# ANTIPROLIFERATIVE ACTIVITY AND POLYPHENOL ANALYSIS IN TOMATO (SOLANUM LYCOPERSICON)

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**Abstract**. Hepatocellular carcinoma (HCC) is among the top three causes of cancer death worldwide, with a poor response to pharmacological treatment. Doxorubicin, an antineoplastic chemotherapeutic medication used frequently to treat advanced HCC owing to its antitumor effects, has demonstrated insufficient efficacy, with a 15-20 % response rate. *Solanum lycopersicum*, or tomato, originated from the Solanaceae family. This study intended to investigate the polyphenol distribution in *S. lycopersicum* and antiproliferative activity against liver cancer cell lines. Antiproliferative effects were determined using the MTT assay and cell morphology analysis using the AO/PI double staining. Caffeic and gallic acids displayed the most abundant phenolic acids in *S. lycopersicum* extract. The ethanolic extract induced cytotoxicity against the HepG2 cell line with an $IC_{50}$ value of 48.0 µg/mL. The percentage of cells in the G2/M phase reduced significantly ($p < 0.05$) from 18.8 % to 6.2 % at higher concentration (72 g/mL) for 48 hours of treatment. There was a significant ($p < 0.05$) two-fold increment in cytochrome c level in the cytosol of HepG2 cells following exposure to 24 µg/mL of *S. lycopersicum* extract for 24 hours. The present study showed that the ethanolic extract of *S. lycopersicum* inhibits the initiation and progression of liver cancer cell lines due to its high phenolic content. These findings show that plant phenolics have both preventative and therapeutic cancer-fighting potential, necessitating additional research.

**Keywords:** *S. lycopersicum*, antiproliferative activity, HepG2, polyphenol, apoptosis

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## Introduction

The living cells constantly produce free radicals through the respiratory chain during energetic metabolisms. These reactive oxygen species (ROS) can be either detrimental or beneficial to our bodies. The human body has several strategies to neutralise the effects of free radicals and oxidative stress, based on enzymatic (e.g., SOD, CAT, and GPx) and nonenzymatic antioxidant molecules (e.g., lipoic acid, glutathione, L-arginine, and coenzyme Q10) that act as endogenous antioxidants. Besides these, exogenous antioxidants obtained from the diet could promote more protection and increase DNA repair to avoid the earlier development of chronic diseases [1]. Dietary polyphenols are naturally occurring compounds primarily found in fruits, vegetables, and medicinal herbs. These molecules include phenolic, flavonoids, vitamins, terpenoids, and other secondary metabolites that protect against oxidative damage through various mechanisms such as antioxidants, anticarcinogenic, and anti-inflammatory activities [2].

The major component of dietary polyphenols is flavonoids. It can be classified into multiple classes: flavones, flavonols, flavanols, flavanones, isoflavones, and anthocyanidin. Each flavonoid group can act as an antioxidant by several mechanisms: destroying ROS formation, inhibiting the lipid peroxidation induced by prooxidants, and scavenging the lipid peroxidation radicals. Chemoprevention using herbal and plant-derived compounds is innovative in cancer research, concentrating on hindering carcinogenesis through initiation, promotion, and progression stages [3].

Tomato, or *Solanum lycopersicon*, is an essential vegetable in the world that belongs to the Solanaceae family. It is usually eaten fresh or as processed foodstuffs. Antioxidants in tomatoes include carotenoids, antioxidant vitamins, and phenolic compounds [4]. Chemopreventive actions of phytochemicals may be exhibited through several mechanisms: i) action of antioxidant enzymes, ii) cell cycle arrest, iii) induction of cell death and necrosis, iv) autophagy, and v) inhibition of the expression of genes suppressing cancer progression. Epidemiological studies have revealed that *S. lycopersicum* is a potent cancer chemopreventive agent mainly accredited to its antioxidant properties [5]. Hence, this study aims to assess the antioxidant capacities of *S. lycopersicum* and its polyphenol distributions (phenolic acids, flavanols, flavonols, and flavanones) against the proliferation of liver cancer lines (HepG2).

