

Sesquiterpene Curzerenone Activates Apoptotic Signaling and Attenuates PI3K/AKT/mTOR Signaling in HepG2 Cells by Triggering Oxidative Stress

Pharmacognosy Magazine

22(1) 395–406, 2026

© The Author(s) 2025

Article reuse guidelines:

in.sagepub.com/journals-permissions-india

DOI: 10.1177/09731296251348104

journals.sagepub.com/home/phm

Yujian Tian¹, Zhongjie Cao² and Renshuan Li³ 

Abstract

Background/Objectives: Curzerenone is a sesquiterpene primarily isolated from *Curcuma zedoaria* rhizomes, identified for its diverse therapeutic efficacy. Recent studies have highlighted curzerenone's potential anti-cancer, inflammatory attenuating, and antioxidant properties. Current research was conducted to investigate the anti-cancer potency of curzerenone on hepatic carcinoma cells.

Materials and Methods: Curzerenone antioxidant potency was initially assessed *in vitro* using ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. Human liver cancer cell line (HepG2) and Vero cell lines were utilized to assess cytotoxic effects via 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. 2',7'-Dichlorofluorescein diacetate (DCFH-DA) staining quantified reactive oxygen species (ROS) generation. Thiobarbituric acid reactive substances (TBARS) assay and antioxidant quantification analyzed curzerenone's impact. Cell adhesion assay evaluated anti-metastatic effects. Apoptotic signaling proteins and phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) proteins were quantified in curzerenone-treated HepG2 cells.

Results: Curzerenone exhibited strong antioxidant activity and tumor cell-specific cytotoxicity while remaining non-cytotoxic to normal Vero cells. Treatment increased intracellular ROS levels, decreased antioxidant levels, and induced lipid peroxidation in HepG2 cells. It elevated apoptotic signaling and attenuated PI3K/AKT/mTOR signaling, preventing cancer cell viability and division while increasing cell death.

Conclusion: Results suggest that curzerenone induces oxidative stress, thereby reducing cancer cell survival and promoting apoptosis in hepatocellular carcinoma cells.

Keywords

Sesquiterpene, curzerenone, antioxidant, human liver cancer cell line hepatocellular carcinoma cells, oxidative stress, apoptosis

Received 05 February 2025; accepted 24 April 2025

Introduction

Hepatic cancers tend to be the most prevalent type of cancer, ranking sixth in global incidence and third in cancer-related demises (Rumgay et al., 2022), with hepatocellular carcinoma (HCC) accounting for 80%–90% of cases (Asrani et al., 2019). It is also the second most significant cause of early cancer-related mortality (Ferlay et al., 2018). Current estimates suggest that the hepatitis B virus is responsible for 56% of liver cancer cases, hepatitis C virus for 20%, and other factors like smoking and alcohol also play significant roles (GBD 2019 Risk Factors Collaborators, 2020; Kulik & El-Serag, 2019; Maucourt-Boulch et al., 2018). The global burden of liver cancer remains substantial, highlighted by

over 9.6 million cancer deaths in 2020 (Sung et al., 2021). By 2025, liver cancer cases could surpass 1 million annually, with rising associations to metabolic syndrome and

Yujian Tian and Zhongjie Cao contributed equally.

¹Department of Hepatobiliary and Pancreatic Surgery, Affiliated Huishan Hospital of Xinglin College, Nantong University, Wuxi Huishan District People's Hospital, Wuxi, Jiangsu, China

²Department of Hepatobiliary and Pancreatic Surgery, Ezhou Central Hospital, Ezhou, Hubei, China

³Department of Oncology Thoracic Surgery, Xianyang Hospital of Yan'an University, Xi'an, Shaanxi, China

Corresponding author:

Renshuan Li, Department of Oncology Thoracic Surgery, Xianyang Hospital of Yan'an University, Xi'an, Shaanxi 712000, China.

E-mail: lirensuan1982@outlook.com



Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (<http://www.creativecommons.org/licenses/by-nc/4.0/>) which permits non-Commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (<https://us.sagepub.com/en-us/nam/open-access-at-sage>).

non-alcoholic steatohepatitis contributing to this trend (Llovet et al., 2021).

HCC management is highly dependent on disease stage and liver function, with early-stage cases treated via surgical resection, liver transplantation, or ablative therapies like radiofrequency or microwave ablation (Schlachterman et al., 2015). Advanced HCC often requires systemic therapies such as targeted therapy, immunotherapy, or chemotherapy, with drugs like pembrolizumab and lenvatinib showing improved survival rates (Liu et al., 2024). Despite advancements, treatments face challenges, including drug resistance, adverse effects, recurrence, and limited efficacy in advanced stages (Forner et al., 2019). Liver transplantation offers the potential for a cure but is hindered by high recurrence and complication rates (Zhou et al., 2009). Other options, such as transarterial chemoembolization (TACE) and sorafenib, provide moderate survival benefits but are limited by toxicity and resistance (Anwanwan et al., 2020; El-Serag et al., 2008).

Natural antioxidant therapies have garnered attention as potential adjunct treatments for HCC because of their capacity to counteract redox imbalance and inflammatory response, which play critical roles in cancer initiation and progression (Hassan et al., 2016; Li, Cao et al., 2023). Research has examined the efficacy of herbal extracts and phytochemicals, such as curcumin, resveratrol, and green tea polyphenols, in pre-clinical and clinical settings. For instance, curcumin inhibits liver cancer cell proliferation and induces apoptosis (Wang et al., 2023); likewise, resveratrol modulates inflammatory responses and enhances antioxidant defenses (Karabekir & Özgörgülü, 2020). Epigallocatechin gallate (EGCG) has demonstrated the ability to suppress tumor growth and metastasis in liver cancer models (Li, Yu et al., 2023). These natural compounds not only provide antioxidant benefits but also may enhance the efficacy of conventional therapies, suggesting a potential role for antioxidant-rich foods and natural supplements in comprehensive liver cancer management (Maiuolo et al., 2021).

Curcuma sp., belonging to Zingiberaceae family, is innate to India and extensively grown in tropical regions (Tung et al., 2019). In traditional Chinese medicine (TCM), it is prescribed for cancer, diabetes, respiratory issues, arthritis, skin conditions, and so on (El-Kenawy et al., 2018). Research indicates that turmeric and its active compounds exhibit several medicinal properties, such as free radical scavenging (Uzunhisarcikli & Aslanturk, 2019), hepatoprotective (Lee et al., 2017), attenuating inflammation (Kinney et al., 2015), anti-cancer (Jia et al., 2014), anti-diabetic (Essa et al., 2019), and neuroprotective effects (Xu et al., 2019), among others. Curzerenone is a bioactive sesquiterpene compound present in *Curcuma zedoaria* and *Curcuma aeruginosa* plants with several pharmacological properties (Poudel et al., 2022). It has been already reported that curzerenone demonstrated anti-cancer activity against lung cancer (Zheng & Chen, 2019) and cervical cancer (Sun et al., 2024) cells. Furthermore, it also showed antioxidant and anti-bacterial properties (Joshi

& Mathela, 2012). However, there are not many reports on the anti-cancer properties of the curzerenone against liver cancer. Therefore, the present has explored the anti-cancer efficacy of curzerenone against HCC cell lines.

Materials and Methods

Chemicals

Curzerenone, Dulbecco's modified Eagle medium (DMEM), and other primary chemicals were obtained from Sigma-Aldrich, USA. All the diagnostic kits for the biochemical markers were purchased from R&D Systems and Abcam, USA, respectively.

Antioxidant Evaluation

Ferric Reducing Antioxidant Power (FRAP) Assay

Curzerenone samples free radical scavenging capacity was evaluated using the FRAP test Benzie and Strain (1996). About 180 μ L of the freshly prepared FRAP reagent and 24 μ L of curzerenone of varied concentrations ranging from 0.5 to 25 μ M, a standard solution, or distilled water as a blank, were added to 96-well plates. After allowing the mixture to incubate at 37°C for 10 min, the final OD was quantified at 593 nm. A standard curve for Trolox was established, and results were reported in mM of Trolox equivalent. The test was conducted with three replicates.

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Assay

Brand-Williams et al.'s (1995) protocol was followed for the DPPH evaluation. About 190 μ L of 0.12 mM DPPH was combined with 10 μ L of curzerenone of varied concentrations ranging from 0.5 to 25 μ M in a 96-well plate. After allowing the mixture to incubate for 30 min, the final OD was quantified at 517 nm. The test was conducted with three replicates.

