and complete humidity [19]. They were detached using 0.05% trypsin/ethylene diamine tetra acetic acid and counted using trypan blue and hemocyto meter when reached ~90% confluence and then resuspended at a concentration of 4×104 cells/cm^2 to add into a 96-well plate (250 µL/well) via a channel pipette. Some wells were kept cell-free as blanks (controls) for background absorption and comparison. The cell viability was assessed by the MTT colorimetric assay which is based on the reduction of MTT by the mitochondrial succinate dehydrogenase of intact cells to a purple formazan product. Briefly, 100 µL aliquots of the exponentially growing Vero cells containing 5×104 cells/mL were added to each well of 96-well flat-micro titer plates and incubated with various concentrations of the selected plant‟s crude extract dissolved in 0.1% dimethyl sulfoxide (DMSO). Four replicate wells were used in each point in the experiments. After 48 hrs incubation at 37°C, MTT solution (5.0 mg/mL in PBS) was added and incubated for another 4 hrs at 37°C in a 5% CO_2 incubator. The resulting MTT-formazan product was dissolved by the same volume of lysis buffer (10% SDS–0.1 M HCl) and the incubation was continued overnight at 37°C [20, 21]. The amount of formazan was determined by measuring the absorbance at 570 nm using a Bio-Rad 550 ELISA microplate reader. Positive control: Taxol, a well-known cytotoxic drug, was used as the positive control in the assay. This allowed for the comparison of the cytotoxic effects of Taxol with the aqueous extract from Asam Gelugur.

Results and discussion

Toxicity studies are conducted to determine the toxicity of a substance. This is important because substances that have therapeutic potential may also cause adverse effects. In this study, cytotoxicity testing, which measures the degree of a substance having destructive action on cells. The cells used for the test were Vero, which is a kidney-like cell line. Cytotoxicity was assessed by 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) assay. The assay is a colourmetric assay, based on mitochondrial succinate dehydrogenase potential to reduce MTT. Reduction of MTT can occur in metabolically active cells, therefore, the level of MTT reduction is an indication of cell viability. Dose-response curves were generated for each test substance and the median inhibitory concentration (IC_{50}) was determined by linear regression.

Table 1 illustrates the survival rates of Vero cell lines following a 24-hour treatment with TS006. The concentrations of the extract, measured in µg/mL, are correlated with the percentage of cell viability, indicating the impact on the cells. It elucidates the impact of varying concentrations of TS006, on the viability of vero cell lines after a 24-hr treatment period. Notably, at the concentration of 0.0 µg/mL, the cell viability remained high at 99.99%, indicating minimal cytotoxicity. As the concentration increased, a fluctuation in cell viability was observed. At lower concentrations, 7.8125 µg/mL and 15.625 µg/mL, the mean viability percentage was slightly elevated (107.15% and 97.85%, respectively). This suggests a potential stimulatory effect or a mild response to the extract. However, as the concentration reached 31.25 µg/mL, a slight decrease in viability to 96.69% was noted, indicating a possible concentration-dependent cytotoxic effect. Interestingly, at higher concentrations (62.5 µg/mL, 125 µg/mL, 250 µg/mL, and 500 µg/mL), the mean viability percentage increased, reaching a peak at 500 µg/mL with 118.75%. This unexpected increase may warrant further

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ISSN: 2958-3101 print

investigation to understand the underlying mechanisms and whether it represents a hormetic response. The standard errors of the mean provide insight into the reliability of the results. The variations observed in the data suggest a degree of heterogeneity in the cell response to different concentrations of TS006. Overall, these findings underscore the importance of a comprehensive dose-response analysis to discern the nuanced effects of the aqueous extract of *Garcinia Cambogia* on Vero cell viability.

Table 1: The survival of the vero cell line after 24 hours of treatment with TS006

Concentration (µg/mL)	Viability (%)	
	Mean	S.E.M
0.00	99.99	0.004
7.8125	107.15	4.69
15.625	97.85	6.78
31.25	96.69	4.02
62.5	104.55	1.81
125	108.96	1.86
250	109.01	3.01
500	118.75	1.11

Table 2 presents the survival of Vero cell lines after a 24-hour treatment with Taxol, a cytotoxic drug utilized as a positive control. The concentrations of Taxol ranged from 0.0 µg/mL to 1.0 µg/mL. A comparison with the result TS006 which depicted the effects of TS006, reveals distinct patterns in the cytotoxic response. At the lowest concentration (0.0 µg/mL), Taxol displayed no cytotoxicity, maintaining cell viability at 100%, similar to the TS006. However, as the concentration increased, a notable decrease in cell viability was observed. At concentrations of 0.00001 µg/mL and 0.0001 µg/mL, the mean viability percentage dropped to 91.64% and 88.70%, respectively, indicating the onset of cytotoxic effects.