## Materials and Methods

### *Sample Preparations*

The powder of *S. lycopersicum* was extracted for 24 hours with 80 % ethanol at room temperature. Following drying by evaporation at 50 °C, the dried sample (50 mg) was dissolved in dimethyl sulfoxide (DMSO) to provide a 1 mg/mL working solution.

### *Free Radical Scavenging Activity (FRSA)*

The FRSA was achieved referring to a technique described by Lim and Tee [6]. The percentage of radical scavenging activities (SA) was determined using the following formula:

$$\% \text{ SA} = \frac{\text{A}_{\text{control}} - \text{A}_{\text{sample}}}{\text{A}_{\text{control}}} \times 100 \quad (1)$$

where $A_{control}$ = absorbance of DPPH alone and $A_{sample}$ = absorbance of DPPH along with different concentrations of extracts.

***Beta-carotene Bleaching Assay (BCBA)***

The BCBA was executed following the method described [7]. At a wavelength of 470 nm, the absorbance was measured every 15 minutes for two hours. DMSO was used as a blank, and butylated hydroxytoluene (BHT) was used as a control. The bleaching rate (R) of β-carotene is measured with below equation:

$$\text{R} = \text{In}\left[\frac{\text{Ab0}}{\text{Abt}}\right]/120 \quad (2)$$

where ln = natural logarithm, Ab0 = absorbance at 0 minute and Abt = absorbance measured every 15 minutes for two hours (120 minutes).

Additionally, the antioxidant activity (AOX) was measured using the equation below:

$$\% \text{ AOX} = \frac{\text{DR}_{\text{control}} - \text{DR}_{\text{sample}}}{\text{DR}_{\text{control}}} \times 100 \quad (3)$$

where $DR_{control}$ = degradation rate of β-carotene in the control and $DR_{sample}$ = degradation rate of β-carotene in the sample.

***Total Phenolic (TPC) and Flavonoid (TFC) Contents***

The TPC was measured referring to the technique adapted from Velioglu et al. [8] with slight modifications. The optical density was monitored at 725 nm. The TPC was expressed in mg of gallic acid equivalents GAE/g dry weight (DW) samples.

The TFC was quantified from the quercetin standard calibration curve, plotted at 0.02 to 0.1 mg/mL. The flavonoids contents were reported as mg of quercetin equivalents (QE)/g DW samples [4].

***MTT Assay***

HepG2 and 3T3 normal fibroblast cells were acquired from the ATCC in Virginia, United States. The cells were grown to maturity in 75 $cm^2$ flasks containing RPMI-1640 supplemented with 10 % FBS and 1 % Pen Strep, and place in 5 % carbon dioxide incubator.

The antiproliferative activity was assessed using cell viability by the MTT assay. The cells 1 × $10^5$ cells/mL were exposed to the extract in various concentrations (6.25–200 µg/mL) and treated with doxorubicin (0.20–25 µg/mL). The absorbance of the coloured solution was measured at 570 nm using a plate reading spectrophotometer after 72 hours of extract and drug exposure. The calculation for the percentage (%) of cytotoxicity was as follows:

$$\% \text{ cytotoxixity} = \frac{\text{OD}_{\text{sample}}}{\text{OD}_{\text{negative control}}} \times 100 \quad (4)$$

where $OD_{sample}$ = absorbance values of the sample and $OD_{negative\ control}$ = absorbance values of the no treatment control

### *Apoptosis Determination by Acridine Orange/Propidium Iodide (AO/PI) Double Staining*

About $1 \times 10^5$ cells/mL of HepG2 cells were seeded onto six-well plates for 24 hours. After that, the cells were preserved with *S. lycopersicum* extract at the $IC_{50}$ concentration and RPMI with 1 % DMSO as a negative control. The 10 µL of cells were mixed with 5 µL of AO/PI and detected under a fluorescence microscope [9].

### *Analysis of DNA Fragmentation*

The HepG2 cells were treated with *S. lycopersicum* extract at the $IC_{50}$ concentration, whereas treatment with doxorubicin, at 8 µg/mL, and RPMI medium with 1 % DMSO served as a positive and negative control, respectively. The test is performed following the manufacturer's kit assay. After 72 hours, the fragmented nuclei were observed using FluorChem 5500 Chemiluminescent.

