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RESEARCH PAPER

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

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ABSTRACT

Plant-derived medicinal extracts have found application in preparation of bioactive compounds which can be utilized in preparation of biomedical-based sustainable nanomaterials. This study involves extraction and characterization of two large constituents (dihydrocapsaicin (8-methyl-N-vanillylnonanamide and farnesyl phenyl sulfone (2E, 6E-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)sulfonyl benzene) in the seeds of the Iraqi green chili pepper (Capsicum annuum). A profile of cold maceration extraction of 10 days that depends on the solvent. The acetic acid was found to be more selective and effective to yield a percentage of high amount of dihydrocapsaicin (86) and farnesyl phenyl sulfone (10). Ethanol on the contrary was not as selective to give less dihydrocapsaicin (79%) and higher farnesyl phenyl sulfone (18%). Mechanical downsizing of nanoscale is obtained through ball milling which produces downsizing particles with high surface area and enhanced physicochemical properties. The structural confirmation tests were performed using FT-IR, UV-Visible spectroscopy, and GC-MS. In addition to this, field-emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM) were used to support the existence of nanoscale crystalline structures that have a unique 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.

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INTRODUCTION

Chili peppers (Capsicum spp.) are appreciated as food additives and as good sources of biologically active metabolites with a variety of pharmacological potential. The most widely studied of those is the capsaicin (or dihydrocapsaicin), which has strong antioxidant, anti-inflammatory and anti-cancer properties [1-3]. Its amphiphilic structure, a vanillyl group (4-hydroxy-3-methoxybenzyl) attached with an amide bond to a hydrophobic fatty acid chain (1, Fig. 1) increases its absorption into the membrane and interacts with the TRPV1 receptor, which modulates the ion transport, responses to oxidative stress and apoptosis among malignant cells [4-6].

In addition to dihydrocapsaicin (8-methyl-N-vanillylnonanamide), chili pepper seeds have other bioactive compounds, such as the sulfonylated terpenoid farnesyl phenyl sulfone [(2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl) sulfonyl)benzene] (2, Fig. 1). The backbone of it is an unsaturated terpenoid, which gives it high lipophilicity and free radical scavenging activity [3, 4]. The sulfone (SO_2) group provides chemical stability, polarity, and possible pharmacological activity. Sulfone molecules have been of great interest in medicinal chemistry as molecules exhibiting sulfone conjugations have been shown to increase metabolic stability and drug-like behavior, which ought to make the derivatives good potential therapeutic agents [7].

Agricultural byproducts like the seeds of chili peppers are usually discarded even though they are biochemically rich. By exploiting these materials, there is a sustainable way of achieving the recovery of natural products and value addition. The physicochemical and biological performance of isolated compounds can be further improved when these compounds are processed at the nanoscale. Aromatic fragment, conjugation double bonds, as well as hydrogen-bonding groups facilitate formation of supramolecular scaffold, improve the crystalline structure, and enable controlled surface features, leading to improve the key factors for biomedical applications by enhancing solubility, stability, and cellular internalization [8-10].

Plant-derived nanoparticles (phytonanoparticles) have become famous green and biocompatible drug delivery platforms, antimicrobial therapy systems, wound healing, and anticancer application systems in the last several years. Selective phytochemical caping agents reduce toxicity, and they are appropriately targeted in addition to efficient cellular internalization due to their nanoscale size, high surface to volume ratio, and targeted action. Remarkably, the synthesis of gold, silver, and zinc oxide nanoparticles prepared by plant extracts have exhibited the selective anticancer activity via the regulation of oxidative stress and the programmed cell death [11, 12].

The most widespread primary malignancy of the liver is called hepatocellular carcinoma (HCC), and is one of the main causes of cancer deaths on a global scale. It is also problematic in terms of late diagnosis, bad prognosis, high recurrence and due to resistance to chemotherapy [13]. HepG2 Cells This is a cell line derived in a human hepatocellular cancer and used extensively as an in vitro model to elucidate the biology of liver cancer and screen possible therapies [14]. Such cells have numerous differentiated hepatic functions including the secretion of albumin and the activity of drug-metabolizing enzymes, which have made them very useful in the assessment of natural compounds with anticancer properties. Earlier research has indicated that capsaicin promotes the induction of apoptosis in HepG2 cells through the effects of mitochondrial dysfunction, caspase activation, NF-kB and STAT3 inhibition [15, 16].

