J Nanostruct 16(2): 1565-1575, Spring 2026

RESEARCH PAPER

Extraction, Characterization, and Anticancer Activity of Dihydrocapsaicin and Farnesyl Phenyl Sulfone Nanoparticles from Iraqi Hot Pepper Seeds

Muhannad Abdul-Redha Al-Saedy *, Noor Kadhim Ahmed, Shaimaa Ibrahim Khalil, Nedhal M. Khamis, Nuha A. Imran, Shurooq M. Wsain

Department of Chemistry, College of Science, Mustansiriyah University, Iraq

ARTICLE INFO

Article History:
Received 10 December 2025
Accepted 22 February 2026
Published 01 April 2026

Keywords:
Anti-cancer activity
Capsaisonides
Farnesyl phenyl sulfone
Medicinal plant extracts
Nano particles
HepG2

ABSTRACT

This study reports the extraction and characterization of two major constituents, dihydrocapsaicin (8-methyl-N-vanillylnonanamide and farnesyl phenyl sulfone (2*E*, 6*E*-3,7,11-trimethyldodeca-2,6,10-trien-1-yl) sulfonyl benzene) from the seeds of Iraqi green chili pepper (*Capsicum annuum*). A solvent-dependent profile of extraction by 10-day cold maceration with a variety of solvents. Acetic acid was highly effective and more selective, yielding a higher percentage of dihydrocapsaicin (86%) and farnesyl phenyl sulfone (10%). Whereas, Ethanol was lower selective to provide less dihydrocapsaicin (79%) and more farnesyl phenyl sulfone (18%). Mechanical downsizing using ball milling generate nanoscale particles with enhanced surface area and improved physicochemical properties. Structural confirmation were done by FT-IR, UV-Visible spectroscopy, and GC-MS. In addition, field-emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM) confirmed nanoscale crystalline features with well-defined surface topology. The in vitro assessment of the anticancer properties of the prepared nanoformulations was conducted against the human hepatocellular carcinoma (HepG2) cells line using normal human dermal fibroblast neonatal cells (HDFn) as control. The extracts exhibited a dose-dependent cytotoxicity toward HepG2 cancer cells indicating their promise as natural anticancer agents. These results underscore the use of chili pepper seeds as a sustainable agricultural byproduct and a potential source of nanostructured plant-derived molecules in pharmaceutical and nutraceutical therapy especially in the treatment of liver cancer.

How to cite this article
Al-Saedy M., Ahmed N., Khalil S. Extraction, Characterization, and Anticancer Activity of Dihydrocapsaicin and Farnesyl Phenyl Sulfone Nanoparticles from Iraqi Hot Pepper Seeds. J Nanostruct, 2026; 16(2):1565-1575. DOI: 10.22052/JNS.2026.02.008

INTRODUCTION

Chili peppers (Capsicum spp.) are valued as culinary components and as rich sources of biologically active metabolites with diverse pharmacological potential. Among these, capsaicin (or dihydrocapsaicin) is the most extensively studied due to its potent antioxidant, anti-inflammatory, and anticancer effects [1-3]. Its amphiphilic structure, comprising a vanillyl group (4-hydroxy-3-methoxybenzyl) linked via an

* *Corresponding Author Email: dr.muhannad.alsaedy@uomustansiriyah.edu.iq*

amide bond to a hydrophobic fatty acid chain (1, Fig. 1), enhances membrane permeability and enables interaction with the TRPV1 receptor, thereby influencing ion transport, oxidative stress responses, and apoptosis in malignant cells [4-6].

Beyond dihydrocapsaicin (8-methyl-N-vanillylnonanamide), chili pepper seeds contain additional bioactive constituents, including farnesyl phenyl sulfone [(2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)sulfonyl)benzene] (2, Fig. 1), a sulfonylated terpenoid derivative. Its unsaturated terpenoid backbone imparts high lipophilicity and free radical, scavenging capability, while the sulfone (SO_2) group contributes to chemical stability, polarity, and potential pharmacological activity [3, 4]. In medicinal chemistry, sulfone-containing molecules are particularly valued for enhancing metabolic stability and improving drug-like properties, making such derivatives promising candidates for therapeutic development [7].

Despite their biochemical richness, agricultural byproducts such as chili pepper seeds are often discarded. Utilizing these materials provides a sustainable route for natural product recovery and value-added applications. When isolated compounds are processed at the nanoscale, their physicochemical and biological performance can be further enhanced. Aromatic systems, conjugated double bonds, and hydrogen-bonding functionality promote supramolecular assembly, crystallinity, and tailored surface properties, improving solubility, stability, and cellular uptake, all critical parameters for biomedical use [8-10].

In recent years, plant-derived nanoparticles (phytonanoparticles), have gained significant attention as green, biocompatible platforms for drug delivery, antimicrobial therapy, wound healing, and anticancer treatment. Their nanoscale dimensions and high surface to volume ratio enable efficient cellular internalization and targeted action, while natural phytochemical capping agents minimize toxicity. Notably, gold, silver, and zinc oxide nanoparticles synthesized using plant extracts have demonstrated selective anticancer activity through oxidative stress modulation and induction of programmed cell death [11, 12].

Hepatocellular carcinoma (HCC), the most common primary liver cancer, is a major cause of cancer-related mortality worldwide. Its treatment is challenged by late diagnosis, poor prognosis, high recurrence, and frequent resistance to chemotherapy [11]. HepG2 cells, derived from a human hepatocellular carcinoma, serve as a widely used in vitro model for studying liver cancer biology and screening potential therapeutics [12]. These cells retain many differentiated hepatic functions, such as albumin secretion and drug-metabolizing enzyme activity, making them highly relevant for evaluating natural compounds with anticancer potential. Previous studies have shown that capsaicin induces apoptosis in HepG2 cells via mitochondrial dysfunction, caspase activation, and inhibition of NF-κB and STAT3 pathways [13, 14].