### *Determination of Polyphenol by HPLC*

The polyphenol analyses of *S. lycopersicum* were determined by HPLC-DAD-MS [10]. The determination of hydroxybenzoic acids (gallic and vanillic acids), flavan-3-ols (epigallocatechin gallate) and flavanones (naringin) were performed at 280 nm, hydroxycinnamic acids (chlorogenic, ferullic, p-coumaric, trans-ferullic and caffeic acids) were performed at 320 nm, and flavonols (quercetin and kaempferol) were performed at 360 nm and 280 nm, respectively.

### *Statistical Analysis*

The experimentations were completed in three replicates of independent experiments. Results were reported as mean ± standard deviation (S.D) using the SPSS, version 25.0. The Independent-Sample T-test was used to compare means for cytotoxic activity with the untreated group. The level of statistical significance was set at the level of $p < 0.05$.

## Results and Discussion

### *DPPH Free Radical Scavenging Activity (FRSA)*

This FRSA measures the extract's capability to donate an electron to scavenge the DPPH radicals, thus blanching its purple hue. The extract with the maximum Effective Concentration ($EC_{50}$) value exhibits the smallest FRSA activity and vice versa. At various doses (10–80 µg/mL), ascorbic acid (AA) served as a positive control. Ascorbic acid is a remarkably effective scavenger of alkoxy radicals, even at a lower concentration. Contrarily, the sample extract was prepared at different concentrations from 1 to 8 mg/mL in DMSO. *S.*

*lycopersicum* (0.45 mg/mL ± 0.05) had substantially ($p < 0.05$) low $EC_{50}$ to scavenge the stable DPPH radical (Figure 1).

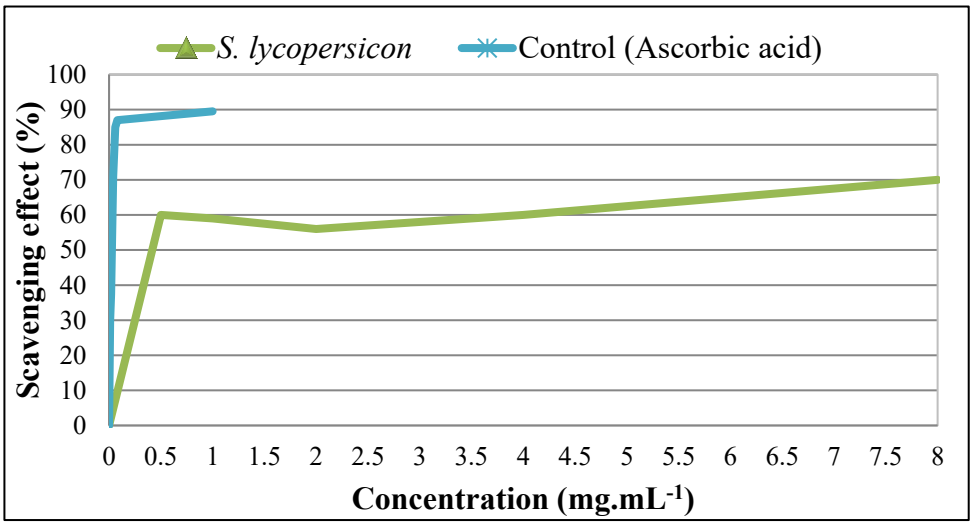


**Figure 1:** Scavenging activity of *S. lycopersicum* extract.

### *Beta-Carotene Bleaching (BCB) Activity*

The antioxidant activity (AOA) of *S. lycopersicum* (71.60 ± 2.10 %) assayed using BCB assay is shown in Table 1.