In this regard, the current work aimed to isolate and describe nanostructures of dihydrocapsaicin and farnesyl phenyl sulfone from Iraqi hot pepper

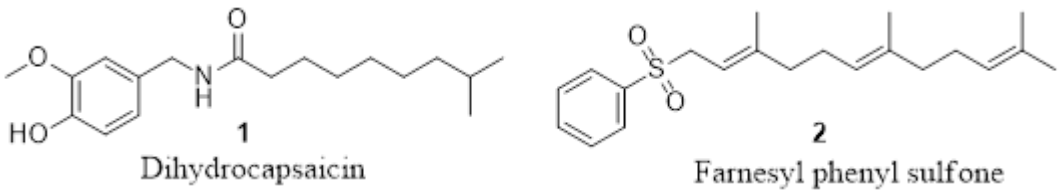

Fig. 1. The structure of Dihydrocapsaicin and Farnesyl phenyl sulfone.

seeds (Capsicum annuum). Cold maceration was performed using ethanol and acetic acid to extract these compounds (3), followed by purification and characterization through spectroscopic and microscopic methods. Finally, the cytotoxic properties of the extracts were evaluated in vitro on HepG2 liver cancer cells, with normal human dermal fibroblast neonatal cells (HDFn) serving as controls. This paper highlights chili pepper seeds as an untapped agricultural waste and a potential source of nanostructural bioactive compounds with possible applications in anticancer therapy and nutraceutical production.

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

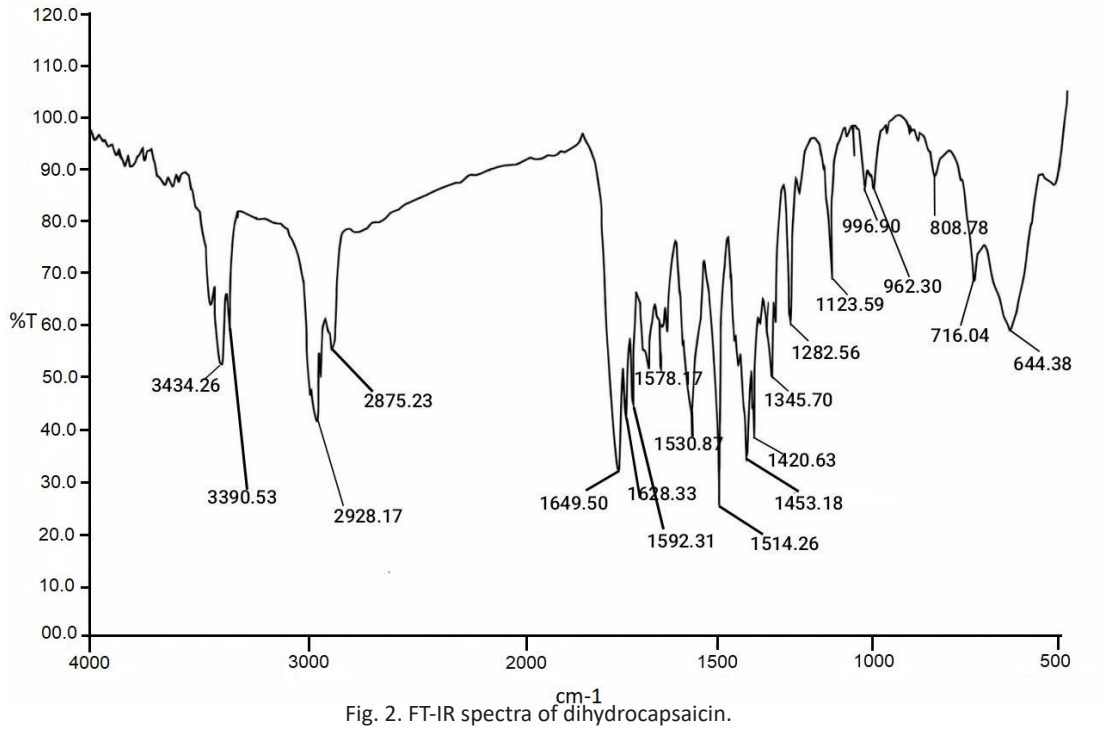

Fig. 2. FT-IR spectra of dihydrocapsaicin.