Table 2: The survival of the Vero cell line after 24 hours of treatment with Taxol

Concentration (µg/mL)	Viability (%)	
	Mean	S.E.M
0.0	100.00	0.004
0.00001	91.64	15.72
0.0001	88.70	12.53
0.001	85.91	8.01
0.01	86.44	9.74
0.10	70.21	9.70
1.00	50.77	5.81

The decreasing trend continued with concentrations of 0.001 µg/mL, 0.01 µg/mL, and 0.1 µg/mL, reaching a mean viability percentage of 85.91%, 86.44%, and 70.21%, respectively. These concentrations demonstrate a dose-dependent cytotoxic response of Taxol on Vero cells. The highest concentration of Taxol (1.0 µg/mL) exhibited a substantial decrease in cell viability to 50.77%, emphasizing the potent cytotoxic nature of the drug. This stark reduction in viability contrasts with the unexpected increase observed at the highest concentration of TS006. The standard errors of the mean in tables indicate the variability in the experimental results. The comparison highlights the contrasting behaviors of Taxol and TS006. While Taxol demonstrates clear dose-dependent cytotoxicity, the effects of TS006 appear more nuanced, with fluctuations in cell viability at different concentrations. These distinct responses underscore the importance of considering specific compounds and their concentrations when assessing cytotoxicity in experimental settings.

Table 3 displays the median IC_{50} values for TS006 and Taxol. The IC_{50} values provide insight into the concentration at which each sample inhibits 50% of cell viability. Here, the results reveal notable differences between the two substances. TS006 shows an IC_{50} value of ≥500 µg/mL. This higher IC_{50} value suggests that

ISSN: 2789-1895 online
ISSN: 2958-3101 print

a relatively high concentration of the aqueous extract is required to achieve a 50.0% reduction in cell viability. It indicates a lower cytotoxic potency compared to Taxol. In contrast, Taxol exhibits a significantly lower IC_{50} value of 0.0581±0.0303 µg/mL. This implies that Taxol is highly potent, with a relatively small concentration needed to exert a 50.0% inhibitory effect on cell viability. The narrow range of the standard error of the mean for Taxol further indicates the consistency and precision of these results across independent experiments. The comparison between the two IC_{50} values underscores the distinct cytotoxic profiles of TS006 and Taxol. Taxol, a known cytotoxic drug, demonstrates a potent inhibitory effect at a low concentration, while TS006, exhibits a less potent cytotoxicity, requiring a substantially higher concentration for a similar impact. These findings contribute to our understanding of the differential cytotoxic potential of natural extracts compared to synthetic drugs, providing valuable information for further exploration and potential therapeutic applications.

Table 3: Median inhibitory concentration (IC_{50}) values of TS006 and Taxol

Test samples	IC_{50} (µg/mL)
TS006 (aqueous extract *Garcinia combogia*)	≥500
Taxol	0.0581±0.0303

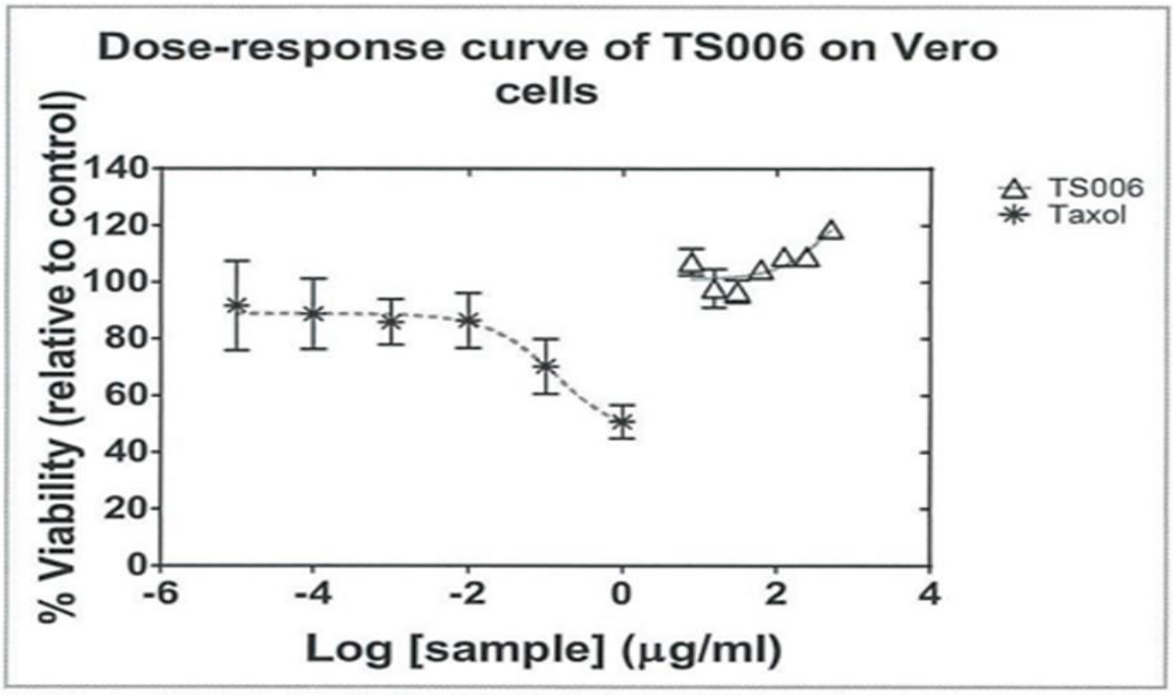

Figure 1: Dose-response curves of TS006 and Taxol on Vero cell line. Data are mean±S.E.M.