**Table 1:** Total phenolic content (TPC) and antioxidant activities (AOA) of *S. lycopersicum* extract (mean ± SD of triplicate determinations).

| **Antioxidant analyses** | ***S. lycopersicum* (mean ± SD)** |
|---|---|
| TPC (mg GAE/g DW) | 1.70 ± 0.10 |
| TFC (mg RE/g DW) | 0.35 ± 0.19 |
| AOA (%) | 71.60 ± 2.10 |
| $EC_{50}$ (mg/mL) | 0.45 ± 0.05 |

Figure 2 shows the degradation rate (DR) of *S. lycopersicum* extract, in which the rate of BCB can be overcome with the presence of antioxidants. The DR were measured at 470 nm of the *S. lycopersicum* extract in comparison with BHT (300 µg/mL). The DR rate of BHT was stable, demonstrating the efficacy of BHT in stopping the degradation of β-carotene, followed by *S. lycopersicum* extract, and control which oxidised quickly. The extract with the slowest of β-carotene DR demonstrated the maximum antioxidant activity. Results show that the *S. lycopersicum* exhibited significantly ($p < 0.05$) high AOA but lower than the standard (BHT).

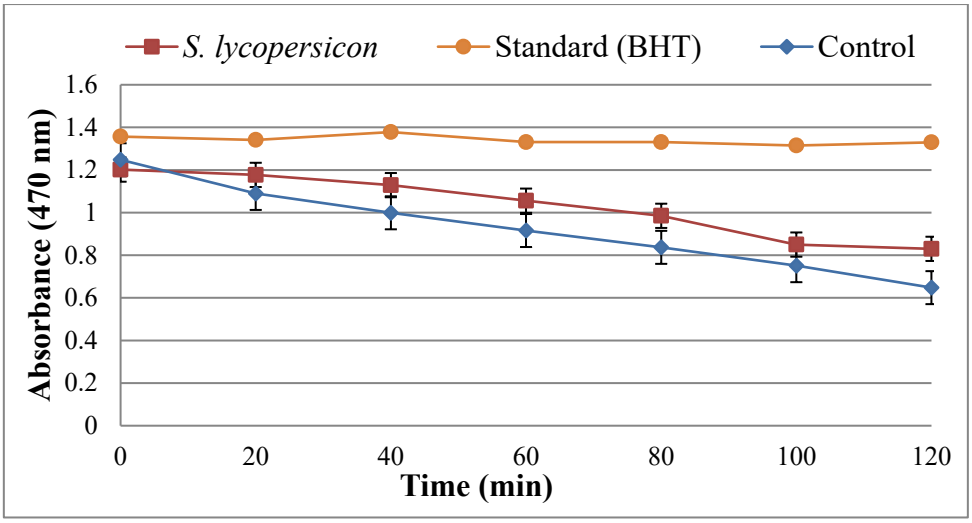


**Figure 2:** Degradation rate of *S. lycopersicum* extract.

### *Total Phenolic and Flavonoid Content*

Table 1 shows the TPC and TFC values reported as mg GAE/g in DW samples. Gallic acid (GA) was utilised as a control, and the amount of phenolic compounds was determined from a calibration graph using the straight-line equation y = 0.2503x + 1.4771 ($R^2$ = 0.8827). Statistical analysis indicated that *S. lycopersicum* contains substantial polyphenol (1.70 ± 0.10 mg GAE/g DW). A previous study by Francisco et al. [11] reported that the TPC of tomatoes was measured from 0.37 to 0.86 mg GAE/g FW.

The TFC values were measured using the aluminum chloride colourimetric method. The amount of TFC was represented as quercetin equivalent (mg QE/g DW). Table 1 shows that *S. lycopersicum* demonstrated a high TFC of 0.35 ± 0.09 mg QE/g DW compared to a previous study that reported TFC in tomatoes were around 0.06 mg QE/g FW samples [12].

### *Cytotoxicity Activity*

The cytotoxic behaviour of *S. lycopersicum* was tested on the liver (HepG2) adenocarcinoma and normal mouse fibroblast (3T3). Increased of *S. lycopersicum* extract concentration against HepG2 cells leads to a higher cytotoxic activity. Figure 3(a) shows that *S. lycopersicum* extract strongly inhibits the HepG2 cancer cell line proliferation with the $IC_{50}$ values of 48.0 µg/mL. It was verified that an increase in the concentration of *S. lycopersicum* extract cells leads to a higher cytotoxic activity against HepG2 cells which agrees with the previous study by Mutalib et al. [13]. On the other hand, doxorubicin (chemotherapy drug) strongly induced cytotoxicity in HepG2 at a concentration of 0.35 µg/mL. Nevertheless, doxorubicin also displayed a significant antiproliferative effect against 3T3 at an $IC_{50}$ value of 8.0 µg/mL (Figure 3(b)) compared to the sample extract with minimum cytotoxic effect on the proliferation of 3T3 cells ($IC_{50}$ greater than 200.0 µg/mL). Doxorubicin treatment currently used in chemotherapy shows nonspecific cytotoxicity, affecting normal cells and cause serious side effects to the cancer patient due to the unpredictable cytotoxic properties. Our findings suggest that *S. lycopersicum* extract displayed selective killing properties against HepG2 cells and was not toxic to normal cells. Natural products have displayed selective benefits against cancer cells compared to normal