Cell Culture

Human liver cancer cell line (HepG2) and Vero cells are typically cultured in 10% fetal bovine serum supplemented DMEM and incubated at 37°C in a humidified incubator with 5% CO₂. When the cells reached 70%–80% confluence, they were passaged by washing with phosphate-buffered saline (PBS), treating with trypsin to detach the cells, and then resuspending them in fresh medium before being split at a ratio of 1:3 for subculturing into new flasks.

MTT Assay

HepG2 and Vero cells were cultured in 96-well plates at a cell density of 5×10^3 cells/well and treated with curzerenone (0.5–25 μ M). Control cells were treated only with DMEM

medium. The cells were incubated in a 5% CO₂ humidified chamber at 37°C for 24 h. Following treatment, the cells were treated with MTT solution (5 mg/mL dissolved in PBS) for 4 h. DMSO was added to dissolve the formazan crystals, and the final OD was quantified at 550 nm. The 50% cytotoxic concentration (CC₅₀) was determined using GraphPad Prism software based on the dose-response curves generated from the absorbance readings (Mosmann, 1983).

Intracellular Reactive Oxygen Species Quantification

HepG2 cells (5 × 10⁵ cells/well) were treated with 7.5 μM curzerenone or doxorubicin (standard drug) and incubated for another 24 h. Control cells were treated only with DMEM medium. ROS levels were measured using 10 μM DCFH-DA (10 μM concentration prepared in DMSO), incubated for 30 min in the dark, and fluorescence was recorded. For visualization, cells grown on coverslips were similarly treated and examined using a confocal laser scanning microscope (Eruslanov & Kusmartsev, 2010).

Thiobarbituric Acid Reactive Substances Assay

HepG2 cells were cultured in 6-well plates and treated with curzerenone and doxorubicin for 24 hours. The cells were then lysed with a protease inhibitor-containing buffer, and the lysate was centrifuged to obtain the supernatant. The supernatant was mixed with TBA solution (prepared by dissolving 0.67% (w/v) thiobarbituric acid in 0.05 M NaOH), heated at 95°C for 1 h, cooled, and the absorbance of the pink complex was measured at 532 nm using a spectrophotometer (de Godoi et al., 2017; Ohkawa et al., 1979).

Quantification of Antioxidants

Superoxide dismutase (SOD) evaluation was performed as per the protocol of Marklund and Marklund (1974). The cell lysates were centrifuged at 6,000 rpm for 15 min, and the obtained supernatant was treated with a reaction mixture containing xanthine (100 mM), xanthine oxidase (0.1 U/mL), and nitroblue tetrazolium (NBT) (100 μM) and the OD was quantified at 560 nm, results were indicated as SOD activity in units per milligram of protein. For the glutathione assay, Ellman's (1959) protocol was followed; the supernatant was treated with DTNB, the final OD was quantified at 412 nm, and glutathione levels were expressed as μmol/mg protein.

Cell Adhesion Assay

A cell adhesion assay was performed with HepG2 cells treated with curzerenone (7.5 μM) and doxorubicin (2 μg) for 24 h. Control cells were treated only with DMEM medium. After treatment, 100 μL of cell suspension (1 × 10⁶ cells/mL) was loaded onto Matrigel-coated plates and incubated at

37°C in a humidified CO₂ (5%) chamber for 2 h. Suspended cells were removed by gentle washing with PBS, and attached cells were fixed with 4% formaldehyde, stained with 0.2% crystal violet for 15 min, and photographed at 10× magnification for analysis (Wu et al., 2013).

Assessment of Apoptotic Signaling

Apoptotic proteins Bax, Bcl2, caspases 3, 9, and cyclin D1 were measured in curzerenone and doxorubicin-treated HepG2 cells using an ELISA kit (R&D Systems). The assay was performed in triplicates as per the guidelines provided in the kit manual.

Assessment of Phosphatidylinositol 3-Kinase/Protein Kinase B/Mammalian Target of Rapamycin (PI3K/AKT/mTOR) Signaling

PI3K, AKT, and mTOR protein were measured in the HepG2 cells treated with curzerenone and doxorubicin using the ELISA kits obtained from Abcam. The assay was done as per the guidelines prescribed in the kit manual, and the final absorbance was estimated at 450 nm.

Statistics

GraphPad Prism 8.0 (USA) was used to analyze the results obtained. Data are expressed as Mean ± SD. Group comparisons were made using one-way analysis of variance (ANOVA), and within-group differences were evaluated using Tukey's test.

Results

Antioxidant Efficacy of Sesquiterpene Curzerenone

The antioxidant potency of curzerenone at different concentrations was assessed using FRAP and DPPH assays, and the data were illustrated in Figure 1. Curzerenone potency to reduce ferric ions to ferrous ions was measured and represented as Trolox equivalents in Figure 1A. Curzerenone effectively reduced ferric ions to ferrous ions, indicating its antioxidant capacity. The minimum dose of 0.5 μM curzerenone showed 22 ± 2 mM TE, while the maximum dose of 25 μM curzerenone exhibited 78 ± 1.2 mM TE. Figure 1B illustrates the free radical scavenging ability of curzerenone, quantified by DPPH assay. 3.8 ± 0.005 mM TE was observed at the 0.5 μM concentration, and 8.4 ± 0.009 mM TE was recorded at the 25 μM concentration of curzerenone treatment.

Cytotoxic Potency of Sesquiterpene Curzerenone

Figure 2 depicts the cytotoxic effect of curzerenone on HepG2, HCC cells, and Vero, normal kidney epithelial cells. Curzerenone treatment rendered a significant cytotoxic effect

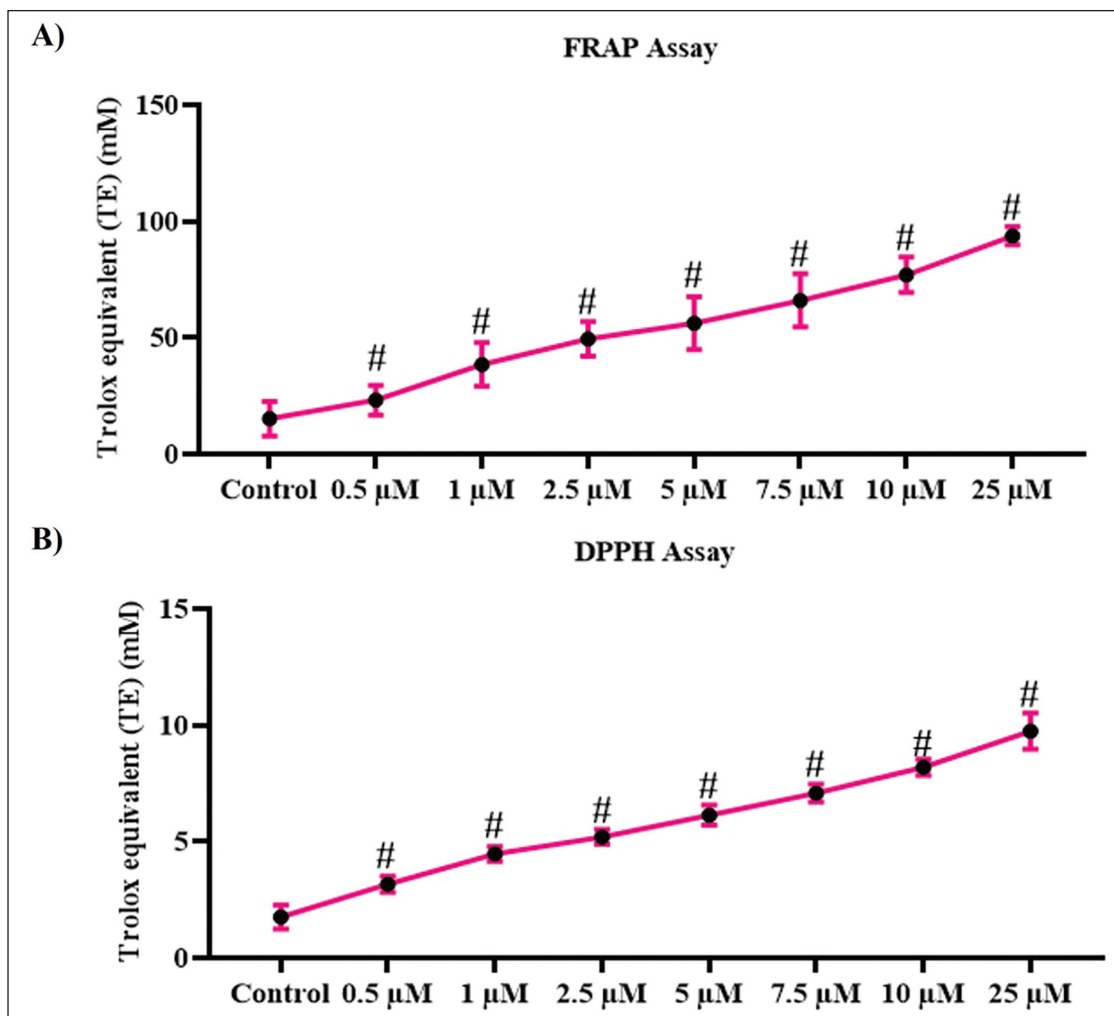


Figure 1. Antioxidant Efficacy of Sesquiterpene Curzerenone.