In this context, the present study aimed to extract and characterize a nanostructure of dihydrocapsaicin and farnesyl phenyl sulfone from Iraqi hot pepper (Capsicum annuum) seeds. Extraction was performed using ethanol and acetic acid by cold maceration [3], followed by purification and characterization through spectroscopic and microscopic techniques. Finally, the cytotoxic effects of the extracts were evaluated in vitro against HepG2 liver cancer cells using normal human dermal fibroblast neonatal cells (HDFn) as control. This work highlights chili pepper seeds as an underutilized agricultural byproduct and a promising source of nanostructured bioactive compounds with potential applications in anticancer therapy and nutraceutical development.

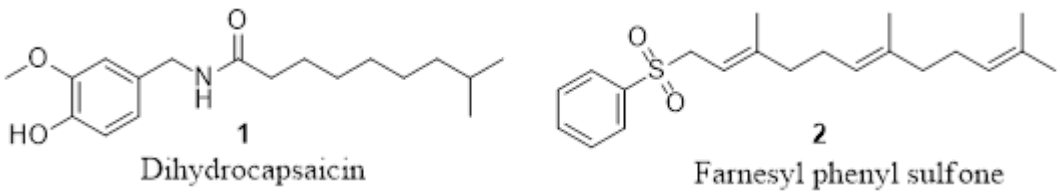

Fig. 1. The structure of Dihydrocapsaicin and Farnesyl phenyl sulfone.

MATERIALS AND METHODS

Chemicals and Reagents

Dimethyl sulfoxide (DMSO), trypsin, fetal bovine serum (FBS), RPMI-1640 medium, phosphate-buffered saline (PBS), sodium bicarbonate, and acetic acid were purchased from Sigma-Aldrich (USA). Absolute ethanol was obtained from Hemadia (India). The MTT cell viability assay kit was purchased from Intron Biotechnology (Korea).

Human hepatocellular carcinoma cells (HepG2, HB-8065™) and normal human dermal fibroblast neonatal cells (HDFn, CRL-11233™) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Dulbecco's Modified Eagle's Medium (DMEM) was purchased from Lonza/Clonetics Corporation (USA). The supplier validated and tested cell lines before distribution. The cells were tested as per ATCC certification to ensure that they were not contaminated with bacteria, fungi, and mycoplasma. The cell lines were also tested with significant viral contaminants, such as human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV). Inverted light microscopy was a regular routine in our laboratory used to monitor cell morphology and growth properties. The cells were kept in a sterile condition and applied within the recommended amount of passages to guarantee the reproducibility of the experiment and the avoidance of biological variability.

Sample Collection

Fresh Iraqi green chili peppers (*Capsicum annuum*) were obtained from al-Muqdadiyah market in Diyala, Iraq, where they are locally cultivated. Seeds were manually removed, washed twice with distilled water, and air-dried at room temperature for seven days.

Formation of nanoparticles [15]

A total of 200 g chili pepper seeds were initially ground in a traditional herb-crushing machine (LEEVOT) at 28,000 rpm with the power output of 1200 W in three separate cycles, 5 minutes each, to produce a fine micro powder. The resulting powder was further reduced in size by a locally made ball-milling system. To conduct this process, 100 g of the pre-ground chili pepper seed powder was put in a 1 L milling container with 100 stainless-steel balls of different sizes (5-15 mm). The milling cycles were comprised of 15 min milling at 400 rpm and a cooling period of 15 min. The number of consecutive cycles that were done was 16 which corresponds to an effective 4 h milling to produce the Nano powder of chili pepper seeds.

Extraction of Bioactive Compounds

Bioactive compounds were extracted using the cold maceration method [16, 17]. Equal portions of seed powder were soaked separately in either ethanol or glacial acetic acid for four consecutive days without agitation. Each mixture was then transferred to a 100 mL round-bottom flask, tightly sealed, and placed on a magnetic stirrer (without heating) for 10 days. The Crude mixture was filtered, and extracted with acetic acid to produce about 86% dihydrocapsaicin and 10% of the sulfonated compound. While, ethanol extraction produced about 79% dihydrocapsaicin and 18% of the sulfonated compound. The isolated dihydrocapsaicin and farnesyl phenyl sulfone were further purified by flash chromatography over silica gel, eluting with petroleum ether/EtOAc (0-30%) provided the targeting compounds.

Characterization

The identity of the purified isolated compounds was confirmed using Fourier Transform Infrared Spectroscopy (FT-IR), Ultraviolet–Visible Spectroscopy (UV-Vis), and Gas Chromatography-Mass Spectrometry (GC-MS). While, the nanoscale morphology of ball-milling powder of the chili pepper seeds was confirmed using Field-emission scanning electron microscopy (FESEM) and Transmission electron microscopy (TEM).

Cell Culture Maintenance

HepG2 and HDFn cells were maintained according to ATCC guidelines [18, 19]. Cells were cultured in DMEM supplemented with 10% fetal bovine serum and incubated at 37°C in a humidified atmosphere containing 5% CO_2. Cells were passaged at 70–80% confluence using trypsin–EDTA and used within the recommended passage range.

Cytotoxicity Assay (MATT)[20]

Cytotoxicity was evaluated using the MTT assay. HepG2 and HDFn cells were seeded in 96-well plates at 5×10^3 cells/well in 200 µL complete DMEM and incubated for 24 h to allow attachment. The medium was then replaced with fresh medium containing pepper seed nanoparticle extract at 25, 50, 100, 200, and 400 mg/mL, and cells were

incubated for a further 24 h.

Following treatment, 10 µL MTT reagent was added to each well and incubated for 3 h at 37°C to allow formazan crystal formation. The medium was carefully removed and the crystals were dissolved using 100 µL dissolution solution. Absorbance was measured using a microplate reader at 630 nm. Cell viability (%) was calculated relative to untreated control cells.