The dose-response curves for Taxol and TS006 provide crucial insights into their cytotoxic effects on Vero cells. The discussion revolves around the patterns observed in the viability percentages at varying concentrations, shedding light on their relative potency and efficacy. Taxol, a well-known cytotoxic drug, exhibits a dose-dependent decrease in cell viability. At lower concentrations (0.00001 to 0.001 µg/mL), there is a gradual decline in cell viability, indicating the initiation of cytotoxic effects. The curve steepens between 0.001 and 0.1 µg/mL, demonstrating an escalating impact on cell survival. Notably, at concentrations beyond 0.1 µg/mL, the viability drops more rapidly, reaching the lowest point at 1.0 µg/mL. This steep decline signifies the potent cytotoxic nature of Taxol, consistent with its established role as an anticancer agent. Contrastingly, TS006 displays a less steep dose-response curve. At concentrations up to 500 µg/mL, there is a gradual reduction in cell viability, suggesting a milder cytotoxic effect. The curve indicates a more gradual decline compared to Taxol, emphasizing the lower cytotoxic potency of TS006. The viability percentage

ISSN: 2789-1895 online
ISSN: 2958-3101 print

remains relatively high even at the highest concentration tested (500 µg/mL), reinforcing the extract's lower impact on cell survival. Comparing the two dose-response curves reveals the stark contrast in cytotoxic potency between Taxol and TS006. Taxol demonstrates a potent and rapid decrease in cell viability, typical of cytotoxic drugs designed for cancer treatment. In contrast, TS006 exhibits a milder and more gradual reduction in viability, characteristic of many natural extracts. This preliminary result indicates a dose-dependent cytotoxic effect of Taxol on Vero cells. Lower concentrations demonstrated minimal impact, mirroring the drug's selective nature. However, higher concentrations revealed a notable reduction in cell viability, suggesting a potential risk to normal renal cells. The IC_{50} value for the aqueous extract from *Garcinia combogia* surpassed 500 µg/mL, signifying lower toxicity compared to Taxol's 0.0581 µg/mL. This substantial difference highlights the potential safety of *Garcinia combogia*, positioning it favorably for inclusion in nutraceuticals and herbal supplements. The obtained results unveiled a notable difference in the IC_{50} values. The aqueous extract from *Garcinia combogia* demonstrated an IC_{50} value exceeding 500 µg/mL, significantly higher than Taxol's 0.0581 µg/mL. This higher IC_{50} value suggests a lower level of cytotoxicity for the extract, positioning it as a potentially safer alternative. The discussion delves into the implications of the observed lower cytotoxicity of the aqueous extract from *Garcinia combogia*. The historical use of *Garcinia cambogia* aligns with the study's findings, substantiating its potential safety in contemporary nutraceuticals and herbal supplements. The identified HCA content is likely a key contributor to its observed effects. However, this study is limited by its reliance on the Vero cell line as a model, which may not accurately reflect the complex interactions and cytotoxicity profiles found in human tissues. Moreover, the analysis focused exclusively on the cytotoxicity of *Garcinia cambogia* extract without isolating or examining the specific effects of its primary bioactive compound, HCA, thereby limiting understanding of the compound's distinct contributions. The lack of *in vivo* experiments or clinical trials further constrains the applicability of the findings to practical settings.

Conclusion: Garcinia combogia aqueous extract exhibits low cytotoxicity, supporting its safe utilization in nutraceuticals and herbal supplements. This study contributes substantively to the ongoing discourse on the safety and efficacy of herbal supplements, paving the way for future investigations into the myriad benefits offered by *Garcinia combogia*. This study contributes to the ongoing dialogue on optimizing Taxol administration, ensuring its efficacy in cancer therapy while safeguarding the integrity of vital organs.

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Acknowledgments: The authors gratefully acknowledge the Faculty of Science, University Malaya for providing the facilities and Vannajan Sanghiran Lee for her instrumental support. Special thanks to the Research Management Centre, University College MAIWP International for financial support by awarding the Tun Ahmad Sarji Research Fund.
Author contribution: NAS conceived, and designed the study. NAS & NSN collected data. RM & AKA contributed to data analysis. NSN performed data analysis and interpretation. NAS, RM, VSL & AKA drafted and reviewed the manuscript for intellectual context. All authors approved the final version of the manuscript and agreed to be accountable for its contents.
Conflict of interest: The authors declare the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
Ethical issues: The authors observed ethical issues including plagiarism, informed consent, data fabrication or falsification, and double publication or submission.
Data availability statement: The raw data that support the findings of this article are available from the corresponding author upon reasonable request.
Author declarations: The authors confirm that they have followed all relevant ethical guidelines and obtained any necessary IRB and/or ethics committee approvals.