Notes: (A) Ferric reducing antioxidant power (FRAP) assay, (B) 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Antioxidant efficacy of different concentrations of curzerenone ranging from 0.5 to 25 μM . Results were analyzed with one-way analysis of variance (ANOVA) followed by the Tukey test using GraphPad Prism statistical software. Values are expressed as the Mean $\pm$ Standard Error of Mean (SEM), and “#” indicates the statistical significance at $p < .05$ from the control group.

on HepG2 cells, depending on the dosage of treatment. About $3\% \pm 0.07\%$ of dead cells were quantified with 0.5 μM treatment, whereas it is increased to $17\% \pm 0.05\%$ and $26\% \pm 0.06\%$, with 2.5 and 5 μM treatment respectively. The IC_{50} dose was obtained with 7.5 μM curzerenone treatment, which exhibited $48\% \pm 0.09\%$ of cell death. The cell death was further increased to $55\% \pm 0.04\%$ and $63\% \pm 0.05\%$ with 10 and 25 μM curzerenone treatment (Figure 2A). Curzerenone treatment exhibited a minimal cytotoxic effect on normal kidney epithelial cells even at the maximum dose of 25 μM , which rendered $63\% \pm 0.05\%$ of cell death in HepG2 cells exhibited only $14.5\% \pm 0.05\%$ of cell death in Vero cells (Figure 2B).

Intracellular ROS Triggering Efficacy of Sesquiterpene Curzerenone

The efficacy of curzerenone to enhance intracellular ROS synthesis was quantified in the HepG2 cells (Figure 3). An increase in green fluorescence emission was witnessed in the HepG2 cells treated with curzerenone and doxorubicin than in the control cells. The standard chemotherapeutic drug doxorubicin exhibited enhanced green fluorescence, which was quantified to be $135\% \pm 0.06\%$, whereas the curzerenone-treated cells exhibited $59\% \pm 0.08\%$, and the control untreated HepG2 cells exhibited minimum fluorescence intensity of $3\% \pm 0.04\%$.

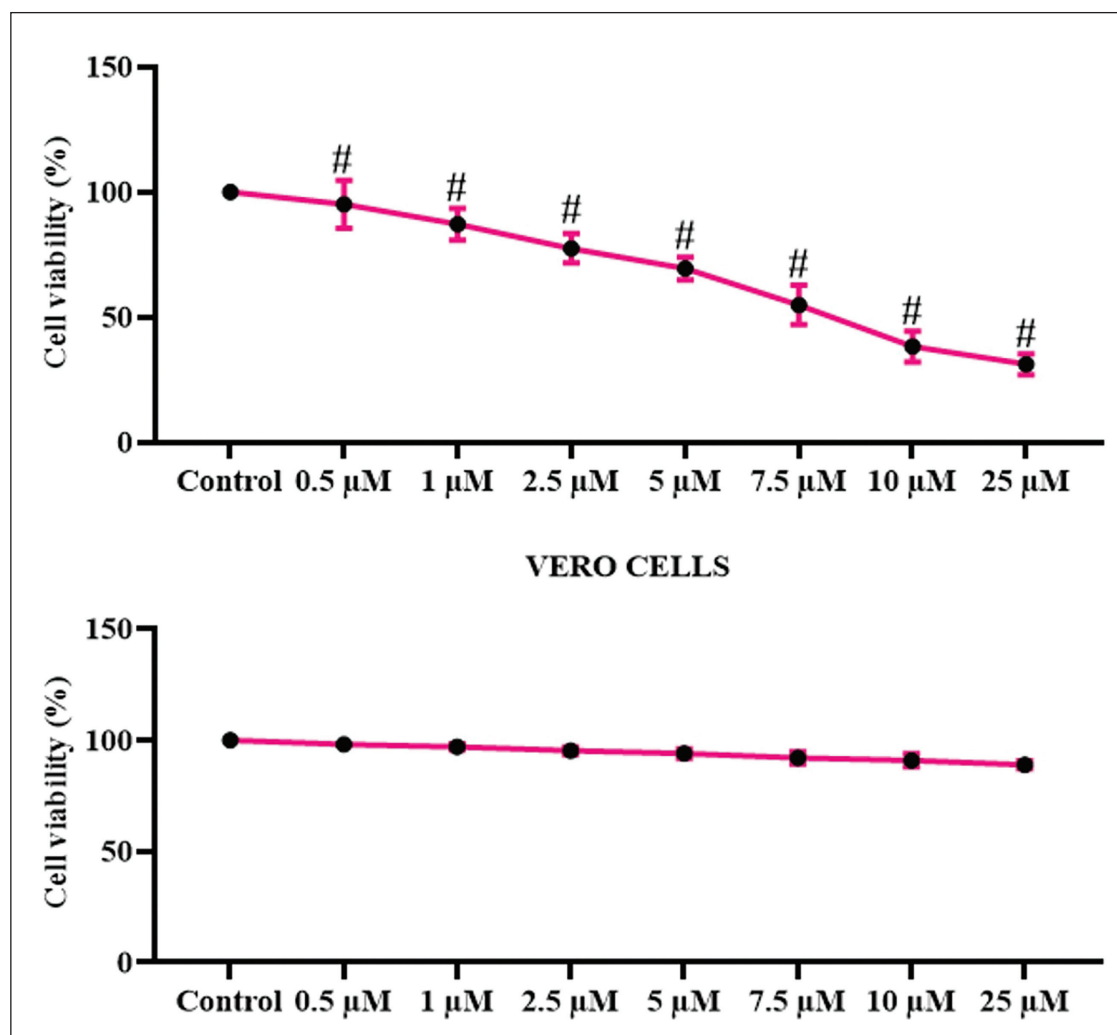


Figure 2. Cytotoxic Potency of Sesquiterpene Curzerenone.

Notes: Hepatocellular carcinoma cells human liver cancer cell line (HepG2) cells and normal kidney epithelial Vero cells were subjected to curzerenone cytotoxicity analysis. Cells were treated with curzerenone doses ranging from 0.5 to 25 μ M for 24 h. Results were analyzed with one-way analysis of variance (ANOVA) followed by Tukey test using GraphPad Prism statistical software. Values are expressed as the Mean $\pm$ Standard Error of Mean (SEM), and “#” indicates statistical significance at $p < .05$ from the control group.

Antioxidant Attenuating Potency of Sesquiterpene Curzerenone

Figure 4 illustrates the impact of sesquiterpene curzerenone on the antioxidant status of HepG2 cells. Lipid peroxidation triggered by curzerenone in HepG2 cells was quantified, and the results were depicted in Figure 4A. Both curzerenone and doxorubicin treatment considerably increased the lipid peroxidation in HepG2 cells. 76.4 ± 0.04 and 39.7 ± 0.06 nMol/mg protein of MDA were quantified in the doxorubicin and curzerenone treated cells, whereas only 18.2 ± 0.05 nMol/mg protein of MDA were measured in the control untreated cells. Curzerenone treatment attenuated the antioxidant levels in the HepG2 cells. 27.6 ± 0.05 units/mg protein of glutathione and 27.3 ± 0.04 units/mg protein of SOD

were quantified in the curzerenone-treated cells, whereas the control cells exhibited 42.3 ± 0.06 units/mg protein and 46.5 ± 0.05 units/mg protein of glutathione and SOD, respectively. Doxorubicin-treated cells exhibited 21.2 ± 0.04 units/mg protein of glutathione and 14.5 ± 0.05 units/mg protein of SOD.