Statistical Analysis

The experiments were conducted in three repetitions and three independent repetitions. Statistical data is presented in terms of mean and standard deviation (SD). GraphPad Prism software (GraphPad Software, San Diego, CA, USA) was the statistical analysis program [21]. The experimental group differences were compared through one-way analysis of variance (ANOVA) [22] and multiple comparison post-hoc test (Tukey)[23]. The level of significance taken was $p < 0.05$. The nonlinear regression analysis (log[inhibitor] vs. normalized response, variable slope model) was used to obtain dose-response curves and IC 50 values [24].

Ethical Statement

This study did not involve human participants or live animals. Human cell lines used in all the experimental processes are commercially made and taken from certified cell repositories. Hence, no ethical consent was needed in this research. All laboratory safety and biosafety protocols of the institution were followed during all experimental procedures.

RESULTS AND DISCUSSION

Solvent Extraction Efficiency

The extraction efficiency of ethanol and acetic acid solvents was compared to determine optimal recovery of bioactive compounds. Ethanol extraction yielded approximately 79% dihydrocapsaicin and 18% farnesyl phenyl sulfone, whereas acetic acid extraction produced approximately 86% dihydrocapsaicin and 10% farnesyl phenyl sulfone.

The superior balanced extraction performance of ethanol may be attributed to its intermediate polarity, allowing simultaneous extraction of both moderately polar and lipophilic compounds. The

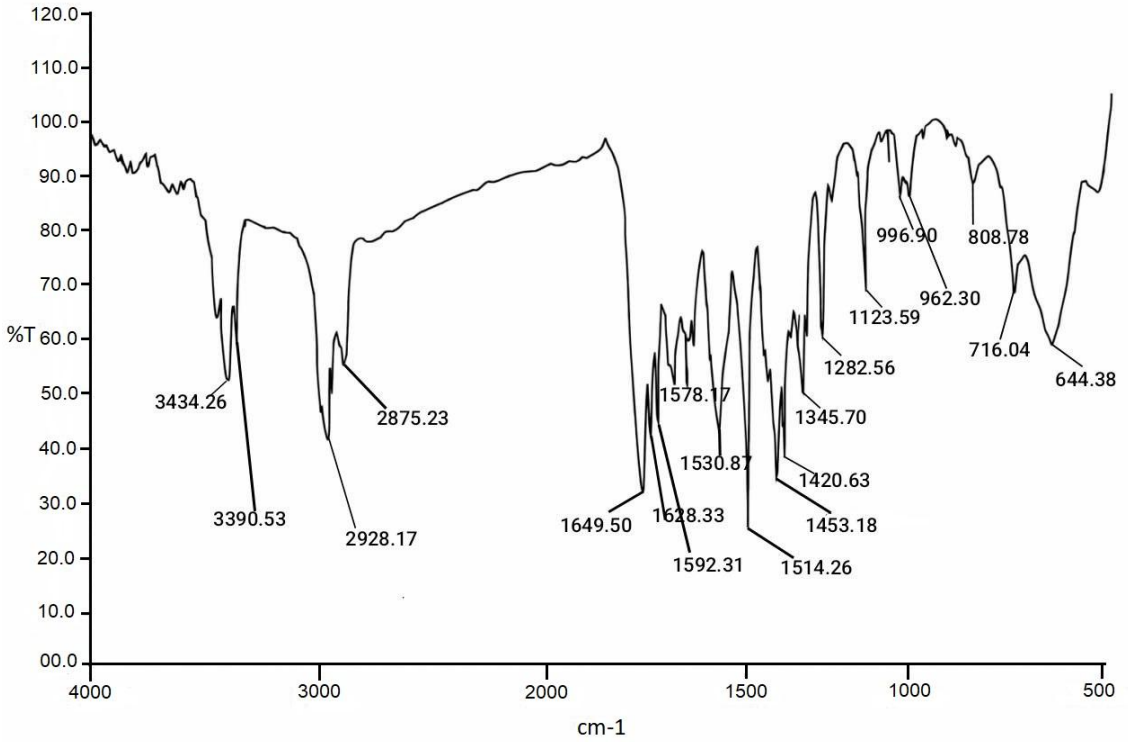

Fig. 2. FT-IR spectra of dihydrocapsaicin.

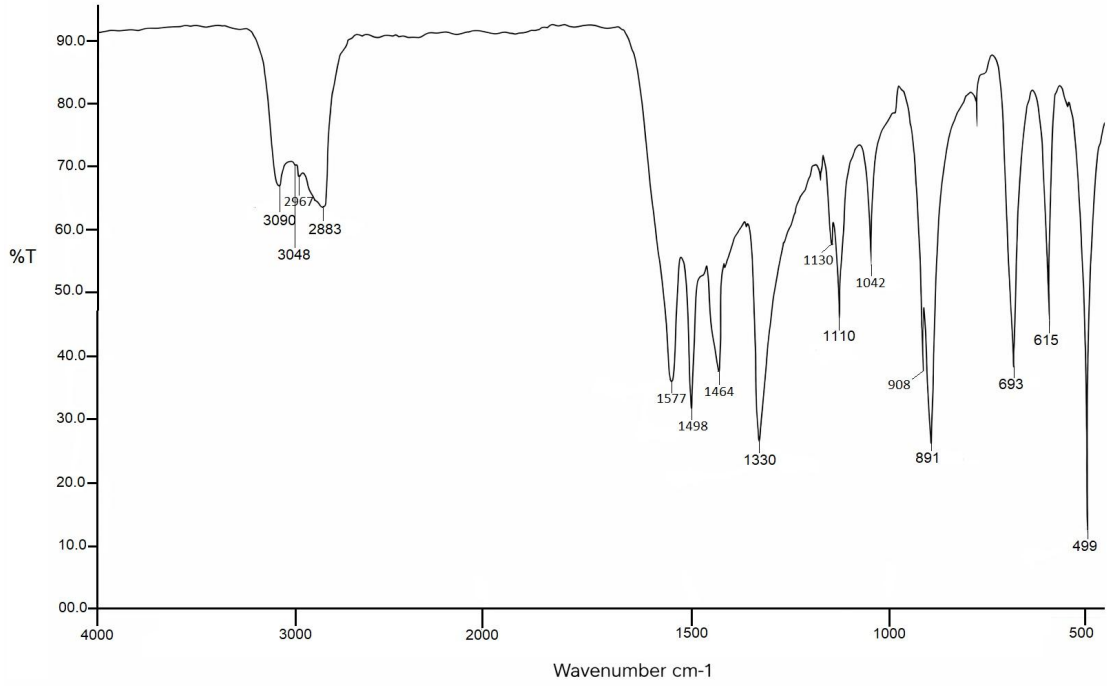

Fig. 3. FT-IR spectra of farnesyl phenyl sulfone.

co-extraction of dihydrocapsaicin and sulfonated derivatives may contribute to synergistic biological activity, as combined phytochemicals often demonstrate enhanced therapeutic effects compared with isolated compounds.