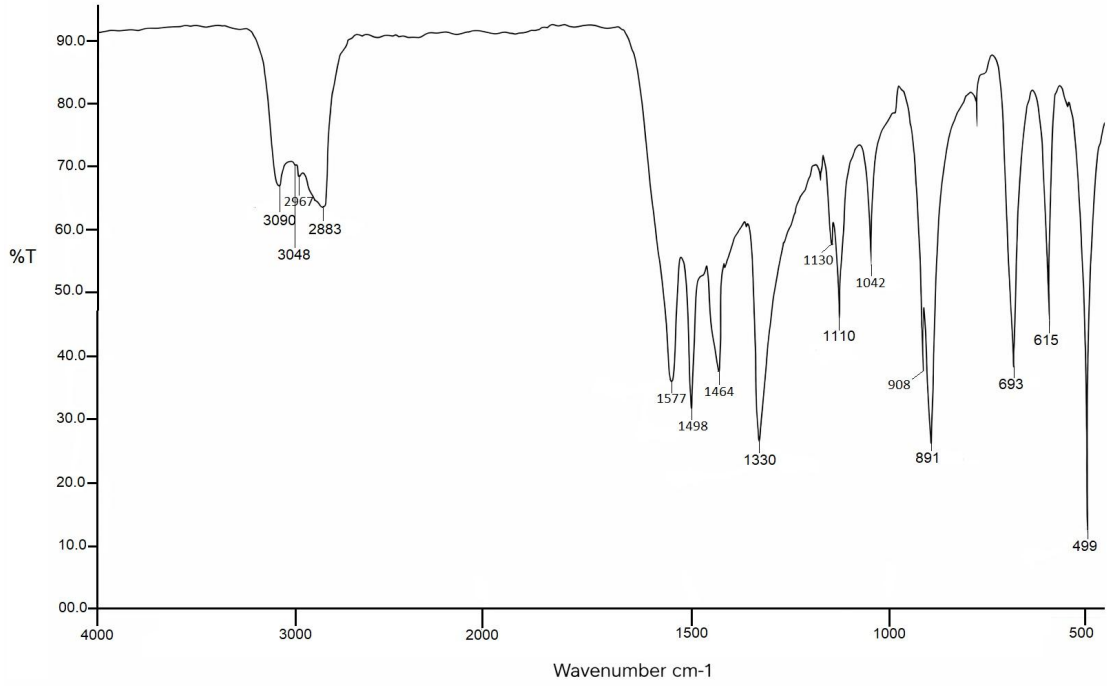

Fig. 3. FT-IR spectra of farnesyl phenyl sulfone.

optimal recovery of bioactive compounds. Ethanol extraction gave a higher percentage of dihydrocapsaicin of about 79% and farnesyl phenyl sulfone of about 18% whereas the acetic acid extraction showed a more specific extract of dihydrocapsaicin of about 86% and farnesyl phenyl sulfone of about 10%. This selectivity can be explained by the fact that acetic acid is more polar and acidic and thus it will increase the solubilization of dihydrocapsaicin and minimize the co-extraction of other less compatible lipophilic compounds. On the contrary, the intermediate polarity of ethanol enables the extract to obtain both moderately polar and lipophilic constituents simultaneously. Whereas ethanol enhances co-extraction which could promote synergistic biological activity, more selective acetic acid can be beneficial in cases where enrichment of a select bioactive is required.

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

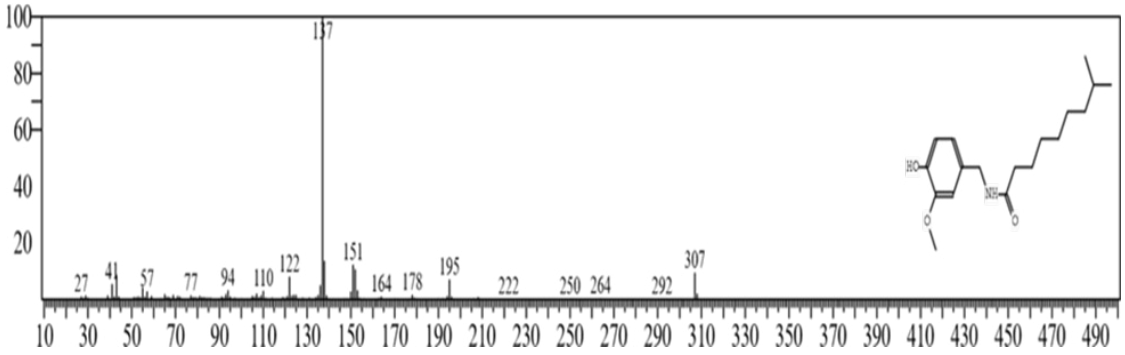

Fig. 4. GC-mass spectra of the dihydrocapsaicin extract.