cells by targeting tumour cells by regulating cell death pathways such as extrinsic and intrinsic apoptosis and autophagic pathways [14].

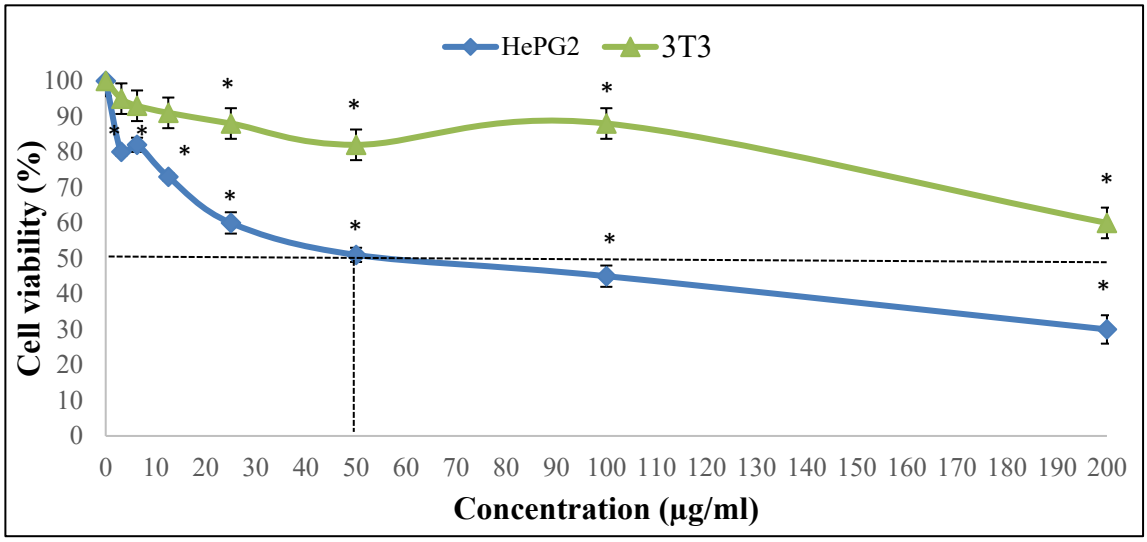


**Figure 3(a):** Dose dependent inhibition of *S. lycopersicum* on proliferation of HepG2 (liver) cancer and 3T3 cells (normal mouse fibroblast), assessed by MTT assay. Data represent the mean values ± SD, ≤ 0.05 versus untreated cells.