Characterizations and interpretation of spectral measurements

FT-IR spectra interpretation of the dihydrocapsaicin (8-methyl-N-vanillylnonanamide), and farnesyl phenyl sulfone (2E, 6E-3,7,11-trimethyldodeca-2,6,10-trien-1-yl) sulfonyl)benzene) extract.

FT-IR Spectral Analysis

The structures of the extracted compounds were verified using Fourier-transform infrared (FT-IR) spectroscopy, UV–Visible spectroscopy, and gas chromatography–mass spectrometry (GC–MS). In addition, field-emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM) revealed well-defined nanoscale crystalline features.

The FT-IR spectrum of dihydrocapsaicin (8-methyl-N-vanillylnonanamide) (Fig. 2) exhibited some characteristic absorption bands corresponding to the phenolic hydroxyl group, the amide N–H and C=O functionalities, aromatic and aliphatic C–H stretching, and the etheric C-O-C linkage.

More specifically, a broad absorption band at 3464 cm^{-1} was attributed to phenolic O-H stretching, which overlapped with N-H stretching vibrations observed at 3390 cm^{-1}. Aromatic C-H stretching appeared at 3080 cm^{-1}, and the aliphatic C-H stretching bands at 2928 and 2875 cm^{-1} were consistent with methyl and methylene groups. A weak band at 1649 cm^{-1} indicated the amidic C=O functionality. The peaks at 1628 and 1592 cm^{-1} were attributed to C=C stretching vibrations of aromatic ring. Whereas, the peak at 1514 along with the peak at 1453 cm^{-1} are caused by a combination of N-H bending and C-N stretching vibrations. The characteristic etheric C-O-C stretching band at 1282 cm^{-1} further supported the structural identity of dihydrocapsaicin.

The FT-IR spectrum of the farnesyl phenyl sulfone (2E, 6E-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)sulfonyl)benzene), (Fig. 3), displayed characteristic absorption bands consistent with a conjugated aromatic system bearing a sulfonyl substituent. An aromatic C-H stretching band

appeared at 3090 cm^{-1}, overlapping with an olefinic C-H stretching band at 3048 cm^{-1}. Aliphatic C-H stretching vibrations corresponding to methyl and methylene groups were observed at 2967 and 2883 cm^{-1}. The C=C stretching modes of both aromatic and aliphatic fragments were recorded at 1577, 1498, 1464 cm^{-1}. Additionally, bands appeared at 1330 and 1110 cm^{-1} belong to the asymmetric and symmetric stretching vibration of SO_2 group.

GC-mass spectra interpretation of the Dihydrocapsaicin and Farnesyl phenyl sulfone

The mass spectrum of dihydrocapsaicin showed a molecular ion peak at *m/z* 307, corresponding to the measured molecular weight of the compound. This value is in agreement with the theoretical exact mass calculated from its chemical structure $[C_{18}H_{29}NO_3]$ (307.21 g/mol), confirming the identity and purity of the isolated dihydrocapsaicin (Fig. 4).

The mass spectrum of ((2E, 6E)-3, 7, 11-trimethyldodeca-2, 6, 10-trien-1-yl) sulfonyl) benzene displayed a distinct molecular ion peak at *m/z* 346, consistent with the theoretical exact mass of the compound $[C_{21}H_{30}O_2S]$ (346.20 g/mol), thereby confirming its structural identity and successful isolation (Fig. 5).

UV-Viss spectra of the dihydrocapsaicin extract

The UV–Visible absorption spectrum of the dihydrocapsaicin extract displayed two prominent peaks at 194.8 nm and 280.4 nm, corresponding to π→π* and n→π* electronic transitions, respectively, as shown in (Fig. 6). These transitions are characteristic of the dihydrocapsaicin structure and reflect its conjugated aromatic system and

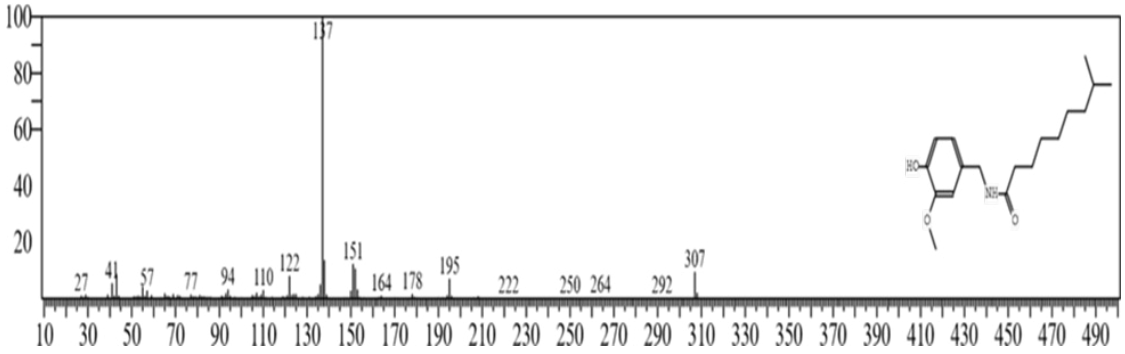

Fig. 4. GC-mass spectra of the dihydrocapsaicin extract.