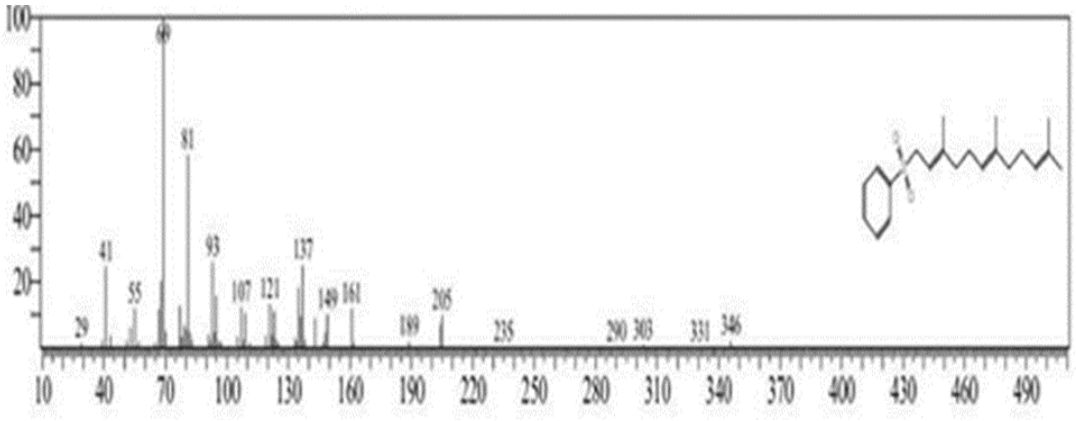

Fig. 5. GC-mass spectra of the farnesyl phenyl sulfone extract.

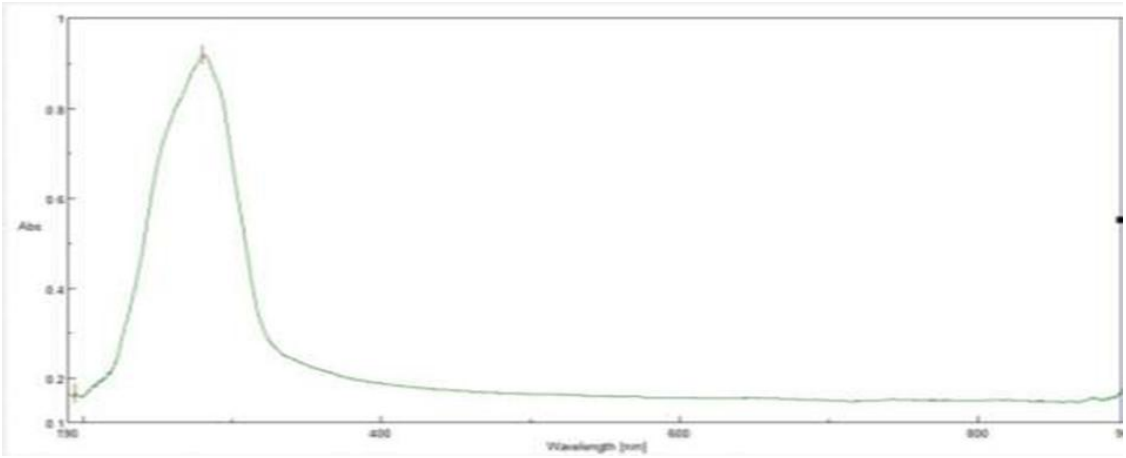

Fig. 6. The ultra Uv-Vis spectra of dihydrocapsaicin.