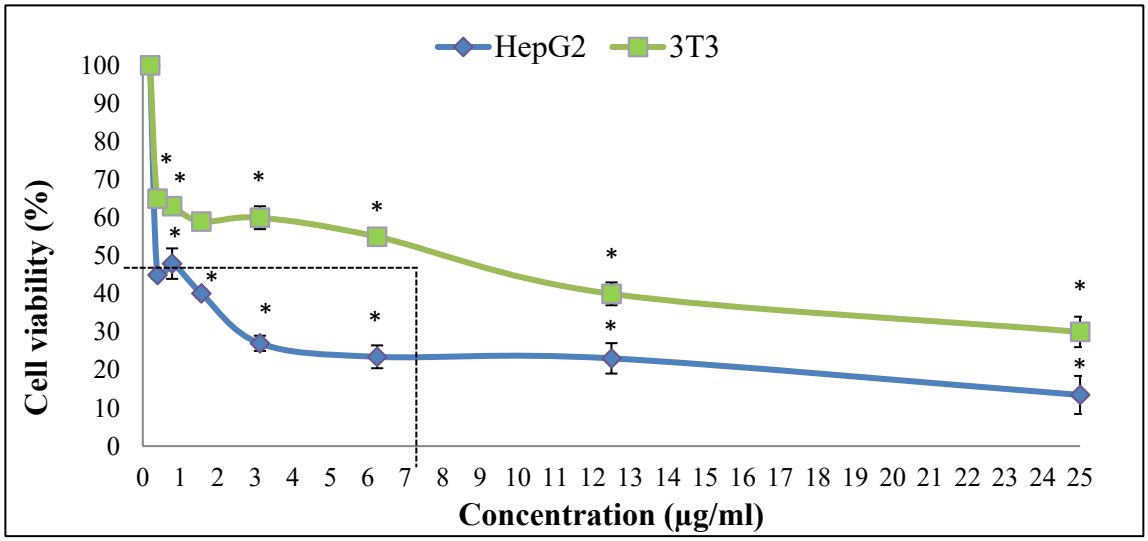


**Figure 3(b):** Dose dependent inhibition of doxorubicin on proliferation of HepG2 (liver) cancer and 3T3 cells (normal mouse fibroblast), assessed by MTT assay. Data represent the mean values ± SD, ≤ 0.05 versus untreated cells.

### *Fluorescent Microscopy*

Apoptotic and viable HepG2 liver cells treated with *S. lycopersicum* extract were observed under a fluorescent microscope. Healthy viable cells were visualised as round and large, with intact green nuclear structures (Figure 4A). In contrast, cells treated with *S.*

*lycopersicum* extract contracted and exhibited fragmented nuclei, confirming dose-dependent apoptosis (Figures 4C and 4D).

Furthermore, the treated cells were visible asymmetrically with membrane blebbing and nuclear margination. Early apoptosis features were seen for HepG2 cells treated with doxorubicin (Figure 4(B)). Acridine orange entered the fragmented DNA and appeared with nuclear condensation, representing moderate programmed cell death. The late phase of apoptosis displayed with the appearance of an apoptotic body detected with red fluorescence signifies the hallmark of late apoptosis, which can be seen clearly in HepG2 treated at a higher concentration (72 µg/mL) of *S. lycopersicum* extract (Figure 4D).