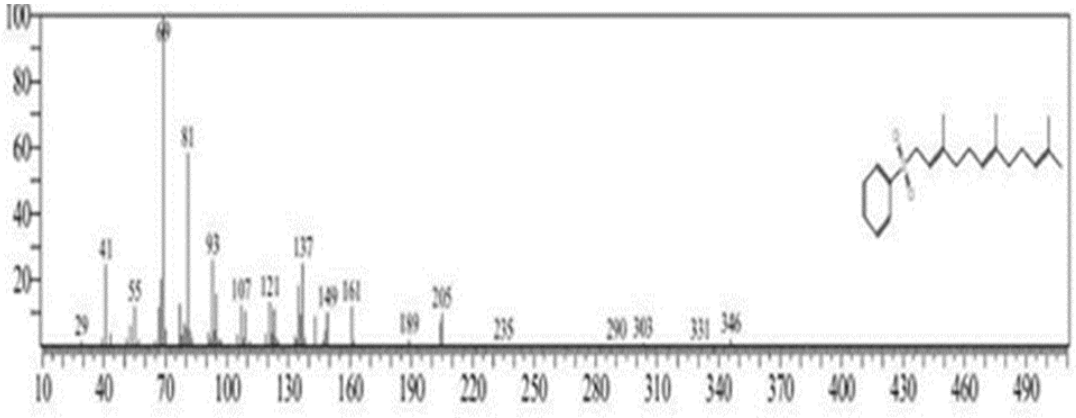

Fig. 5. GC-mass spectra of the farnesyl phenyl sulfone extract.

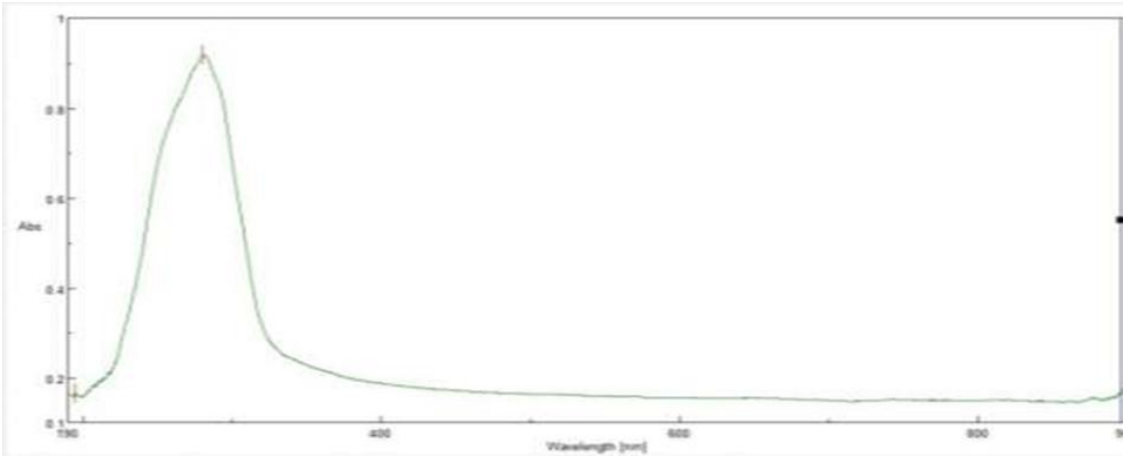

Fig. 6. The ultra Uv-Vis spectra of dihydrocapsaicin.

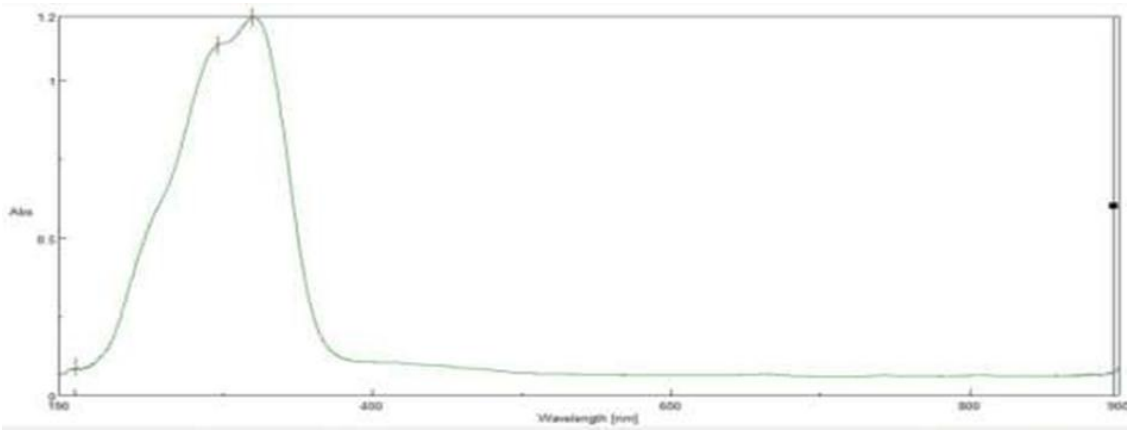

Fig. 7. The Uv-Vis spectra of the farnesyl phenyl sulfone.

non-bonding electron interactions.

In contrast, the spectrum of the farnesyl phenyl sulfone extract exhibited three distinct absorption peaks at 201.2 nm, 296.0 nm, and 319.6 nm. These were assigned to π→π*, n→π*, and charge-transfer (CT) transitions, respectively (Fig. 7). The presence of a CT band indicates an enhanced degree of electron delocalization within the molecule, consistent with its conjugated terpenoid–sulfonyl structure.