Inhibitory Potency of Sesquiterpene Curzerenone Against HepG2 Cell Adhesion

The impact of curzerenone on HepG2 cell adhesion, which is a key phenomenon of cancer cell proliferation and metastasis, is illustrated in Figure 5. Curzerenone effectively inhibited the adhesion of HepG2 cells, which was evidenced with the microscopic analysis.

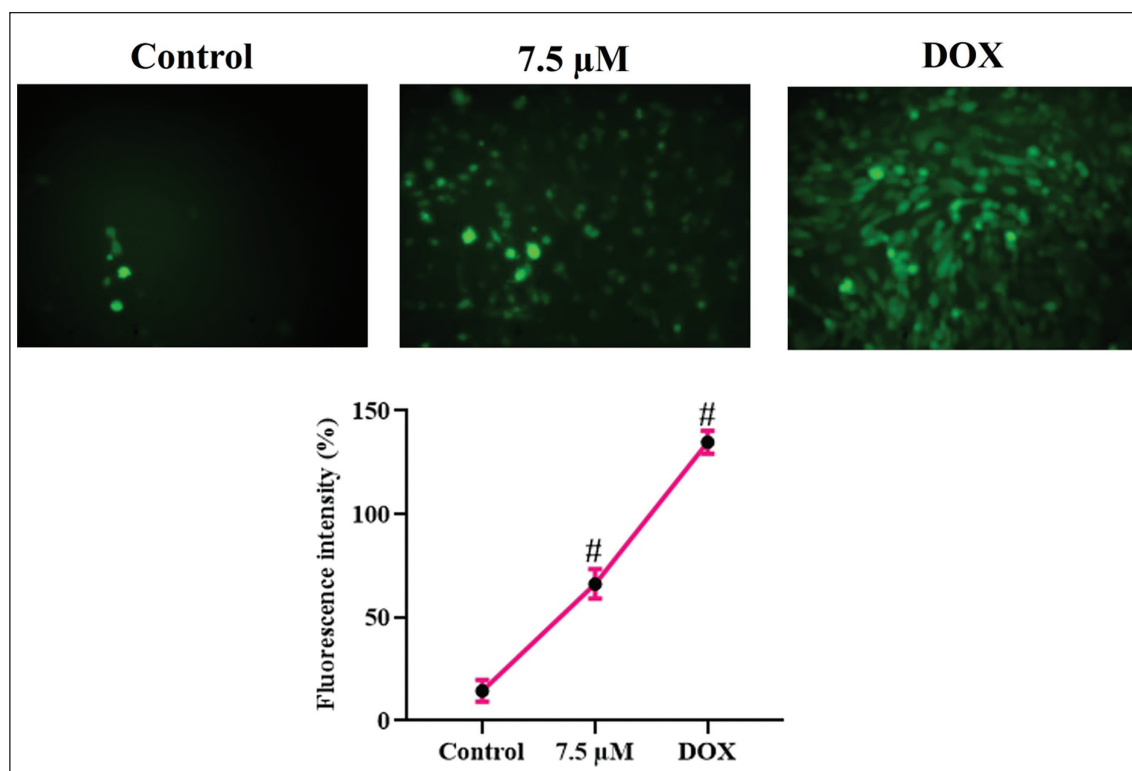


Figure 3. Intracellular Reactive Oxygen Species (ROS) Triggering Efficacy of Sesquiterpene Curzerenone.

Notes: Human liver cancer cell line (HepG2) cells treated with curzerenone and doxorubicin were subjected to ROS staining with 2',7'-dichlorofluorescein diacetate (DCFH-DA) stain. The intensity of green fluorescence emission was measured. Results were analyzed with one-way analysis of variance (ANOVA) followed by the Tukey test using GraphPad Prism statistical software. Values are expressed as the mean $\pm$ standard error of mean (SEM), and “#” indicates statistical significance at $p < .05$ from the control group. Control: Control HepG2 cells; 7.5 μ M: 7.5 μ M of sesquiterpene-treated HepG2 cells; DOX: 2 μ g of doxorubicin (DOX)-treated HepG2 cells.

Apoptosis Stimulating Efficacy of Sesquiterpene Curzerenone on HepG2 Cells

Figure 6 depicts curzerenone impact on apoptotic proteins in HepG2 cells. Curzerenone and doxorubicin treatment significantly elevated the levels of apoptotic stimulating protein Bax, and it considerably decreased the levels of apoptotic attenuating protein Bcl2. It also significantly decreased the levels of cyclin D1 protein, which regulates the cell cycle progression. The caspase levels were also increased in the curzerenone and doxorubicin-treated cells than in control cells. Significant enhancement in both caspases 3 and 9 was noted in the curzerenone-treated cells.

Attenuating Effect of Sesquiterpene Curzerenone on PI3K/AKT/mTOR Signaling in HepG2 Cells

Figure 7 illustrates the curzerenone efficacy of attenuating PI3K/AKT/mTOR signaling in HepG2, which is often a targeted signaling pathway of anti-cancer drugs. Curzerenone and chemotherapeutic drug doxorubicin treatment attenuated the PI3K, AKT, and mTOR proteins in HepG2 cells. HepG2

cells treated with curzerenone exhibited a decrease in PI3K, AKT, and mTOR proteins than the control untreated cells.

Discussion

ROS has been widely examined in relation to several ailments, particularly cancers (Cairns et al., 2012; Chio & Tuveson, 2017). As natural byproducts of cellular processes, including oxygen metabolism, ROS levels can be modulated as a potential anti-cancer strategy (Sena & Chandel, 2012). Research indicates that ROS possesses a “double-edged sword” role in cancer (Forman & Zhang, 2021; Okon & Zou, 2015). Minimal concentrations of ROS facilitate processes such as cancer cell survival and drug resistance (Gorrini et al., 2013). Conversely, high ROS levels can be detrimental, leading to cell death. Cancer cells frequently acclimate to increased ROS levels by activating antioxidant pathways, thereby improving their capacity to neutralize and remove ROS (Schafer et al., 2009). Additionally, cancer cells exhibit heightened sensitivity to therapies that induce ROS, as many chemotherapeutic agents are known to increase ROS

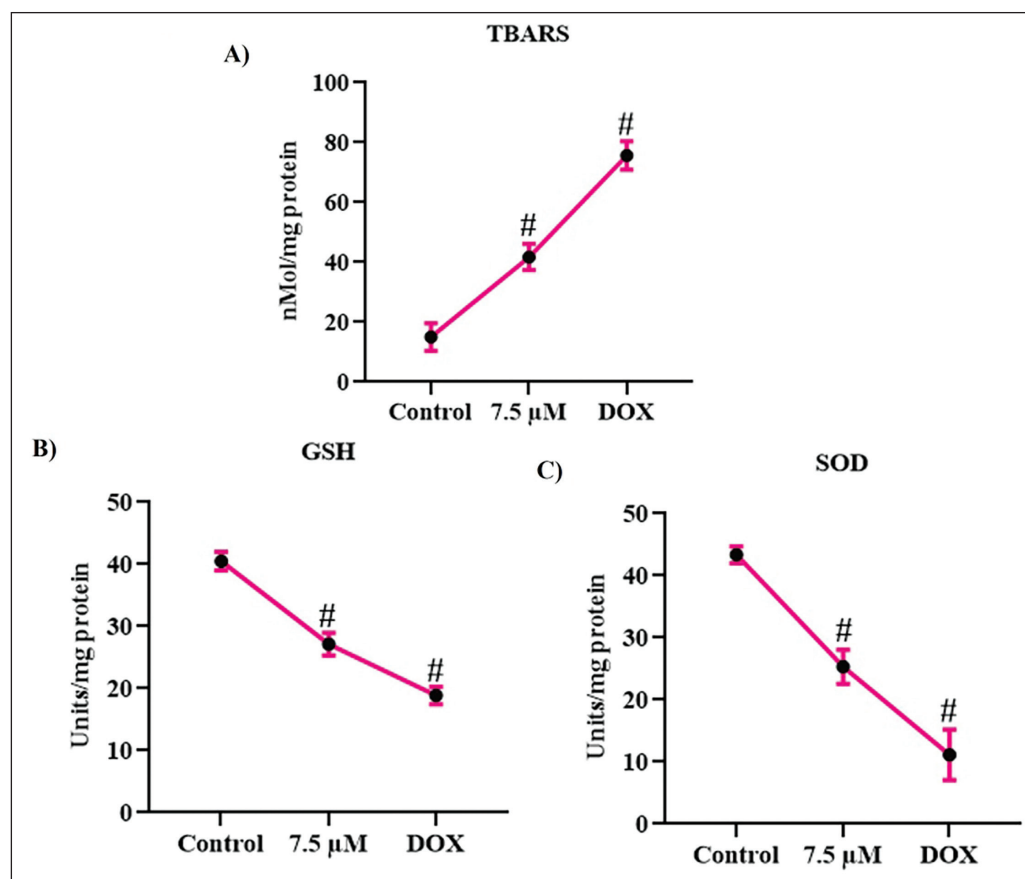


Figure 4. Antioxidant Attenuating Potency of Sesquiterpene Curzerenone.