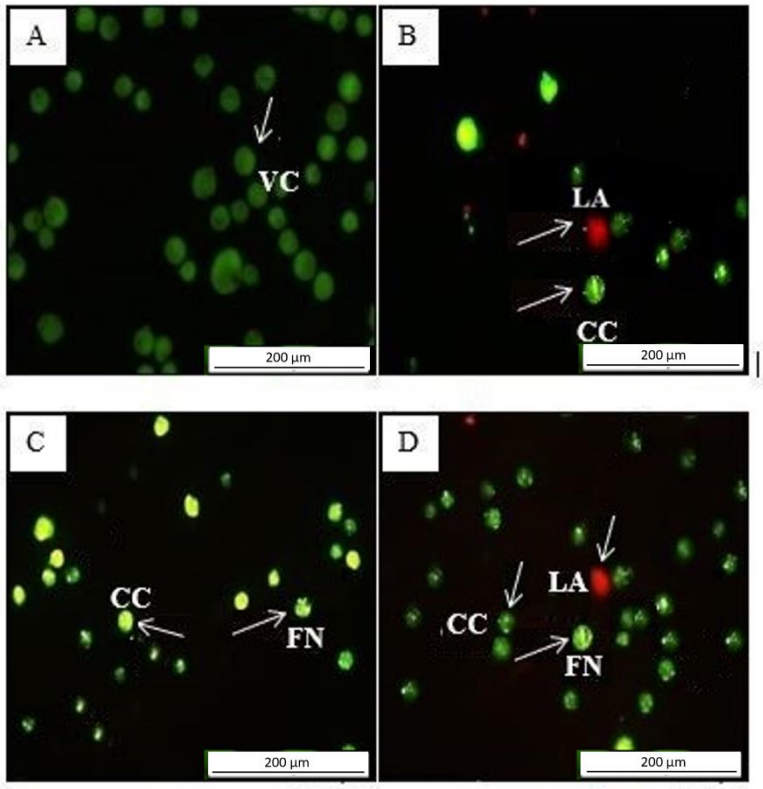


**Figure 4:** Fluorescent micrographs of HepG2 liver cells treated with *S. lycopersicum* extract (48 and 72 µg/mL). Morphological changes include: (A) Untreated HepG2 displays typical cell structure, and the viable cells (VC) fluoresce with green colour and have rounded complete nuclei, (B) HepG2 cells preserved with 8 µg/mL of doxorubicin showed chromatin condensation (CC), late apoptosis (LA), (C) HepG2 treated with *S. lycopersicum* extract (48 µg/mL) showing chromatin condensation (CC) and fragmented nuclei (FN) and (D) HepG2 treated with *S. lycopersicum* extract (72 µg/mL) appeared as chromatin condensation (CC), fragmented nuclei (FN), and late apoptosis (LA) events.

### *DNA Ladder Assay*

Figure 5 shows the internucleosomal DNA fragment observed under the UV illuminator in HepG2 cells treated with *S. lycopersicum* extract at $IC_{50}$ and $IC_{75}$ concentrations, respectively (Lane 2 and 3). The DNA band was not detected in the control (untreated) cultures, as displayed in Lane 1. The commercial doxorubicin-induced DNA

fragmentation at the lowest concentration (8 µg/mL), as revealed in Lane 4. These findings indicate that the cytotoxic action of *S. lycopersicum* extract caused significant DNA damage, resulting in programmed cell death in liver cells.

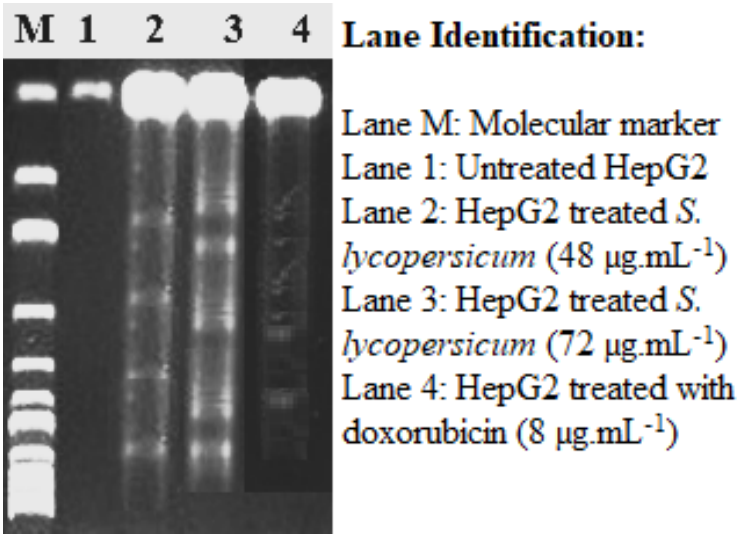


**Figure 5:** Internucleosomal DNA fragment observed under the UV illuminator in HepG2 cells

### *Phytochemical Compositions*

### *a) Phenolic Acids Composition*

Phenolic acid contents extracted from *S. lycopersicum* extract are shown in Table 2. The most abundant phenolic acids examined in *S. lycopersicum* were caffeic and gallic, followed by chlorogenic and vanillic acids. Meanwhile, ferulic acid and trans-ferulic were found in a moderate amount that ranged from 1.54 to 1.99 µg.g$^{-1}$ DW. However, *p*-coumaric acid was found in a minor quantity in *S. lycopersicum*.