Study the constitutional morphological qualities of the particles surface

The structural features of the synthesized nanoparticles were examined using Field Emission Scanning Electron Microscopy (FESEM) and Transmission Electron Microscopy (TEM). FESEM micrographs demonstrated that the hot pepper seed-derived nanoparticles exhibited a relatively uniform morphology with minimal aggregation. The particles appeared predominantly spherical to semi-spherical in shape, with an average particle diameter of approximately 100 nm (Fig. 8). The uniform surface topology indicates effective nanoparticle formation and stable dispersion. Further structural confirmation was obtained using Transmission Electron Microscopy (TEM). As illustrated in Fig. 8, the nanoparticles demonstrated well-defined internal structures with particle sizes ranging from approximately 50

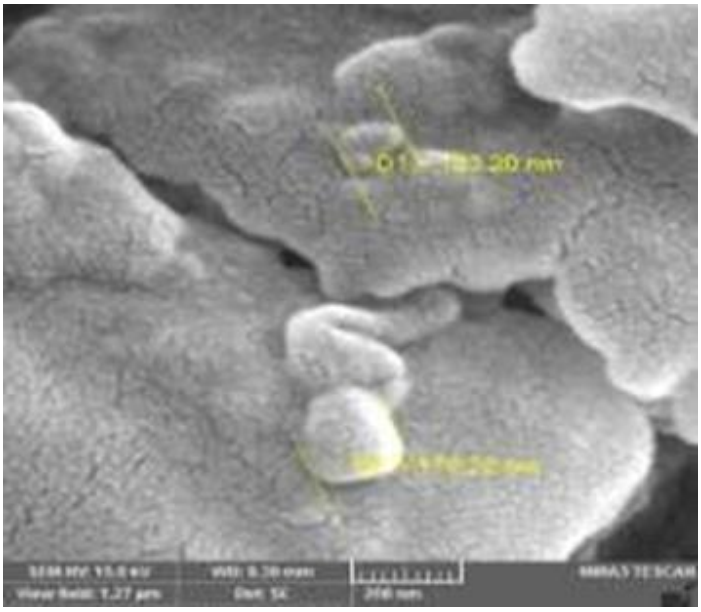

Fig. 8. FESEM interpretation for Nano-particles of the hot pepper seeds.

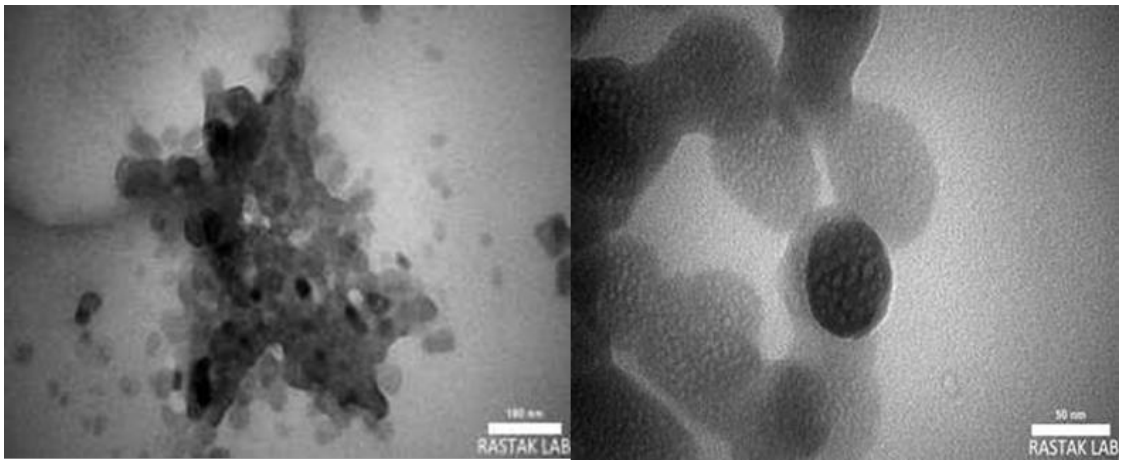

Fig. 9. TEM interpretation for Nano-particles of the hot pepper seeds.

to 100 nm (Fig. 9).

The nanoscale size distribution observed by TEM supports the FESEM findings and confirms successful nanoparticle synthesis suitable for biological applications. Nanoparticles within this size range are known to exhibit enhanced cellular uptake through endocytosis, particularly in cancer cells with higher metabolic activity. The nanoscale size also increases surface area, which may enhance the delivery and intracellular release of bioactive phytochemicals.

Anti-HepG2 Activity of Pepper Seed Extract

The anticancer activity of pepper seed-derived nanoparticle extract was tested in vitro on human hepatocellular carcinoma cells (HepG2) using normal human dermal fibroblast neonatal (HDFn) cells as the non-cancerous control. The MTT assay was used to determine cell viability after 24 h of exposure to different extract concentrations (25-

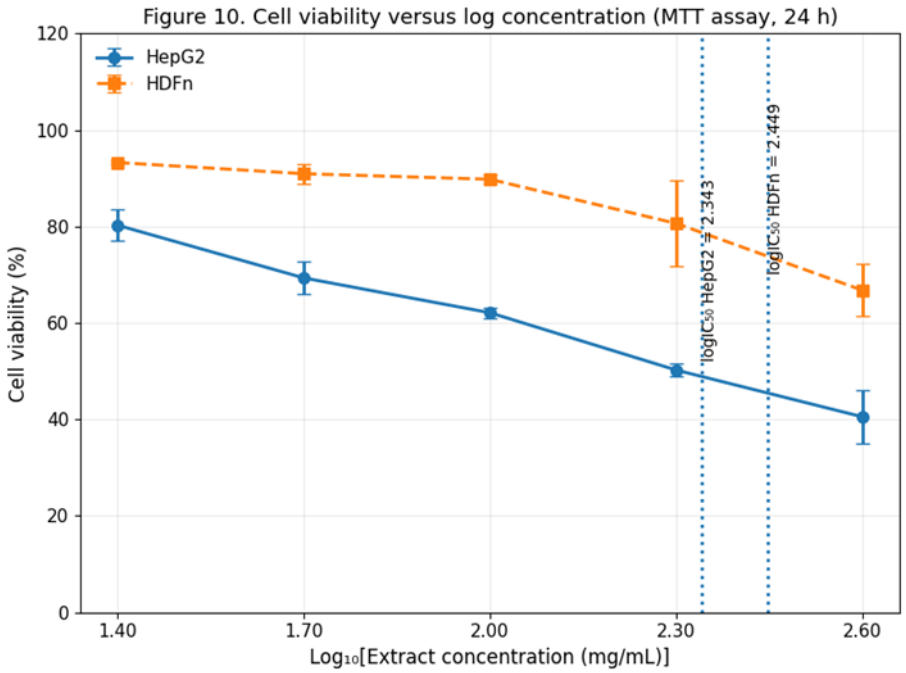

Fig. 10. Cell viability (%) vs Log_{10} concentration (with mean ± SD error bars).