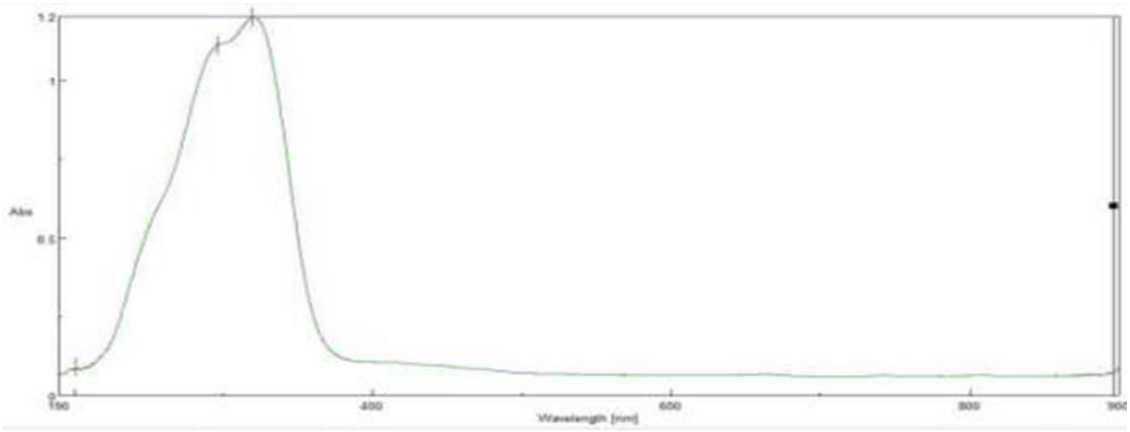

Fig. 7. The Uv-Vis spectra of the farnesyl phenyl sulfone.

successful isolation (Fig. 5).

UV-Viss spectra of the dihydrocapsaicin extract

The UV–Visible absorption spectrum of the dihydrocapsaicin extract displayed two 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 typical of the structure of the dihydrocapsaicin structure and stipulate the conjugated aromatic system and the non-bonding electron interactions. In contrast, farnesyl phenyl sulfone extract had three different absorption peaks with 201.2 nm, 296.0 nm and 319.6 nm. They were attributed to π→π*, n→π*, and charge-transfer (CT) transitions, respectively (Fig. 7). The formation of a CT band demonstrates increased level of electron delocalization of the molecule which is in accordance with the conjugated terpenoid-sulfonyl structure.

Study the constitutional morphological qualities of the particles surface

FESEM and Transmission Electron Microscopy (TEM) were used to study the structural characteristics of the synthesized nanoparticles. FESEM micrographs confirmed that the nanoparticles of hot pepper seed were relatively uniform with little aggregation. The particles were majorly spherical to semi-spherical shaped with an average diameter of the particles of about 100

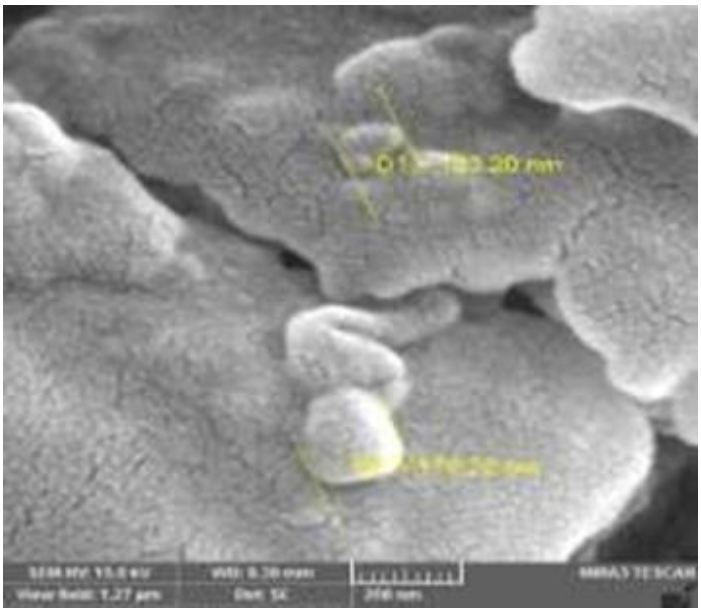

Fig. 8. FESEM interpretation for Nano-particles of the hot pepper seeds.