**Table 2:** Phenolic acids composition (µg.g$^{-1}$ DW[a]) of *S. lycopersicum.*

| PA | Concentration (µg.g$^{-1}$) | RT (min) | Area (mAU) |
|---|---|---|---|
| Chlorogenic | 33.63 ± 0.32 | 16.212 | 5762.43 |
| Ferulic | 1.99 ± 3.92 | 19.743 | 1346.50 |
| *p*-coumaric | 0.60 ± 0.18 | 19.557 | 558.06 |
| Caffeic | 179.77 ± 3.08 | 17.635 | 3848.47 |
| Vanillic | 4.75 ± 0.13 | 17.768 | 1645.29 |
| Trans-ferulic | 1.54 ± 6.14 | 19.729 | 1328.53 |
| Gallic | 161.35 ± 0.34 | 17.633 | 5555.28 |

[a]Dry weight = mean ± S.D (*n*=3)

Plant phenolics have been demonstrated to affect carcinogenesis via scavenging free radical activity and modulation of cellular signaling pathways including DNA damage repair, cell proliferation, apoptosis, and invasion [15]. Both caffeic and gallic acids have been shown to have a wide range of biological effects, including scavenging free radicals, primarily linked to modulating carcinogenesis.

### *b) Flavonoids Composition*

#### *Flavan-3-ols*

Table 3 shows the amount of epigallocatechin gallate (flavan-3-ols) in *S. lycopersicum*. Higher content of EGCG was found in *S. lycopersicum* with values of 9.22 ± 2.30 μg.g$^{-1}$ DW. EGCG has been found to affect both levels of carcinogenesis; initiation and promotion phase. EGCG is reported to be present in fruits such as berries, grapes, plums, and peaches, typically at exceedingly small concentrations (less than 10 μg.g$^{-1}$ FW) but in abundance in tea (68–3950 μg.g$^{-1}$ FW) [16].

#### *Flavonols*

Kaempferol and quercetin concentrations from the flavonols group found in *S. lycopersicum* were displayed in Table 3. Kaempferol concentration (2.58 ± 2.40 μg.g$^{-1}$ DW) was reported to be lower than quercetin (3.15 ± 0.56 μg.g$^{-1}$ DW). The kaempferol levels in tomatoes were detected between 0.2–0.6 μg.g$^{-1}$ FW, slightly lower than our findings [17]. In the same study, the tomato contained 2.7–9.3 μg.g$^{-1}$ FW of quercetin, which is in accordance with the present study. The flavonols found in food, predominantly kaempferol, quercetin, and myricetin, have been described in association with their antioxidant potential [18]. The three flavonols have been extensively investigated for their health benefits, mainly for their antioxidant and anti-inflammatory activities and modulation of signaling pathways accountable for flavonols' beneficial properties [2].

#### *Flavanones*

Table 3 displays the concentration of naringin in *S. lycopersicum* (21.58 ± 1.30 μg.g$^{-1}$ DW). Flavanones have been shown to inhibit the proliferation of various cancer cells while having a minimum effect on normal cells. This suggests that the moderate flavanones content in *S. lycopersicum* makes them an ideal candidate to develop as potential flavonoid-based chemopreventive agents.

**Table 3:** Flavonoids composition (μg.g$^{-1}$ DW[a]) of *S. lycopersicum*.

| Phenolic acid | Concentration (μg.g$^{-1}$) | RT (min) | Area (mAU) |
|---|---|---|---|
| **Flavan-3-ols** | | | |
| Epigallocatechin gallate | 90.22 ± 2.30 | 16.016 | 6330.92 |
| **Flavonols** | | | |
| Quercetin | 3.15 ± 0.56 | 23.277 | 323.13 |
| Kaempferol | 2.58 ± 2.40 | 24.669 | 869.55 |
| **Flavanones** | | | |
| Naringin | 21.58 ±1.30 | 19.561 | 546.58 |

[a]Dry weight = mean ± S.D ($n$ = 3)

## Conclusions

It can be concluded that *S. lycopersicum* has significant cytotoxicity against HepG2 cells. This is due to its antioxidant characteristics and high polyphenolic content. The

profiling of its polyphenolic contents has identified the principal polyphenols implicated in the antioxidant and anticancer pathways as prospective candidates for developing anticancer medicines.

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## Author Contributions

MAM and NAAAK made substantial contributions to conception, design, and acquisition of data. MAM, NNNR, SGHT and SHA contributed for the analysis, interpretation of data and revised the manuscript. ASS was reviewed and edited the manuscript. All authors participated in the initial draft of the manuscript. All authors read and approved the final manuscript.

## Disclosure of Conflict of Interest

The authors declare no conflict of interest.

## Compliance with Ethical Standards

The work is compliant with ethical standards.

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