Table 1. Effect of hot pepper seed extract on HepG2 cell viability (MTT assay).

Concentration (mg/mL)	Log(Conc.)	Viability (%) (Mean ± SD), n = 3
25	1.30	80.25 ± 3.22
50	1.6989	69.33 ± 3.37
100	2.00	62.11 ± 1.05
200	2.30	50.23 ± 1.25
400	2.60	40.55 ± 5.45

Table 2. Effect of hot pepper seed extract on HDFn cell viability (MTT assay).

Concentration (mg/mL)	Log(Conc.)	Viability (%) (Mean ± SD), n = 3
25	1.30	93.25 ± 0.35
50	1.6989	90.93 ± 2.03
100	2.00	89.78 ± 1.00
200	2.30	80.63 ± 8.81
400	2.60	66.78 ± 5.38

Table 3. Selectivity analysis: comparison of HepG2 vs HDFn cell viability.

Concentration (mg/mL)	HepG2 viability (%) (Mean ± SD)	HDFn viability (%) (Mean ± SD)	p-value
25	80.25 ± 3.22	93.25 ± 0.35	0.01881
50	69.33 ± 3.37	90.93 ± 2.03	0.001687
100	62.11 ± 1.05	89.78 ± 1.00	5.116×10^{-6}
200	50.23 ± 1.25	80.63 ± 8.81	0.02495
400	40.55 ± 5.45	66.78 ± 5.38	0.004048

*Values are mean ± SD (n = 3). p-values were calculated using a two-tailed Welch's t-test comparing HepG2 vs HDFn at each concentration.

400mg/mL).

As Table 1 summarizes and Fig. 10 shows, when cells of HepG2 were treated with the extract, a significant concentration-dependent decrease in cell viability was observed. Concentrations of 25-100 mg/mL showed progressive reduction of HepG2 viability, which showed moderate cytotoxicity. When the concentration was escalated up to 200 and 400mg/mL, a significant decrease in viability was found, thus showing a great anti-proliferative effect on liver cancer cells.

In contrast, HDFn cells were also much more tolerant to the same conditions of treatment. The ability to retain cell viability in normal cells was found to be higher at lower concentrations and decreased slowly at higher doses as indicated in Table 2, due to lower susceptibility as compared to cancer cells.

Table 3 provides direct statistical comparison of HepG2 and HDFn cells at similar concentrations. The analysis using t-test (two tailed) showed that cancerous and normal cells differ significantly at all concentrations tested, with p-values of 0.0188 (25mg/ml), 0.00169 (50mg/ml), 5.12×10^{-6} (100mg/ml), 0.0249(200mg/ml) and 0.00405(400mg/ml). Such findings substantiate the selectivity of the cytotoxic activity of the extract to the malignant cells ($p < 0.05$).

The dose-response curves (log[inhibitor] vs. response, variable slope model) were analyzed through nonlinear regression analysis, which revealed that IC50 of HepG2 cells and HDFn cells are about 220 mg/mL and 281 mg/mL, respectively, which clarifies that normal cells are more resistant to the extract.

The selectivity that was observed is possibly due to the nanoscale characteristics of the extract and the presence of bioactive capsaicinoid and sulfonated compounds. The cancer cells are normal cells characterized by a higher level of nanoparticle uptake, a disturbed redox system, and a higher vulnerability to oxidative stress, which is likely to be the cause of elevated cytotoxic response of cancer cells in comparison to the normal fibroblasts.

CONCLUSION

This research indicates that the Iraqi green chili pepper (Capsicum annuum) seeds have great potential of being used as a sustainable source of bioactive constituents to formulate nanostructured medicinal products. The successful extraction and characterization of the major constituents, dihydrocapsaicin (8-methyl-N-vanillylnonanamide) and farnesyl phenyl sulfone ((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)sulfonyl benzene), highlight the chemical richness of this agricultural byproduct. Maceration extraction was demonstrated to be solvent-dependent with the highest recovery rate with ethanol to yielded (79%) capsaicine and (18%) farnesyl phenyl sulfone, whereas acetic acid selectively extracted dihydrocapsaicin (86%), and decreased the content of the sulfone to only (10%). A biological assessment of the nanoparticle-enriched extracts has shown that it possesses strong anticancer properties on hepatocellular carcinoma cells. Concentrations of 25, 50 and 100 mg/mL were shown to have a significant growth inhibitory effect with no observed cytotoxicity to normal healthy cells. The enhanced activity is attributed to improved cellular uptake and increased surface interaction of Nano-scaled powder. Taken together, these results indicate the selective therapeutic capabilities of chili pepper seed-derived nanomaterials and provide a solid basis to their further application as natural, targeted anti-cancer agents.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the support provided by Mustansiriyah University and

University of Diyala for facilitating this research. The authors thank the laboratories and research facilities of their institutions for providing the necessary infrastructure, instrumentation, and technical assistance required to complete this work. Special appreciation is extended to the staff and analytical laboratories for their support during the experimental and characterization studies.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