Notes: (A) Thiobarbituric acid reactive substances (TBARS) assay, glutathione (GSH) assay, (C) superoxide dismutase (SOD) assay were performed in human liver cancer cell line (HepG2) cells treated with curzerenone and doxorubicin. Results were analyzed with one-way analysis of variance (ANOVA) followed by the Tukey test using GraphPad Prism statistically software values are expressed as the mean $\pm$ standard error of mean (SEM), and “#” indicates statistical significance at $p < .05$ from the control group. Control: Control HepG2 cells; 7.5 μ M: 7.5 μ M of sesquiterpene-treated HepG2 cells; DOX: 2 μ g of doxorubicin (DOX)-treated HepG2 cells.

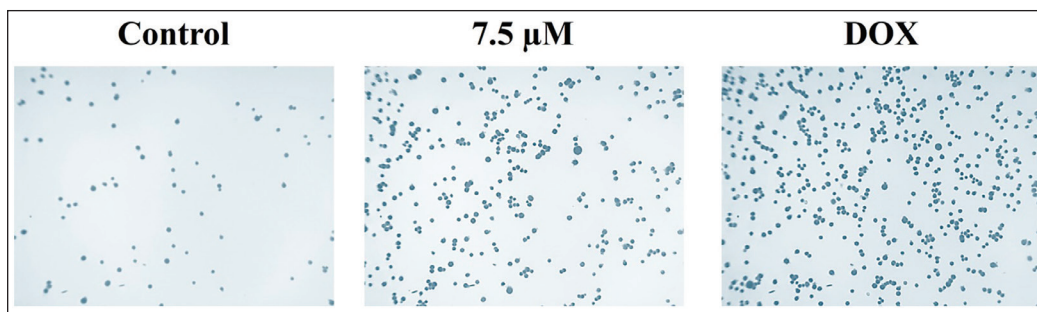


Figure 5. Inhibitory Potency of Sesquiterpene Curzerenone Against HepG2 Cell Adhesion.

Notes: Human liver cancer cell line (HepG2) cells treated with curzerenone and doxorubicin were subjected to a cell adhesion assay. Control: Control HepG2 cells; 7.5 μ M: 7.5 μ M of sesquiterpene-treated HepG2 cells; DOX: 2 μ g of doxorubicin (DOX)-treated HepG2 cells.

production within these cells (Dogan et al., 2008; Nakamura & Takada, 2021; Siomek et al., 2006). The phytochemical compound curzerenone anti-cancer effect on HCC cells via generating ROS was examined.

Multiple studies have demonstrated the significant free radical scavenging activity of extracts from the rhizomes of *C. zedoaria*. Previous research employed various assays, including DPPH, ABTS, and reducing power assays, to

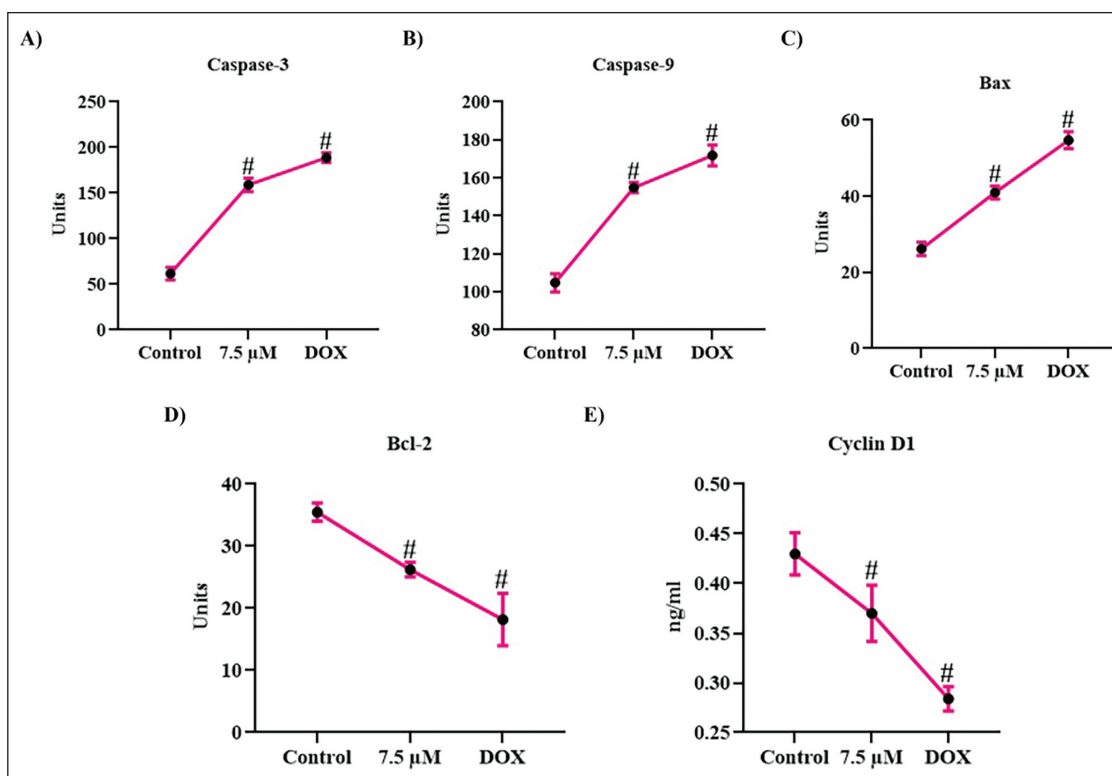


Figure 6. Apoptosis Stimulating Efficacy of Sesquiterpene Curzerenone on HepG2 Cells.

Notes: Human liver cancer cell line (HepG2) cells treated with curzerenone and doxorubicin were subjected to quantification of apoptotic proteins. (A) Caspase 3, (B) caspase 9, (C) Bax, (D) Bcl2, (E) cyclin D1. Results were analyzed with one-way analysis of variance (ANOVA) followed by the Tukey test using GraphPad Prism statistical software. Values are expressed as the Mean $\pm$ Standard Error of Mean (SEM), and “#” indicates statistical significance at $p < .05$ from the control group. Control: Control HepG2 cells; 7.5 µM: 7.5 µM of sesquiterpene-treated HepG2 cells; DOX: 2 µg of doxorubicin (DOX)-treated HepG2 cells.

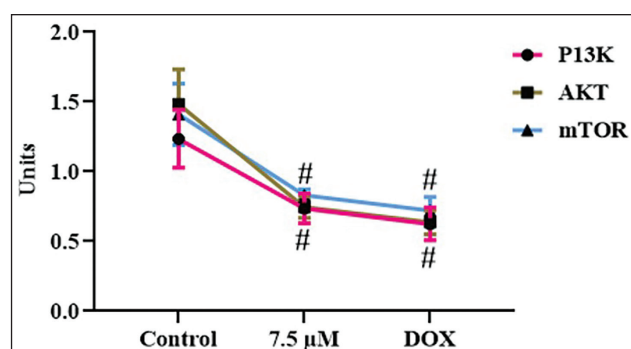


Figure 7. Attenuating Effect of Sesquiterpene Curzerenone on PI3K/AKT/mTOR Signaling in HepG2 Cells.