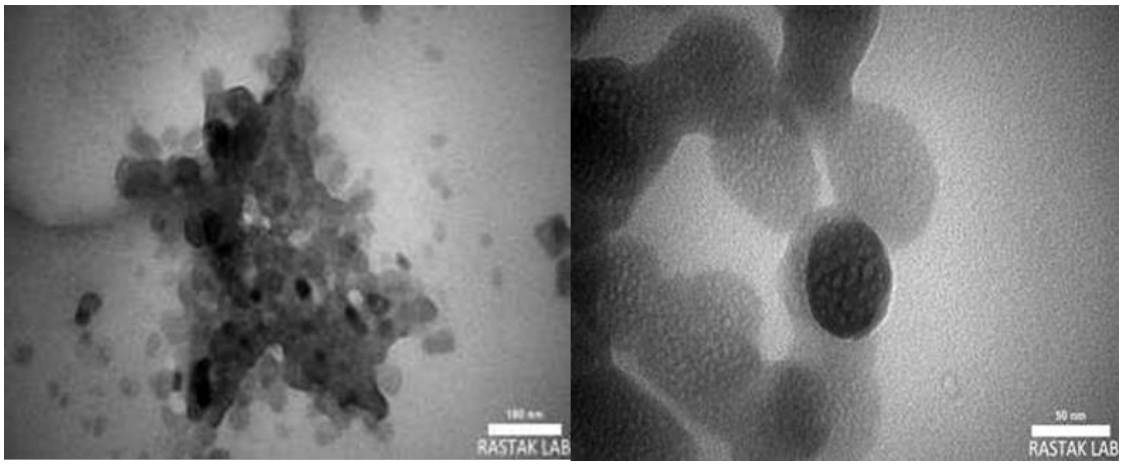

Fig. 9. TEM interpretation for Nano-particles of the hot pepper seeds.

nm (Fig. 8). The topology of the uniform surface forms the evidence of the successful formation of the nanoparticles and the equilibrium dispersion. Additional structural validation was done through Transmission Electron Microscopy (TEM). The nanoparticles had a distinct internal structure as shown by the nanoparticles in Fig. 8 where the particle size was about 50-100 nm (Fig. 9).

The size distribution seen by TEM is in the nanoscale and it justifies the findings of FESEM and proves that there was a successful production of nanoparticles that can be used in biology. It has been established that nanoparticles in this size regime have increased cellular uptake by endocytosis, especially in cancer cells that are more metabolically active. Nanoscale size is also associated with an increase in the surface area and this could facilitate the delivery and intracellular

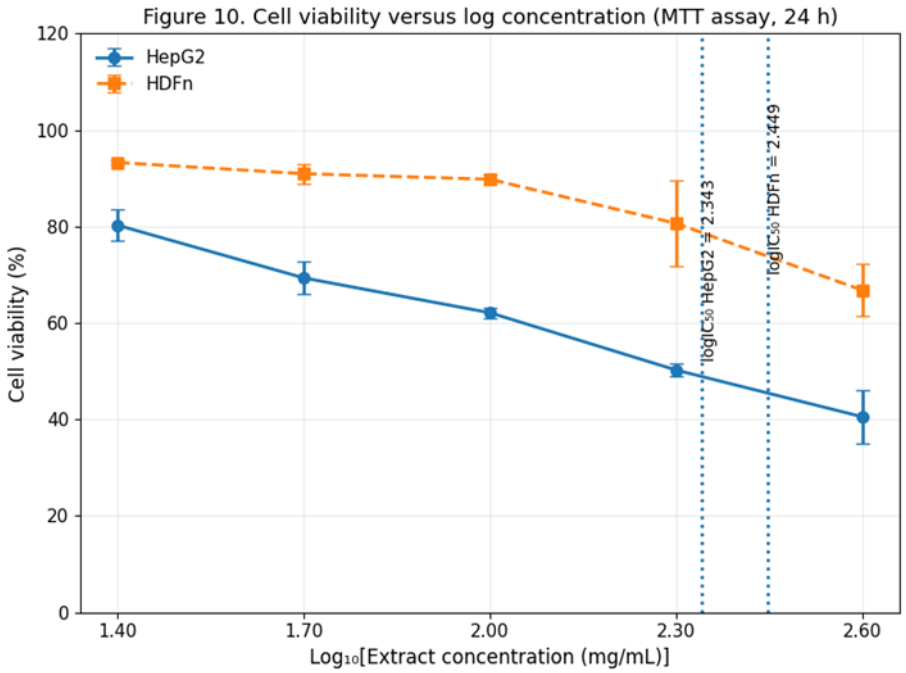

Fig. 10. Cell viability (%) vs Log_{10} concentration (with mean ± SD error bars).

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.

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 (25-400mg/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.

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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.