REFERENCES

1. Duranova H, Valkova V, Gabriny L. Chili peppers (Capsicum spp.): the spice not only for cuisine purposes: an update on current knowledge. Phytochem Rev. 2021;21(4):1379-1413.
2. Hernández-Pérez T, Gómez-García MdR, Valverde ME, Paredes-López O. Capsicum annuum (hot pepper): An ancient Latin-American crop with outstanding bioactive compounds and nutraceutical potential. A review. Comprehensive Reviews in Food Science and Food Safety. 2020;19(6):2972-2993.
3. Hudáková T, Šemeláková M, Očenáš P, Kožurková M, Krochtová K, Sovová S, et al. Chili pepper extracts, capsaicin, and dihydrocapsaicin as potential anticancer agents targeting topoisomerases. BMC Complementary Medicine and Therapies. 2024;24(1).
4. Maharjan A, Vasamsetti BMK, Park J-H. A comprehensive review of capsaicin: Biosynthesis, industrial productions, processing to applications, and clinical uses. Heliyon. 2024;10(21):e39721.
5. Ilie M, Caruntu C, Tampa M, Georgescu S-R, Matei C, Negrei C, et al. Capsaicin: Physicochemical properties, cutaneous reactions and potential applications in painful and inflammatory conditions (Review). Exp Ther Med. 2019.
6. Wu L, Xu S, Cheng X, Zhang L, Wang Y, Wu J, et al. Capsaicin inhibits the stemness of anaplastic thyroid carcinoma cells by triggering autophagy-lysosome mediated OCT4A degradation. Phytother Res. 2022;36(2):938-950.
7. P A. A Review on Diverse Biological Activities of Benzoxazole Molecule. World Journal of Pharmacy and Pharmaceutical Sciences. 2017:1779-1794.
8. Shrestha S, Wang B, Dutta P. Nanoparticle processing: Understanding and controlling aggregation. Advances in Colloid and Interface Science. 2020;279:102162.
9. Joudeh N, Linke D. Nanoparticle classification, physicochemical properties, characterization, and applications: a comprehensive review for biologists. Journal of Nanobiotechnology. 2022;20(1).
10. Kunjiappan S, Sankaranarayanan M, Karan Kumar B, Pavadai P, Babkiewicz E, Maszczyk P, et al. Capsaicin-loaded solid lipid nanoparticles: design, biodistribution, in silico modeling and in vitro cytotoxicity evaluation. Nanotechnology. 2020;32(9):095101.
11. Edo GI, Mafe AN, Ali ABM, Akpoghelie PO, Yousif E, Isoje EF, et al. Green Biosynthesis of Nanoparticles Using Plant Extracts: Mechanisms, Advances, Challenges, and Applications. BioNanoScience. 2025;15(2).
12. Thatyana M, Dube NP, Kemboi D, Manicum A-LE, Mokgalaka-Fleischmann NS, Tembu JV. Advances in Phytonanotechnology: A Plant-Mediated Green Synthesis of Metal Nanoparticles Using Phyllanthus Plant Extracts and Their Antimicrobial and Anticancer Applications. Nanomaterials. 2023;13(19):2616.
13. Amin N, Anwar J, Sulaiman A, Naumova NN, Anwar N. Hepatocellular Carcinoma: A Comprehensive Review. Diseases. 2025;13(7):207.
14. Yokoyama Y, Sasaki Y, Terasaki N, Kawataki T, Takekawa K, Iwase Y, et al. Comparison of Drug Metabolism and Its Related Hepatotoxic Effects in HepaRG, Cryopreserved Human Hepatocytes, and HepG2 Cell Cultures. Biological and Pharmaceutical Bulletin. 2018;41(5):722-732.
15. Arzumanian VA, Kiseleva OI, Poverennaya EV. The Curious Case of the HepG2 Cell Line: 40 Years of Expertise. Int J Mol Sci. 2021;22(23):13135.
16. Donato MT, Tolosa L, Gómez-Lechón MJ. Culture and Functional Characterization of Human Hepatoma HepG2 Cells. Methods in Molecular Biology: Springer New York; 2014. p. 77-93. http://dx.doi.org/10.1007/978-1-4939-2074-7_5
17. Alsafy MMM, Al-Hinai N, Alzebdeh KI, El-Shafey E-SI, Nassar MMA. Characterization of extracted bio-nano particles from date palm agro-residues. Journal of Materials Research and Technology. 2024;30:4939-4949.
18. Waqas M, Ahmed D, Qamar MT. Surfactant-mediated extraction of capsaicin from Capsicum annuum L. fruit in various solvents. Heliyon. 2022;8(8):e10273.
19. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Latha LY. Extraction, Isolation And Characterization Of Bioactive Compounds From Plants' Extracts. African Journal of Traditional, Complementary and Alternative Medicines. 2010;8(1).
20. Rj H. Availability and Standardization of Cell Lines at the American Type Culture Collection: Current Status and Prospects for the Future. Cell-Culture Test Methods: ASTM International100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 19428-2959; 1983. p. 114-126.
21. Leuchtenberger A. American Type Culture Collection Catalogue of Fungi/Yeasts. 17th edition, 1987. Herausgegeben von S. C. Jong und M. J. Gantt. 532 Seiten. American Type Culture Collection, Rockville, Maryland, USA 1987. Food / Nahrung. 1990;34(4):344-344.
22. van Meerloo J, Kaspers GJL, Cloos J. Cell Sensitivity Assays: The MTT Assay. Methods in Molecular Biology: Humana Press; 2011. p. 237-245.
23. Hock S. GraphPad, INPLOT and INSTAT, INDIGIT, Software from GraphPAD, 10855 Sorrento Valley Rd, Suite 204B, San Diego, CA 92121, USA, IBM/MS-DOS Compatible. Adv Mater. 1992;4(4):308-309.
24. Meyer DL. George W. Snedecor and William G. Cochran. Statistical Methods. Ames, Iowa: The Iowa State University Press, 1967, Pp. xiv + 593. $8.50. Psychometrika. 1968;33(4):507-508.
25. Tukey JW. Comparing Individual Means in the Analysis of Variance. Biometrics. 1949;5(2):99.
26. Motulsky H, Christopoulos A. Fitting Models to Biological Data Using Linear and Nonlinear Regression. Oxford University PressNew York, NY; 2004.