Notes: Human liver cancer cell line (HepG2) cells treated with curzerenone and doxorubicin were subjected to quantification of phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) signaling proteins. Results were analyzed with one-way analysis of variance (ANOVA) followed by the Tukey test using GraphPad Prism statistical software. Values are expressed as the Mean $\pm$ Standard Error of Mean (SEM), and “#” indicates statistical significance at $p < .05$ from the control group. Control: Control HepG2 cells; 7.5 µM: 7.5 µM of sesquiterpene-treated HepG2 cells; DOX: 2 µg of doxorubicin (DOX)-treated HepG2 cells.

highlight the scavenging potential of essential oils derived from these *Curcuma* extracts and *C. zedoaria* (Angel et al., 2013; Sudipta et al., 2012). Additionally, Sumathi et al. (2013) utilized DPPH, ABTS, and hydrogen peroxide inhibition to further substantiate the radical scavenging activity (Sumathi et al., 2013). Our results also correlate with the above findings. FRAP and DPPH assays performed on curzerenone depicted its strong antioxidant capacity.

Even though numerous therapeutic agents have been approved to treat cancer, patients still face challenges in accessing effective treatments due to drug resistance, which significantly limits the clinical efficacy of even the most advanced therapies (Lopez & Banerji, 2017; Scott et al., 2023). This resistance may arise from the enhanced activity of detoxification mechanisms, such as glutathione-S-transferase, which facilitates the removal of reactive compounds using glutathione (Townsend & Tew, 2003). Additionally, glutathione peroxidase plays a role in converting hydrogen peroxide to water and reducing lipid peroxides to alcohol, thereby mitigating the harmful effects of ROS. Therefore, anti-cancer drugs have been designed to target glutathione for chemotherapy or to serve as detoxifying agents in chemoprevention strategies

(Lubos et al., 2011). In our study, we assessed the capacity of curzerenone to generate intracellular ROS and inhibit antioxidant synthesis. Curzerenone effectively increased the levels of ROS and attenuated the antioxidants GSH and SOD. Increased thiobarbituric acid reactive substances (TBARS) levels in the curzerenone-treated HepG2 cells confirm the ROS-induced cellular damage in HCC cells.

Reactive oxygen species levels are implicated in the facilitation of tumors, but an elevation in ROS levels can also activate anti-tumor signaling pathways and induce redox imbalance, leading to apoptosis in tumor cells via proapoptotic mechanisms (Redza-Dutordoir & Averill-Bates, 2016; Zhao et al., 2023). Research has shown that free radicals produced in tumor cells can trigger cell death through apoptotic signaling pathways, contributing to tumor inhibition (Wang et al., 2018). Elevated mitochondrial ROS can trigger intrinsic apoptosis. Apoptotic protease activating factor-1 interacts with cytochrome *c* in the cytosol, resulting in the apoptosome that activates caspase 9 (Kumar et al., 2022). Caspase 9, an essential element of the intrinsic apoptotic pathway, activates effector caspases such as caspases 3, 6, and 7, which results in the cleavage of vital cellular proteins and ultimately results in apoptosis (Fan et al., 2005). Curzerenone treatment had significantly elevated the ROS and increased the proapoptotic protein Bax expression and the caspases 3 and 9, which eventually led to apoptosis. The MTT assay results of curzerenone-treated HepG2 cells confirm the induction of apoptosis in HCC cells. Curzerenone also inhibited the cyclin D1 expression, which is a pivotal player in cellular proliferation and progression of several cancers.

The PI3K/AKT/mTOR signaling pathway plays a pivotal role in regulating fundamental cellular processes, including cell growth, proliferation, survival, metabolism, and autophagy, thereby exerting a profound influence on the development and progression of cancer (Hoxhaj & Manning, 2020). The PI3K pathway is modulated by reactive oxygen species (ROS), as ROS-induced oxidation of protein phosphatases activates various signaling pathways. Dysregulation of this pathway, frequently observed in HCC, the most prevalent form of liver cancer, can arise from various genetic and epigenetic alterations, including mutations, amplifications, and aberrant expression of its key components (Grabinski et al., 2012; Ye et al., 2019). The PI3K/AKT pathway integrates signals from external cellular stimuli to regulate essential cellular functions and is frequently aberrantly activated in human cancers. Specifically, aberrant activation of the PI3K/AKT/mTOR pathway can promote uncontrolled cell proliferation by upregulating the expression of genes involved in cell cycle progression and inhibiting apoptosis by modulating the activity of proapoptotic proteins. The pathway's intricate network of interacting proteins makes it a crucial player in cancer biology, and its dysregulation has far-reaching consequences for tumorigenesis and treatment resistance (Golob-Schwarzl et al., 2017). In HCC, the PI3K/AKT/mTOR pathway is commonly activated, offering a significant foundation for the development of targeted therapies.

In liver cancer, the dysregulation of the PI3K/AKT/mTOR pathway can disrupt the delicate balance between cell survival and cell death, favoring the survival and proliferation of cancerous cells (Tian et al., 2023). Given the pivotal role of the PI3K/AKT/mTOR pathway in liver cancer, targeting this pathway has emerged as a promising therapeutic strategy. Inhibitors targeting different components of the PI3K/AKT/mTOR pathway have been developed and are being evaluated. Consequently, researchers have developed anti-HCC therapies aimed at this signaling pathway, with clinical trials demonstrating their effectiveness (Yan et al., 2022). Curzerenone treatment had attenuated the PI3K, AKT, and mTOR signaling proteins in HepG2 cells, which proves curzerenone had effectively suppressed cancer cell survival and progression. Our cell adhesion results further confirmed the tumor cell inhibition potency of curzerenone.

Conclusion

In conclusion, this study demonstrates that curzerenone, a sesquiterpene derived from *C. zedoaria*, possesses notable anti-cancer properties, particularly against HCC cells. The compound's strong antioxidant activity was confirmed through FRAP and DPPH assays, yet it paradoxically elevated intracellular ROS levels and lipid peroxidation in HepG2 cells, leading to oxidative stress. Curzerenone exhibited selective cytotoxicity towards cancer cells, sparing normal epithelial cells, and effectively triggered apoptotic signaling and attenuated the PI3K/AKT/mTOR pathway. These findings indicate that curzerenone's potency to trigger oxidative stress and promote apoptosis can significantly impair HCC cell proliferation and survival, emphasizing its potential as a therapeutic candidate in the treatment of liver cancer. Further *in vivo* studies and investigations using other cancer cell lines are needed to fully elucidate curzerenone's anti-cancer effects.

Abbreviations

AKT: Protein kinase B; ANOVA: Analysis of variance; Bax: BCL2 associated X; Bcl2: B-cell lymphoma 2; DCFH-DA: 2',7'-Dichlorofluorescein diacetate; DMEM: Dulbecco's modified Eagle medium; DMSO: Dimethyl sulfoxide; DNA: Deoxyribonucleic acid; DOX: Doxorubicin; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; ERK: Extracellular signal-regulated kinases; FRAP: Ferric reducing antioxidant power; GSH: Glutathione; HCC: Hepatocellular carcinoma; IC₅₀: Half maximal inhibitory concentration; MAPK: Mitogen-activated protein kinase; MDA: Malondialdehyde; MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; mTOR: Mammalian target of rapamycin; NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells; PBS: Phosphate-buffered saline; PI3K: Phosphatidylinositol 3-kinase; RNA: Ribonucleic acid; ROS: Reactive oxygen species; SEM: Standard error of mean; SOD: Superoxide dismutase; TACE: Trans-arterial chemoembolization; TCM: Traditional Chinese medicine; TBARS: Thiobarbituric acid reactive substances.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval and Informed Consent

Not applicable.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

ORCID iD

Renshuan Li  <https://orcid.org/0009-0006-9832-9841>

References

- Angel, G. R., Vimala, B., & Nambisan, B. (2013). Antioxidant and anti-inflammatory activities of proteins isolated from eight *Curcuma* species. *Phytopharmacology*, 4(1), 96–105.
- Anwanwan, D., Singh, S. K., Singh, S., Saikam, V., & Singh, R. (2020). Challenges in liver cancer and possible treatment approaches. *Biochimica Biophysica Acta Reviews on Cancer*, 1873(1), 188314.
- Asrani, S. K., Devarbhavi, H., Eaton, J., & Kamath, P. S. (2019). Burden of liver diseases in the world. *Journal of Hepatology*, 70(1), 151–171.
- Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay. *Analytical Biochemistry*, 239(1), 70–76.
- Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT—Food Science and Technology*, 28(1), 25–30.
- Cairns, R. A., Harris, I. S., & Mak, T. W. (2011). Regulation of cancer cell metabolism. *Nature Reviews Cancer*, 11, 85–95.
- Chio, I. I. C., & Tuveson, D. A. (2017). ROS in cancer: The burning question. *Trends in Molecular Medicine*, 23, 411–429.
- de Godoi, S. N., Quatrin, P. M., Sagrillo, M. R., Nascimento, K., Wagner, R., Klein, B., Santos, R. C. V., & Ourique, A. F. (2017). Evaluation of stability and *in vitro* security of nanoemulsions containing eucalyptus globulus oil. *Biomed Research International*, 2017, 2723418.
- Dougan, S. J., Habtemariam, A., McHale, S. E., Parsons, S., & Sadler, P. J. (2008). Catalytic organometallic anticancer complexes. *Proceedings of the National Academy of Sciences United States of America*, 105(33), 11628–11633.
- El-Kenawy, A. E., Hassan, S. M., Mohamed, A. M., & Mohammed, H. M. (2018). *Tumeric or Curcuma longa* Linn. in nonvitamin and nonmineral nutritional supplements (pp. 447–453). Elsevier.
- Ellman, G. L. (1959). Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics*, 82(1), 70–77.
- El-Serag, H. B., Marrero, J. A., Rudolph, L., & Reddy, K. R. (2008). Diagnosis and treatment of hepatocellular carcinoma. *Gastroenterology*, 134(6), 1752–1763.
- Eruslanov, E., & Kusmartsev, S. (2010). Identification of ROS using oxidized DCFDA and flow-cytometry. *Methods in Molecular Biology*, 594, 57–72.
- Essa, R., El Sadek, A. M., Baset, M. E., Rawash, M. A., Sami, D. G., Badawy, M. T., Mansour, M. E., Attia, H., Saadeldin, M. K., & Abdellatif, A. (2019). Effects of turmeric (*Curcuma longa*) extract in streptozocin-induced diabetic model. *Journal of Food Biochemistry*, 43(9), e12988.
- Fan, T. J., Han, L. H., Cong, R. S., & Liang, J. (2005). Caspase family proteases and apoptosis. *Acta Biochimica Biophysica Sinica (Shanghai)*, 37(11), 719–727.
- Ferlay, J., Ervik, M., Lam, F., Colombet, M., Mery, L., Piñeros, M., Znaor, A., Soerjomataram, I., & Bray, F. (2018). Global cancer observatory. *Cancer Today*, 23(7), 323–326.
- Forman, H. J., & Zhang, H. (2021). Targeting oxidative stress in disease: Promise and limitations of antioxidant therapy. *Nature Reviews Drug Discovery*, 20(9), 689–709. <https://doi.org/10.1038/s41573-021-00233-1>
- Forner, A., Da Fonseca, L. G., Díaz-González, Á., Sanduzzi-Zamparelli, M., Reig, M., & Bruix, J. (2019). Controversies in the management of hepatocellular carcinoma. *JHEP Reports*, 1(1), 17–29.
- GBD 2019 Risk Factors Collaborators. (2020). Global burden of 87 risk factors in 204 countries and territories, 1990–2019: A systematic analysis for the global burden of disease study 2019. *Lancet*, 396(10258), 1223–1249.
- Golob-Schwarzl, N., Krassnig, S., Toeglhofer, A. M., Park, Y. N., Gogg-Kamerer, M., Vierlinger, K., Schroder, F., Rhee, H., Schicho, R., & Fickert, P. (2017). New liver cancer biomarkers: PI3K/AKT/mTOR pathway members and eukaryotic translation initiation factors. *European Journal of Cancer*, 83, 56–70.
- Gorrini, C., Harris, I. S., & Mak, T. W. (2013). Modulation of oxidative stress as an anticancer strategy. *Nature Reviews Drug Discovery*, 12, 931–947.
- Grabinski, N., Ewald, F., Hofmann, B. T., Stauffer, K., Schumacher, U., Nashan, B., & Jucker, M. (2012). Combined targeting of AKT and mTOR synergistically inhibits proliferation of hepatocellular carcinoma cells. *Molecular Cancer*, 11, 85.
- Hassan, H. A., El-Gharib, N. E., & Azhari, A. F. (2016). Role of natural antioxidants in the therapeutic management of hepatocellular carcinoma. *Hepatoma Research*, 2, 216–223.
- Hoxhaj, G., & Manning, B. D. (2020). The PI3K-AKT network at the interface of oncogenic signalling and cancer metabolism. *Nature Reviews Cancer*, 20(2), 74–88.
- Jia, T., Zhang, L., Duan, Y., Zhang, M., Wang, G., Zhang, J., & Zhao, Z. (2014). The differential susceptibilities of MCF-7 and MDA-MB-231 cells to the cytotoxic effects of curcumin are associated with the PI3K/Akt-SKP2-Cip/Kips pathway. *Cancer Cell International*, 14, 1–4.
- Joshi, S. C., & Mathela, C. S. (2012). Antioxidant and antibacterial activities of the leaf essential oil and its constituents furanodienone and curzerenone from *Lindera pulcherrima* (Nees.) Benth. ex hook. f. *Pharmacognosy Research*, 4(2), 80–84.
- Karabekir, S. C., & Özgörgülü, A. (2020). Possible protective effects of resveratrol in hepatocellular carcinoma. *Iranian Journal of Basic Medical Science*, 23(1), 71–78.
- Kinney, S. R., Carlson, L., Ser-Dolansky, J., Thompson, C., Shah, S., Gambrah, A., Xing, W., Schneider, S. S., & Mathias, C. B. (2015). Curcumin ingestion inhibits mastocytosis and suppresses intestinal anaphylaxis in a murine model of food allergy. *PLoS One*, 10(7), e0132467.

- Kulik, L., & El-Serag, H. B. (2019). Epidemiology and management of hepatocellular carcinoma. *Gastroenterology*, 156(2), 477.e1–491.e1.
- Kumar, S., Dorstyn, L., & Lim, Y. (2022). The role of caspases as executioners of apoptosis. *Biochemical Society Transitions*, 50(1), 33–45.
- Lee, H. Y., Kim, S. W., Lee, G. H., Choi, M. K., Chung, H. W., Lee, Y. C., Kim, H. R., Kwon, H. J., & Chae, H. J. (2017). Curcumin and *Curcuma longa* L. extract ameliorate lipid accumulation through the regulation of the endoplasmic reticulum redox and ER stress. *Scientific Report*, 7(1), 6513.
- Li, D., Cao, D., Cui, Y., Sun, Y., Jiang, J., & Cao, X. (2023). The potential of epigallocatechin gallate in the chemoprevention and therapy of hepatocellular carcinoma. *Frontiers in Pharmacology*, 14, 1201085.
- Li, Y., Yu, Y., Yang, L., & Wang, R. (2023). Insights into the role of oxidative stress in hepatocellular carcinoma development. *Frontiers in Biosciences*, 28(11), 286.
- Liu, Y., Wu, D., Zhang, K., Ren, R., Liu, Y., Zhang, S., Zhang, X., Cheng, J., Chen, L., & Huang, J. (2024). Detection technology and clinical applications of serum viral products of hepatitis B virus infection. *Frontiers in Cellular and Infection Microbiology*, 14, 1402001.
- Llovet, J. M., Kelley, R. K., Villanueva, A., Singal, A. G., Pikarsky, E., Roayaie, S., Lencioni, R., Koike, K., Zucman-Rossi, J., & Finn, R. S. (2021). Hepatocellular carcinoma. *Nature Reviews Disease Primers*, 7(1), 6.
- Lopez, J. S., & Banerji, U. (2017). Combine and conquer: challenges for targeted therapy combinations in early phase trials. *Nature Reviews Clinical Oncology*, 14(1), 57–66.
- Lubos, E., Loscalzo, J., & Handy, D. E. (2011). Glutathione peroxidase-1 in health and disease: From molecular mechanisms to therapeutic opportunities. *Antioxidants & Redox Signaling*, 15(7), 1957–1997.
- Maiuolo, J., Gliozzi, M., Carresi, C., Musolino, V., Oppedisano, F., Scarano, F., Nucera, S., Scicchitano, M., Bosco, F., Macri, R., Ruga, S., Cardamone, A., Coppoletta, A., Mollace, A., Cognetti, F., & Mollace, V. (2021). Nutraceuticals and cancer: Potential for natural polyphenols. *Nutrients*, 13(11), 3834.
- Marklund, S., & Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry*, 47(3), 469–474.
- Maucourt-Boulch, D., de Martel, C., Franceschi, S., & Plummer, M. (2018). Fraction and incidence of liver cancer attributable to hepatitis B and C viruses worldwide. *International Journal of Cancer*, 142(12), 2471–2477.
- Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 65(1–2), 55–63.
- Nakamura, H., & Takada, K. (2021). Reactive oxygen species in cancer: Current findings and future directions. *Cancer Science*, 112(10), 3945–3952.
- Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, 95(2), 351–358.
- Okon, I. S., & Zou, M. H. (2015). Mitochondrial ROS and cancer drug resistance: Implications for therapy. *Pharmacological Research*, 100, 170–174.
- Poudel, D. K., Ojha, P. K., Rokaya, A., Satyal, R., Satyal, P., & Setzer, W. N. (2022). Analysis of volatile constituents in *Curcuma* species, viz. *C. aeruginosa*, *C. zedoaria*, and *C. longa*, from Nepal. *Plants (Basel)*, 11(15), 1932.
- Redza-Dutordoir, M., & Averill-Bates, D. A. (2016). Activation of apoptosis signalling pathways by reactive oxygen species. *Biochimica Biophysica Acta*, 1863, 2977–2992.
- Rumgay, H., Arnold, M., Ferlay, J., Lesi, O., Cabaasag, C. J., Vignat, J., Laversanne, M., McGlynn, K. A., & Soerjomataram, I. (2022). Global burden of primary liver cancer in 2020 and predictions to 2040. *Journal of Hepatology*, 77(6), 1598–1606.
- Schafer, Z. T., Grassian, A. R., Song, L., Jiang, Z., Gerhart-Hines, Z., Irie, H. Y., Gao, S., Puigserver, P., & Brugge, J. S. (2009). Antioxidant and oncogene rescue of metabolic defects caused by loss of matrix attachment. *Nature*, 461(7260), 109–113.
- Schlachterman, A., Craft, W. W. Jr, Hilgenfeldt, E., Mitra, A., & Cabrera, R. (2015). Current and future treatments for hepatocellular carcinoma. *World Journal of Gastroenterology*, 21(28), 8478–8491.
- Scott, E. C., Baines, A. C., Gong, Y., Moore Jr, R., Pamuk, G. E., Saber, H., Subedee, A., Thompson, M. D., Xiao, W., Pazdur, R., & Rao, V. A. (2023). Trends in the approval of cancer therapies by the FDA in the twenty-first century. *Nature Reviews Drug Discovery*, 22(8), 625–640.
- Sena, L. A., & Chandel, N. S. (2012). Physiological roles of mitochondrial reactive oxygen species. *Molecular Cell*, 48, 158–167.
- Siomek, A., Tujakowski, J., Gackowski, D., Rozalski, R., Foksinski, M., Dziaman, T., Roszkowski, K., & Olinski, R. (2006). Severe oxidatively damaged DNA after cisplatin treatment of cancer patients. *International Journal of Cancer*, 119(9), 2228–2230.
- Sudipta, K. M., Lokesh, P., Rashmi, W., Vijay, R., & Ssn, K. (2012). Phytochemical screening and *in vitro* antimicrobial activity of *Bougainvillea spectabilis* flower extracts. *International Journal of Phytomedicine*, 4(3), 375.
- Sumathi, S., Iswariya, G. T., Sivaprabha, B., Dharani, B., Radha, P., & Padma, P. R. (2013). Comparative study of radical scavenging activity and phytochemical analysis of fresh and dry rhizomes of *Curcuma zedoaria*. *International Journal of Pharmaceutical Sciences and Research*, 4(3), 1069.
- Sun, Y., Wang, M., Ling, J., Wu, Q., Han, G., & Zhou, J. (2024). Curzerenone inactivates the nuclear factor-kappa B signaling to suppress malignancy and immune evasion in cervical cancer by targeting CSNK2B. *Human Cell*, 38(1), 35.
- Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, 71(3), 209–249.
- Tian, L. Y., Smit, D. J., & Jücker, M. (2023). The role of PI3K/AKT/mTOR signaling in hepatocellular carcinoma metabolism. *International Journal of Molecular Science*, 24(3), 2652.
- Townsend, D. M., & Tew, K. D. (2003). The role of glutathione-S-transferase in anticancer drug resistance. *Oncogene*, 22(47), 7369–7375.
- Tung, B. T., Nham, D. T., Hai, N. T., & Thu, D. K. (2019). *Curcuma longa*, the polyphenolic curcumin compound and pharmacological effects on liver. *Dietary Interventions in Liver Disease*, 125–134.

- Uzunhisarcikli, M., & Aslanturk, A. (2019). Hepatoprotective effects of curcumin and taurine against bisphenol A-induced liver injury in rats. *Environmental Science and Pollution Research*, 26, 37242–37253.
- Wang, Y., Guo, S. H., Shang, X. J., Yu, L. S., Zhu, J. W., Zhao, A., Zhou, Y. F., An, G. H., Zhang, Q., & Ma, B. (2018). Triptolide induces Sertoli cell apoptosis in mice via ROS/JNK-dependent activation of the mitochondrial pathway and inhibition of Nrf2-mediated antioxidant response. *Acta Pharmacologica Sinica*, 39(2), 311–327.
- Wang, S., Wu, W., Liu, Y., Xing, E., Jiao, J., Li, L., Li, J., & Wang, D. (2023). Curcumin induces apoptosis by suppressing XRCC4 expression in hepatocellular carcinoma. *Nutrition and Cancer*, 75(10), 1958–1967.
- Wu, G. S., Song, Y. L., Yin, Z. Q., Guo, J. J., Wang, S. P., Zhao, W. W., Chen, X. P., Zhang, Q. W., Lu, J. J., & Wang, Y. T. (2013). Ganoderiol A-enriched extract suppresses migration and adhesion of MDA-MB-231 cells by inhibiting FAK-SRC-paxillin cascade pathway. *PLoS One*, 8(10), e76620.
- Xu, L., Ding, L., Su, Y., Shao, R., Liu, J., & Huang, Y. (2019). Neuroprotective effects of curcumin against rats with focal cerebral ischemia-reperfusion injury. *International Journal of Molecular Medicine*, 43(4), 1879–1887.
- Yan, X., Huang, S., Yang, Y., Lu, Z., Li, F., Jiang, L., Jiang, Y., & Liu, J. (2022). Sirolimus or everolimus improves survival after liver transplantation for hepatocellular carcinoma: A systematic review and meta-analysis. *Liver Transplantation*, 28(6), 1063–1077.
- Ye, L., Mayerle, J., Ziesch, A., Reiter, F. P., Gerbes, A. L., & De Toni, E. N. (2019). The PI3K inhibitor copanlisib synergizes with sorafenib to induce cell death in hepatocellular carcinoma. *Cell Death Discovery*, 5, 86.
- Zhao, Y., Ye, X., Xiong, Z., Ihsan, A., Ares, I., Martínez, M., Lopez-Torres, B., Martínez-Larrañaga, M. R., Anadón, A., Wang, X., & Martínez, M. A. (2023). Cancer metabolism: The role of ROS in DNA damage and induction of apoptosis in cancer cells. *Metabolites*, 13(7), 796.
- Zheng, T., & Chen, H. X. (2019). Anticancer effects of curzerenone against drug-resistant human lung carcinoma cells are mediated via programmed cell death, loss of mitochondrial membrane potential, ROS, and blocking the ERK/MAPK and NF- κ B signaling pathway. *Journal of BUON*, 24(3), 907–912.
- Zhou, W. P., Lai, E. C., Li, A. J., Fu, S. Y., Zhou, J. P., Pan, Z. Y., Lau, W. Y., & Wu, M. C. (2019). A prospective, randomized, controlled trial of preoperative transarterial chemoembolization for resectable large hepatocellular carcinoma. *Annals of Surgery*, 249(2), 195–202.